Ministry of Environment
and Food of Denmark
Environmental
Protection Agency

Category approach for selected brominated flame retardants

Preliminary structural grouping of brominated flame retardants

Environmental project No. 1872, 2016

Title:

Category approach for selected brominated flame retardants

Authors:

Eva Bay Wedebye
Nikolai Georgiev Nikolov
Elsa Nielsen
Julie Boberg
Marta Axelstad
Trine Klein Reffstrup
Marianne Dybdahl

Acknowledgements for contributions to the work: Lea Bredsdorff

Published by:

The Danish Environmental Protection Agency
Strandgade 29
1401 Copenhagen K
Denmark
www.mst.dk/english

Year:

2016

ISBN no.

978-87-93435-90-2

Contents

1. Introduction 10

1.1 Non-testing data 10
1.2 (Q)SAR 11
1.3 Grouping and read-across 11
1.4 Preliminary structural grouping of BFRs 12
1.5 Category hypothesis and (Q)SAR predictions for a selected BFR group 12
1.6 Evaluation of experimental data and identification of critical effect 12
1.7 Preliminary category justification and perspectives 12

2. Preliminary structural grouping of BFRs 13

2.1 Data preparation 13
2.2 (Q)SAR predictions 16
2.3 Retrieval of ToxCast information 16
2.4 Grouping 17
2.5 Identified groups from the preliminary structural grouping 18
2.5.1 Group 1 (Aromatic substances) 20
2.5.2 Group 2 (dibromo-(2,3- dibromopropoxy)benzene derivatives) 22
2.5.3 Group 3 (Cycloalkanes) 23
2.5.4 Group 4 (Phthalate acid and its anhydride) 25
2.5.5 Group 5 (Phenols and bisphenols) 26
2.5.6 Group 6 (benzyl ethyl oxygen bridge derivatives) 27
2.5.7 Group 7 (Methoxy dibromobenzene derivatives) 28
2.5.8 Group 8 (Phthalates/Benzoate) 30
2.5.9 Group 9 (TBBPA ethers) 31
2.5.10 Group 10 (small alkyl alkohols) 33
2.5.11 Group 11 (TBBPA esters/acrylate) 34
2.5.12 Group 12 (Phosphates) 35
2.5.13 Group 13 (Triazines) 37
2.5.14 Group 14 (Biphenyles) 38
2.5.15 Group 15 (Diphenyl ethers) 39
2.5.16 Singletons 40

3. (Q)SAR predictions 42

3.1 Definition of the category 42
3.2 Identification of category members 42
3.3 ToxCast/Tox21 43
3.4 (Q)SAR models applied 43
3.5 Results from the (Q)SAR predictions 43
3.5.1 Membership of existing OECD HPV Chemical Categories or US-EPA New Chemical Categories 43
3.5.2 Bioavailability 43
3.5.3 Metabolism / transformation 43
3.5.4 Endocrine endpoints 44
3.5.5 Repro-developmental toxicity 44
3.5.6 Organ toxicity 44
3.5.7 Repeated dose toxicity 44

3.5.8 Genotoxicity and carcinogenicity 44

4. Experimental data 57

4.1 Data collection and evaluation of data 57
4.2 Experimental studies on repeated dose toxicity and carcinogenicity 60
4.2.1 Liver 67
4.2.2 Kidney and urinary bladder 67
4.2.3 Oral cavity, esophagus, forestomach, and small and large intestines 69
4.2.4 Hematopoietic system 69
4.2.5 Spleen 70
4.2.6 Thyroid gland 70
4.2.7 Pancreas 70
4.2.8 Mammary gland 70
4.2.9 Clitoral gland 71
4.2.10 Seminal vesicle 71
4.2.11 Nose 71
4.2.12 Lung 71
4.2.13 Skin and Zymbal gland 72
4.2.14 Harderian gland 73
4.2.15 Eye 73
4.2.16 Mesothelioma 73
4.2.17 Other tumors 73
4.3 Experimental studies on reproductive toxicity 74
4.4 Experimental studies on toxicokinetics 74
4.5 Studies of mode/mechanisms of action 75
4.5.1 Genotoxicity 75
4.5.2 Endocrine activity 76
4.6 Identification of critical effect(s) 76
4.6.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0) 76
4.6.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9) 77
4.6.3 2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA) (CAS RN 36483-57-5) 78
4.6.4 Conclusion on the critical effect, experimental studies 78

5. Category approach for read-across 79

5.1 Future perspectives 81
5.2 Conclusion 81

Appendix 1: Start list with 67 substances

Appendix 2: Start list substances with pictures

Appendix 3: (Q)SAR models

Appendix 4: (Q)SAR based clusterings

Appendix 5: Category members

Appendix 6: Heat maps of(Q)SAR results

Appendix 7: OECD (Q)SAR Application Toolbox profiler alerts explanations

Appendix 8: Literature search document

Appendix 9: Information matrix for category source substances

Foreword

This report describes a project carried out by researchers from the National Food Institute, Technical University of Denmark (DTU).

Preliminary structural grouping of 67 brominated flame retardants and a preliminary category approach for the purpose of endpoint-specific read-across for a selected group of brominated flame retardants has been performed in the project.

The project steering group members were, besides the authors from the DTU, Grete Lottrup Lotus, Dorte Lerche Bjerregaard, Magnus Løfstedt and Elisabeth Paludan from the Danish Environmental Protection Agency (EPA).

The project was financed by the Danish EPA.

Conclusion and Summary

The aim of this project was to attempt grouping of a number of identified brominated flame retardants (BFRs) found in a survey performed in 2014 for the Danish Environmental Protection Agency (Danish EPA 2014). The grouping was performed for 67 brominated flame retardants based on their chemical structures and resulted in 15 preliminary structural groups and 7 substances remaining as "singletons". (Q)SAR predictions for a number of environmental and health effects within these initial groups were generated and investigated.

One of the groups; small linear and branched brominated alkyl alcohols, was chosen for further investigation. The category, defined as having 3-5 carbons, 2-3 bromine atoms and 1-2 alcohol groups comprised 61 members.

(Q)SAR predictions were performed for the members of the category. Predictions for carcinogenic and mutagenic/genotoxic properties indicated that the 61 members in the category of small linear and branched brominated alkyl alcohols have a carcinogenic potential with a possible mutagenic/genotoxic mode of action. The estimated specificities of the applied (Q)SAR models as established by leave-many-out cross-validations are between 85.9% and 95.1%, i.e. the overall false positive rates of the models are around 5%- 14%. From the identified alerts in a number of OECD (Q)SAR Application Toolbox profilers, there was one alert which was identified in all 61 category members, namely the "Aliphatic halogen" alert in the three ISS (Istituto Superiore di Sanita, Rome Italy) profilers for *in vitro* mutagenicity (Ames test), *in vivo* mutagenicity (Micronucleus, in rats and mice) and carcinogenicity (genotoxic and non-genotoxic). From the explanation of the alert contained in the OECD (Q)SAR Application Toolbox there does not seem to be one single mechanistic interpretation of the ISS aliphatic halogen alert in relation to mutagenicity and cancer. The "Aliphatic halogen" alert identified 34% false positives among the mutagenicity training set chemicals (Kazius et al. 2005). According to Benigni et al. (2008 and 2010) it has a positive predictivity (proportion of substances with the alert that are true positive) for carcinogenicity of 74%.

A literature search was performed to collect experimental data on human health effects for the 25 category members with a CAS RN assigned. Relevant experimental data on human health effects were only retrieved for two of the category members identified in the preliminary structural group, i.e. 2,3-dibromo-1-propanol (2,3-DBPA) and 2,2-bis(bromomethyl)-1,3-propanediol (DBNPG). For a third category member identified in the preliminary structural group, i.e. 2,2-bis-(bromomethyl)-3-bromo-1-propanol (TBNPA), relevant experimental data on human health effects were retrieved from the REACH registration dossier.

The critical effect of these three members of the category with relevant experimental data on human health effects is the multiple-organ carcinogenic effect, most probably exerted by a genotoxic mode of action either by the parent compound itself (2,3-DBPA) or by a metabolite of the parent compound (DBNPG and TBNPA). Furthermore, 1,3-dibromo-2-propanol (1,3-DBPA), a REACH pre-registered compound for which no experimental data on human health effects were retrieved, has a notified classification for a possible carcinogenic potential (Carc. 2 H351).

Possible read-across for the critical effect from the three category members with experimental data and the one member with a classification for the identified critical effect to the remaining 57 structurally similar target analogues in the category is supported by the following observations:

a) The experimental data show comparable toxicological effects for the three members of the category identified in the preliminary structural group (2,3-DBPA, DBNPG and TBNPA), i.e. carcinogenic and mutagenic/genotoxic effects.
b) The classifications (harmonized or notified) as Muta. 1B H340 / Muta. 2 H341 and/or Carc. 1B H350 / Carc. 2 H351 for these three members and for 1,3-DBPA.
c) The (Q)SAR predictions for carcinogenic and mutagenic/genotoxic properties indicate that the 61 category members have a carcinogenic potential with a possible mutagenic/genotoxic mode of action. The structural alerts identified in the OECD (Q)SAR Application Toolbox indicate that all members share the same genotoxic/mutagenic mode of action with some variations in their possible mechanisms of action. Some alerts were identified in many or all of the members and/or their metabolites pointing to possible common mechanism(s) of action (e.g. metabolic activation to reactive carbonyl compounds and aldehyde Schiff base formation of DNA adducts and cross-links).

As there is only experimental information for a small number of the members, an even more robust basis for read-across for the category could be pursued by 1) searching the literature for information on carcinogenicity and mutagenicity/genotoxicity on structural analogues outside, but structurally close to the category, 2) experimental testing for mutagenicity/genotoxicity on representative members across the category, as well as 3) further analysis of the underlying mechanisms of action.

Other brominated flame retardants that are metabolised to one of the 61 brominated flame retardants in the category of small linear and branched alkyl alcohols may equally likely as these members themselves possess the critical effect, i.e. the carcinogenic effect, most probably exerted by a mutagenic/genotoxic mode of action.

Konklusion og sammenfatning

Formålet med dette projekt var at forsøge at gruppere et antal bromerede flammehæmmere, som blev identificeret i en undersøgelse foretaget i 2014 for Miljøstyrelsen (Danish EPA 2014). Grupperingen blev foretaget for 67 bromerede flammehæmmere baseret på deres kemiske strukturer og resulterede i 15 præliminære strukturelle grupper og 7 stoffer kategoriseret som "enkeltstoffer". Der blev efterfølgende udarbejdet (Q)SAR forudsigelser for en række miljø- og sundhedseffekter for stofferne i de præliminære grupper.

En af grupperne; små lineære og forgrenede bromerede alkylalkoholer, blev udvalgt til yderligere undersøgelse. Kategorien blev defineret som stoffer med 3-5 kulstofatomer, 2-3 bromatomer og 1-2 alkoholgrupper og den bestod af 61 medlemmer.

For alle medlemmer af kategorien blev der foretaget (Q)SAR forudsigelser for en række sundhedsskadelige effekter. Forudsigelser for kræftfremkaldende og genotoksiske effekter indikerede, at de 61 medlemmer af kategorien havde et potentiale for kræftfremkaldende effekt med en mulig mutagen/genotoksisk virkningsmåde. De anvendte (Q)SAR modeller havde ifølge krydsvalideringsresultater ("leave-many-out") beregnede specificiteter mellem 85,9% and 95,1%, dvs. ifølge disse resultater var de overordnede falsk-positive rater omkring 5%- 14%. Ud af de identificerede strukturelle alerts ved kørsler af "profilers" i OECD (Q)SAR Application Toolbox, var der én alert, som blev identificeret i alle 61 medlemmer af kategorien, nemlig "Aliphatic halogen" i de tre ISS (Istituto Superiore di Sanita, Rome Italy) profilers for "*in vitro* mutagenicity (Ames test)", "*in vivo* mutagenicity (Micronucleus, in rats and mice)" og "carcinogenicity (genotoxic and non-genotoxic)". Ud fra forklaringerne i OECD (Q)SAR Application Toolbox'en for denne ISS alifatisk halogen alert er der tilsyneladende ikke én enkelt mekanistisk fortolkning af alertens relation til den mutagene og kræftfremkaldende effekt. Alerten "Aliphatic halogen" identificerede ifølge Kazius et al. (2005) 34% falsk positive ud af træningssættet af mutagene kemiske stoffer. Ifølge Benigni et al. (2008 and 2010) har alerten en positiv prædiktivitet (andel af stoffer med denne alert som er sande positive) for en kræftfremkaldende effekt på 74%.

Der blev foretaget en litteratursøgning for at indsamle eksperimentelle data vedrørende sundhedsskadelige effekter for de 25 kategorimedlemmer med et tildelt CAS registreringsnummer. Relevante eksperimentelle data blev kun fundet for to kategorimedlemmer fra den præliminære strukturelle gruppe, nemlig for 2,3-dibrom-1-propanol (2,3-DBPA) og 2,2-bis(bromomethyl)-1,3-propandiol (DBNPG). For et tredje kategorimedlem fra den præliminære strukturelle gruppe, 2,2-bis-(bromomethyl)-3-brom-1-propanol (TBNPA), blev relevante eksperimentelle data indhentet fra REACH registreringsdossieret.

Den kritiske effekt for de tre kategorimedlemmer med relevante eksperimentelle data, var en kræftfremkaldende effekt i mange væv og organer, sandsynligvis via en mutagen/genotoksisk virkningsmåde, forårsaget enten af moderstoffet (2,3-DBPA) eller af en metabolit af moderstoffet (DBNPG og TBNPA). Derudover har 1,3-dibrom-2-propanol (1,3-DBPA), et REACH præ-registreret kemisk stof for hvilket der ikke blev fundet eksperimentelle data vedrørende sundhedsskadelige effekter, en notificeret klassificering for muligt kræftfremkaldende potentiale (Carc. 2 H351).

Anvendelse af read-across for den kritiske effekt fra de tre kategorimedlemmer med eksperimentelle data og det ene medlem med en klassificering for den identificerede kritiske effekt til de resterende 57 strukturelle analoger i kategorien understøttes af følgende observationer:

a) De eksperimentelle data viste sammenlignelige toksiske effekter for de tre kategorimedlemmer identificeret i den præliminære strukturelle gruppering (2,3-DBPA, DBNPG and TBNPA), dvs. kræftfremkaldende og mutagene/genotoksiske effekter.
b) Klassificeringerne (harmoniserede eller notificerede) som Muta. 1B H340 / Muta. 2 H341 og/eller Carc. 1B H350 / Carc. 2 H351 for disse tre medlemmer og for 1,3-DBPA.
c) (Q)SAR forudsigelserne for kræftfremkaldende og mutagene/genotoksiske egenskaber indikerer, at de 61 kategorimedlemmer har et kræftfremkaldende potentiale med en mulig mutagen/genotoksisk virkningsmåde. De strukturelle alerts identificeret i OECD (Q)SAR Application Toolbox'en indikerer, at alle medlemmerne udøver deres effekter ved en mutagen/genotoksisk virkemåde med nogle variationer i deres mulige virkningsmekanismer. Nogle alerts blev identificeret i mange eller alle medlemmer og/eller deres metabolitter og peger på mulige fælles virkningsmekanismer (f.eks. metabolisk aktivering til reaktive carbonylstoffer og aldehyd Schiff-base-dannelse af DNA addukter og krydsbinding).

Da der kun er fundet eksperimentelle data for et lille antal af kategorimedlemmerne, ville et mere robust grundlag for read-across for kategorien kunne understøttes ved yderligere information som for eksempel 1) litteratursøgning for eksperimentelle data vedrørende kræftfremkaldende og mutagene/genotoksiske effekter for stoffer udenfor men strukturelt set tæt på kategorien, 2) yderligere testning for mutagene/genotoksiske effekter på repræsentative kategorimedlemmer, og 3) yderligere analyser vedrørende virkningsmekanismer.

Andre bromerede flammehæmmere, som i kroppen nedbrydes til et af de 61 medlemmer i kategorien af små bromerede lineære og forgrenede alkylalkoholer, har sandsynligvis samme potentiale for den kritiske effekt som de 61 kategorimedlemmer, dvs kræftfremkaldende effekt sandsynligvis via en mutagen/genotoksisk virkningsmåde.

1. Introduction

The aim of this project was to attempt grouping of a number of identified brominated flame retardants (BFRs) found in a survey performed in 2014 for the Danish Environmental Protection Agency (Danish EPA 2014). The grouping was performed on the basis of the chemical structures, and trends in (Q)SAR predictions for a number of environmental and health effects within the structural groups were investigated.

From the initial groups, one was chosen and further defined and investigated as a category . All theoretical members of the category were considered in terms of both experimental (where CAS RNs could be identified) and predicted information. It was assessed if a critical health effect seemed to exist based on the available information. Experimental information was available for only three of the category members. A preliminary category approach to perform read across for the identified critical effect from the members with experimental information to the members without experimental information was performed.

With this project the Danish EPA wished to explore the possibility to address BFRs by a grouping approach rather than as individual substances, as there are many BFRs with similar chemical structures and as the regulation of individual substances may be very time demanding. Addressing groups of BFRs in relation to regulation is not new. Polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs), both having 209 theoretically possible congeners, are treated as groups in the RoHS Directive (EU 2011), where there in the homogeneous materials in electric and electronic equipment must be no more than 0.1% in total of either PBDEs or PBBs. Some theoretical members of the chosen group in this project may not currently be on the market or even synthesized. However, they are relevant to include as they could potentially be used in the future to substitute the analogues currently in use.

In the following short introductions to non-testing data, (Q)SAR, grouping and read-across are given. The text builds to a large extent on the REACH guidance chapter R.6, where further information can be found (European Chemicals Agency 2008).

1.1 Non-testing data

Non-test methods are non-experimental methods or approaches that can be used to provide data for the assessment of chemicals. Non-testing data can be generated by three main approaches:

a) (quantitative) structure-activity relationships, (Q)SARs
b) grouping approaches, which include structure analogues and formation of chemical categories for possible read-across
c) expert systems

The development and application of all kinds of non-testing methods is based on the similarity principle, i.e. the hypothesis that similar compounds should have similar biological activities.

Expert systems are compilations of models consisting of combinations of SARs, QSARs and databases and will not be described further in this report, as they were not applied in the project. (Q)SARs, grouping and read-across is further described in the following.

1.2 (Q)SAR

SARs and QSARs, collectively referred to as (Q)SARs, are theoretical models that can be used to predict in a qualitative or quantitative manner the physical-chemical, biological (e.g. toxicological) and environmental fate properties of compounds from knowledge of their chemical structure. The two terms can be defined as follows:

A SAR is a qualitative relationship that relates a chemical (sub)structure ("alert") to the presence or absence of a property or activity of interest. The substructure may consist of adjacently bonded atoms, or an arrangement of non-bonded atoms that are collectively associated with the property or activity. Generally, no applicability domain (AD, see below) is defined for SARs.

A QSAR is a mathematical model (often a statistical correlation) relating one or more quantitative parameters (molecular descriptors) derived from the chemical structure to a quantitative measure of a property or activity. QSARs are quantitative models yielding a continuous or categorical result. Generally, QSARs are associated with a defined AD.

The AD is the physicochemical, structural, or biological space of the training set on which the model was developed, and for which it is applicable to make predictions for new compounds. The AD should be described in terms of the most relevant parameters, i.e. usually those that are descriptors of the model. Ideally, the model should only be used to make predictions within that domain by interpolation and not extrapolation. The accuracy of a model is determined within the defined AD, i.e. a model can have more than one AD definition, with each their accuracy level. The accuracy is determined by robust leave-many-out cross-validation and if possible with external validations with a data set representative of the full AD.

1.3 Grouping and read-across

Category and analogue approaches are techniques for grouping chemicals. Read-across is a technique of filling data gaps in either approach.

The term analogue approach is used when the grouping is based on a very limited number of chemicals, where trends in properties are not apparent.

A chemical category is a set of chemicals whose physical-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic).

Grouping is performed on the basis of chemical structure similarity and possible knowledge of properties in relation to the endpoint(s) of interest. (Q)SAR predictions can be included in relation to the latter. The (Q)SAR information can be employed to predict trends as well as breakpoints in trends, and therefore possible subcategories. As far as possible, the predictions and trends established by (Q)SAR methods should be verified by comparison with experimental data.

Experimental and predicted information for the category members are collected and analysed to identify possible common behaviour or consistent trends. If present, they are generally associated with a common underlying mechanism of action, or a mechanism of action that exhibits intensity changes in a consistent manner across the different members of a category. When identified, the common behaviour or consistent trends can form basis to make read-across from some category members with experimental data ("source substances") to others that lacks data for an endpoint ("target substances"), if the overall data set allows the estimation of the hazard for the missing data points. If so, it is possible to extend the use of measured data to similar untested chemicals.

Knowledge of the expected effect(s) of the category together with information on use and exposure can help in deciding not only whether additional testing of relevance to the predicted effect(s) is

needed, but also the nature and scope of any testing that needs to be carried out. A category test plan can be designed to provide information to characterize the group as a whole rather than to fill every data point for every chemical in the category. This reflects an approach that is more efficient from a testing perspective than test plans for obtaining data on individual chemicals of commercial interest.

1.4 Preliminary structural grouping of BFRs

In this project it was investigated how a number of BFRs identified in the survey for the Danish EPA could be grouped dependent on their chemical structure. Within the initial structural groups, possible trends in (Q)SAR predictions for a large number of environmental and health effects were used to make further endpoint-area specific groupings. Furthermore, it was mapped which of the substances had US EPA ToxCast™ and Tox21 *in vitro* experimental results or were REACH registered to give indications of availability of experimental data for members of the initial structural groups. One group was chosen for further work to explore a possible category approach.

1.5 Category hypothesis and (Q)SAR predictions for a selected BFR group

A working definition of the chosen category was made and all theoretical structural members were identified and predicted by existing (Q)SAR models for relevant health related endpoints. The applicability domains for the most relevant models in relation to the members of the category were mapped to see how well they covered the span of the category members.

1.6 Evaluation of experimental data and identification of critical effect

For all the structural members of the chosen category possible CAS numbers were identified. A literature search in the scientific literature was performed for all the individual members of the group with identified CAS numbers to gather possible experimental information of relevance in relation to a number of health endpoints. The focus of the literature search was to identify a critical health effect, if possible.

1.7 Preliminary category justification and perspectives

Based on the (Q)SAR analysis and evaluation of available experimental data for a possible critical effect, a preliminary justification to do read-across for the critical effect in a category approach was attempted.

2. Preliminary structural grouping of BFRs

2.1 Data preparation

In the Danish Environmental Protection Agency, Environmental Project No. 1536, 2014 "Survey of brominated flame retardants" Table 1 contains BFRs which are either pre-registered under REACH or imported/produced by EU manufacturers. In total, the table contains 65 substances. The substances in Table 1 of the survey are the primary focus of this project. However, a number of additional structures were included to get a broader picture of brominated flame retardants used worldwide and to support the grouping and possible development of meaningful categories. Hence, BFRs listed in Table 2 from the survey report were also included. Table 2 contains 14 BFRs, which were identified in the survey, but which are not pre-registered or registered under REACH but rather manufactured outside the EU. Furthermore, a quick search for BFRs on the Internet and collection of CAS numbers from different sites returned 6 additional BFRs (Fujitsu 2015) not covered by Table 1 or 2 of the survey. These were also included. In total these three sources gave a brutto list of 85 substances.

Structure information in the form of SMILES[1] was retrieved from ChemIDplus or ChemSpider. When information could not be found in these sources, the CAS number was searched in SciFinder, which links to the Chemical Abstracts Service ("CAS"). SMILES cannot be retrieved directly from SciFinder so in five cases the SMILES notations were generated by hand. All the SMILES notations were checked to see if the EPI Suite program could read them and if the program displayed the correct 2D structures. In a few cases where this was not achieved in the EPI Suite program, the SMILES notations were edited manually.

Of the 65 substances in Table 1 of the survey report a total of 48 were included in the project exercise as only aromatic, cycloaliphatic and aliphatic BFRs with a defined chemical structure were included. The reason was that inorganic substances and polymers cannot be subject to the (Q)SAR model systems applied. Furthermore, structure information should be available in order to make (Q)SAR predictions.

The remaining 17 substances in the survey report Table 1 were either confidential (7 substances, i.e. no structure information), polymers (9 substances: CAS RN 148993-99-1, 158725-44-1, 59447-57-3, 68441-62-3, 68928-70-1, 71342-77-3, 88497-56-7, 94334-64-2, 1195978-93-8) or the chemical structure could not be retrieved as it was not unambiguous and a representative SMILES could not be found (1 substance, CAS RN 135229-48-0). However, 5 of the 48 included substances lacked an unambiguously defined 2D chemical structure according to SciFinder (CAS RNs 155613-93-7, 25637-99-4, 32534-81-9, 32536-52-0, 36355-01-8), but for each of these substances a representative SMILES was included in the exercise.

[1] A prerequisite for making predictions with (Q)SAR software is that structural information in the format of e.g. SMILES strings is available for the substances that should be predicted. The Simplified Molecular-Input Line-Entry System (SMILES) is a specification in form of a line notation for describing the structure of chemical species using short text strings.

Of the 14 substances in the survey report Table 2 a total of 13 were included. The remaining substance (CAS RN 168434-45-5) did not have an unambiguous chemical structure and a representative SMILES was not included.

Furthermore, as mentioned above, 6 additional BFRs were included.

This gave a final 'start list' with 67 substances as listed in Table 1 below. The list including information about the source of the substance, possible REACH registration and inclusion in ToxCast/Tox21 etc. is given in Appendix 1. In Appendix 2 the 2D structures of the substances are given together with predictions of bioavailability (Lipinski's rule-of-five) and a few physical-chemical properties.

CAS RN	Substance name	Abbreviated name
1084889-51-9	Octabromotrimethyl- phenyl indane	OBTMPI
1163-19-5	Bis(pentabromophenyl) ether	decaBDE
118-79-6	2,4,6-Tribromophenol	TBP
126-72-7	tris(2,3-dibromopropyl) phosphate	TDBPP
13654-09-6	Decabromo-1,1'-biphenyl	DecaBB
1522-92-5	Tribromoneopentyl alcohol [same substance as CAS RN 36483-57-5]	TBNPA
155613-93-7	1H-Indene, 2,3-dihydro -1,1,3-trimethyl-3-phenyl-, octabromo deriv	OBTMPI
183658-27-7	2-ethylhexyl-2,3,4,5- tetrabromobenzoate	EH-TBB
19186-97-1	Tri[3-bromo-2,2- bis(bromomethyl)propyl]phosphate.	TTBNPP
20566-35-2	2-(2-Hydroxyethoxy)ethyl 2- hydroxypropyl 3,4,5,6-tetrabromophthalate	HEEHP-TEBP
21850-44-2	1,1'-(Isopropylidene) bis[3,5- dibromo-4-(2,3-dibromopropoxy)benzene]	TBBPA-BDBPE
23488-38-2	2,3,5,6-Tetrabromo-p- xylene	TBX
25327-89-3	1,1'-Isopropylidenebis[4- (allyloxy)-3,5- dibromobenzene]	TBBPA- bAE
25495-98-1	Hexabromocyclodecane	HBCYD
25637-99-4	Hexabromocyclododecane	HBCDD
25713-60-4	1,3,5-Triazine, 2,4,6- tris(2,4,6- tribromophenoxy)-	TTBP-TAZ
26040-51-7	Bis(2-ethylhexyl) tetrabromophthalate	BEH-TEBP
26762-91-4	Tribromo-phenyl-allyl-ether, unspecified	AO-TBB2
3072-84-2	2,2'-[(1- Methylethylidene)bis[(2,6- dibromo-4,1-phenyle- le-ne)oxymethylene]]bisoxiran e	TBBPA- BGE
31780-26-4	Dibromostyrene	DBS
3194-55-6	1,2,5,6,9,10- Hexabromocyclododecane	HBCDD
3194-57-8	Cyclooctane, 1,2,5,6- tetrabromo	TBCO

3234-02-4	2,3-Dibromo-2-butene-1,4-diol	DBBD1
32534-81-9	Diphenyl ether, pentabromo derivative	pentaBDE
32536-52-0	Diphenyl ether, octabromo derivative	octaBDE
32588-76-4	N,N'-ethylenebis(3,4,5,6- tetrabromophthalimide)	EBTEBPI
3278-89-5	2-(allyloxy)-1,3,5-tribromobenzene	TBP-AE
3296-90-0	2,2- bis(bromomethyl)propane-1,3-diol	DBNPG
3322-93-8	1,2-Dibromo-4-(1,2- dibromoethyl)cyclohexane	DBE- DBCH
33798-02-6	4,4'-isopropylidenebis[2,6- dibromophenyl] diacetate	TBBPA-bOAc
34571-16-9	1,2,3,4,7,7-Hexachloro-5- (tetrabromo-phenyl)bicyclo[2.2.1]hept-2- ene	HCTBPH
35109-60-5	1,3,5-tribromo-2-(2,3- dibromopropoxy)benzene	DPTE
3555-11-1	Allyl pentabromophenyl ether	PBPAE
36355-01-8	Hexabromo-1,1'-biphenyl	HexaBB
36483-57-5	2,2-dimethylpropan-1-ol, tribromo derivative	TBNPA
37419-42-4	Phenol, 4,4′-(1- methylethyli- dene)bis[2,6dibromo-, dipropanoate (9CI)	TBBPA-BP
37853-59-1	1,1'-[ethane-1,2- diylbisoxy]bis[2,4,6- tribromobenzene]	BTBPE
37853-61-5	Benzene, 1,1′-(1- methylethylidene) bis[3,5-dibromo-4-methoxy	TBBPA-BME
38521-51-6	Benzene, 1,2,3,4,5- pentabromo6- (bromomethyl)	PBBB
39569-21-6	Benzene, 1,2,3,4- tetrabromo-5-chloro-6- methyl-	TBCT
39635-79-5	4,4'-sulphonylbis[2,6- dibromophenol]	TBBPS
4162-45-2	4,4'-isopropylidenebis(2- (2,6- dibromophenoxy)ethanol)	TBBPA- BHEE
42757-55-1	bis[3,5-dibromo-4-(2,3- dibromopropoxy)phenyl] sulphone	TBBPS-BDBPE
497107-13-8	Benzene, 1,1′- [oxybis(methylene)]bis [2,3,4,5,6-pentabromo(9CI)	DBDBE
51936-55-1	7,8-Dibromo-1,2,3,4,11,11- hexachloro-1,4,4a,5,6,7,8,9,10,10a-decahydro-1,4- methanobenzocyclooctene	DBHCTD
52434-90-9	1,3,5-Tris(2,3- dibromopropyl)-1,3,5- triazine-2,4,6(1H,3H,5H)-trione	TDBP-TAZTO
52907-07-0	Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide)	EBDBNDC2
55205-38-4	2-Propenoic acid, 1,1′- [(1-methylethylidene) bis(2,6-dibromo-4,1- phenylene)] ester	TBBPA-BA
55481-60-2	Bis(methyl)tetrabromophtalate	BM-TEBP2
57829-89-7	1-(2,3-Dibromopropyl)-3,5-diallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione	DBP-TAZTO
58495-09-3	Benzene, 1,2,3,4,5- pentabromo6- (chloromethyl)	PBBC

58965-66-5	1,2,4,5-tetrabromo-3,6- Bis(pentabromophenoxy) benzene	4′-PeBPOB-DE208
59447-55-1	(Pentabromophenyl)methyl acrylate	PBB-Acr
607-99-8	2,4,6,-tribromoanisol	TBA
608-71-9	Pentabromophenol	PBP
615-58-7	2,4-dibromophenol	DBP
632-79-1	Tetrabromophthalic anhydride	TEBP-Anh
66710-97-2	2-Propenoic acid, 1,1′[(1- methylethylidene)bis[(2,6- dibromo-4,1phenylene)oxy-2,1-ethanediyl]] ester	TBBPA-BHEEBA
70156-79-5	Benzene, 1,1′- sulfonylbis[3,5-dibromo-4-methoxy	TBPPS-BME
75790-69-1	TBPA, glycol-and propylene-oxide esters	TBPA-esters2
75795-16-3	1,3-Bis(2,3- dibromopropyl)-5-allyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione	BDBP-TAZTO
79-94-7	2,2',6,6'-Tetrabromo-4,4'- isopropylidenediphenol	TBBPA
84852-53-9	1,1'-(Ethane-1,2- diyl)bis[pentabromobenzene]	DBDPE
85-22-3	2,3,4,5,6- Pentabromoethylbenzene	PBEB
87-82-1	Hexabromobenzene	HBB
87-83-2	2,3,4,5,6- Pentabromotoluene	PBT
96-13-9	2,3-Dibromo-1-propanol	2,3-DBPA2

TABLE 1 THE 67 BFRS INCLUDED

2.2 (Q)SAR predictions

(Q)SAR models from Leadscope Predictive Data Miner, MultiCASE CASE Ultra, PASS, EPI Suite and the (Q)SAR Application Toolbox (profilers) were included. The models covered environmental (persistent, bioaccumulative and toxic: PBT and very persistent and very bioaccumulative: vPvB), health and mechanistic endpoints (carcinogenicity, genotoxicity, repro-developmental effects, endocrine activity, skin sensitization, liver toxicity, cardiotoxicity etc.) and a few endpoints for absorption, distribution, metabolism and excretion (ADME). In total, the 67 substances were predicted in >150 (Q)SAR models. To assign PBT and vPvB flags the thresholds applied in the REACH Guidance on Information Requirements and Chemical Safety Assessment R.11 PBT_vPvB assessment (European Chemicals Agency 2014, Tyle et al. 2002) were applied on the basis of predictions of biodegradation (Biowin 2 or 6 showed not ready/readily biodegradable and Biowin 3 < 2.2 meaning ultimate biodegradation in weeks to months), and BCF (B: >2000 and vB: >5000) from EPI Suite and acute aquatic toxicity from DTU models for fish, daphnia and algae. A full list of the included endpoints is given in Appendix 3.

2.3 Retrieval of ToxCast information

The publicly available ToxCast data were downloaded from the United States Environmental Protection Agency (US-EPA) homepage (ToxCastTM Data 2015). The data is organized into different data sets and includes among other things brief descriptions of ToxCast chemicals and assays, files summarizing the screening results from ToxCast (high-throughput data from ~1,800 chemicals), and EPA's analysis of the chemicals screened through the federal Toxicity Testing in the 21st century (Tox21) partnership and archived ToxCast data from older data releases and

publications. The information from ToxCast and Tox21 was used both in the preliminary structural grouping of all BFRs and to support the choice of the selected group and the critical effect.

ToxCast data
Summary files with data for more than 1,800 chemicals and 821 assay endpoints for 20 variables such as the activity or hit call, activity concentrations etc. were downloaded. The following file was used: AllResults_hitc_Matrix_141121.csv. Six of the 67 substances from Appendix 1 were identified in the file (CAS RN 118-79-6, 126-72-7, 26040-51-7, 3194-55-6, 79-94-7, 3296-90-0). They are marked in the column “ToxCast” in Appendix 1.

Tox21 data
EPA’s analysis of the chemicals screened through the federal Toxicity Testing in the 21st century (Tox21) which includes EPA’s activity calls from the screening of 8,599 Tox21 unique substances in the robotic screening was downloaded. The following file was used: ToxCast_Tox21_Level5&6_20141022.csv. Twenty of the 67 substances from Appendix 1 were identified in the file (CAS RNs 118-79-6, 126-72-7, 25327-89-3, 26040-51-7, 3194-55-6, 32534-81-9, 32536-52-0, 3278-89-5, 3322-93-8, 4162-45-2, 608-71-9, 79-94-7, 85-22-3, 87-82-1, 1522-92-5, 3234-02-4, 96-13-9, 21850-44-2, 3296-90-0, 632-79-1). They are marked in the column “Tox21” in Appendix 1.

2.4 Grouping

Leadscope is a predictive data-mining tool for exploring and filtering chemical data sets based on both structural features and associated data. This software contains a predefined library of over 27,000 chemical functional groups (medicinal chemistry building blocks), which can be applied in the analysis of structural similarities within data sets and to perform grouping.

All the generated (Q)SAR predictions and identified training set data, when available, for the 67 substances were imported into the Leadscope program. Only (Q)SAR predictions which were inside the defined applicability domains of the models were imported. Also imported into Leadscope were the ToxCast data for 6 substances and Tox21 data for 20 substances.

The 67 substances were upon import organized by Leadscope based on the internal library of chemical structural features. This structural organization on top level 1 and 2 in Leadscope is shown in Figure 1. The length of the bars indicates the frequency of a structural feature on a logarithmic scale from 1 to 100. A structure can have more than one structural feature.

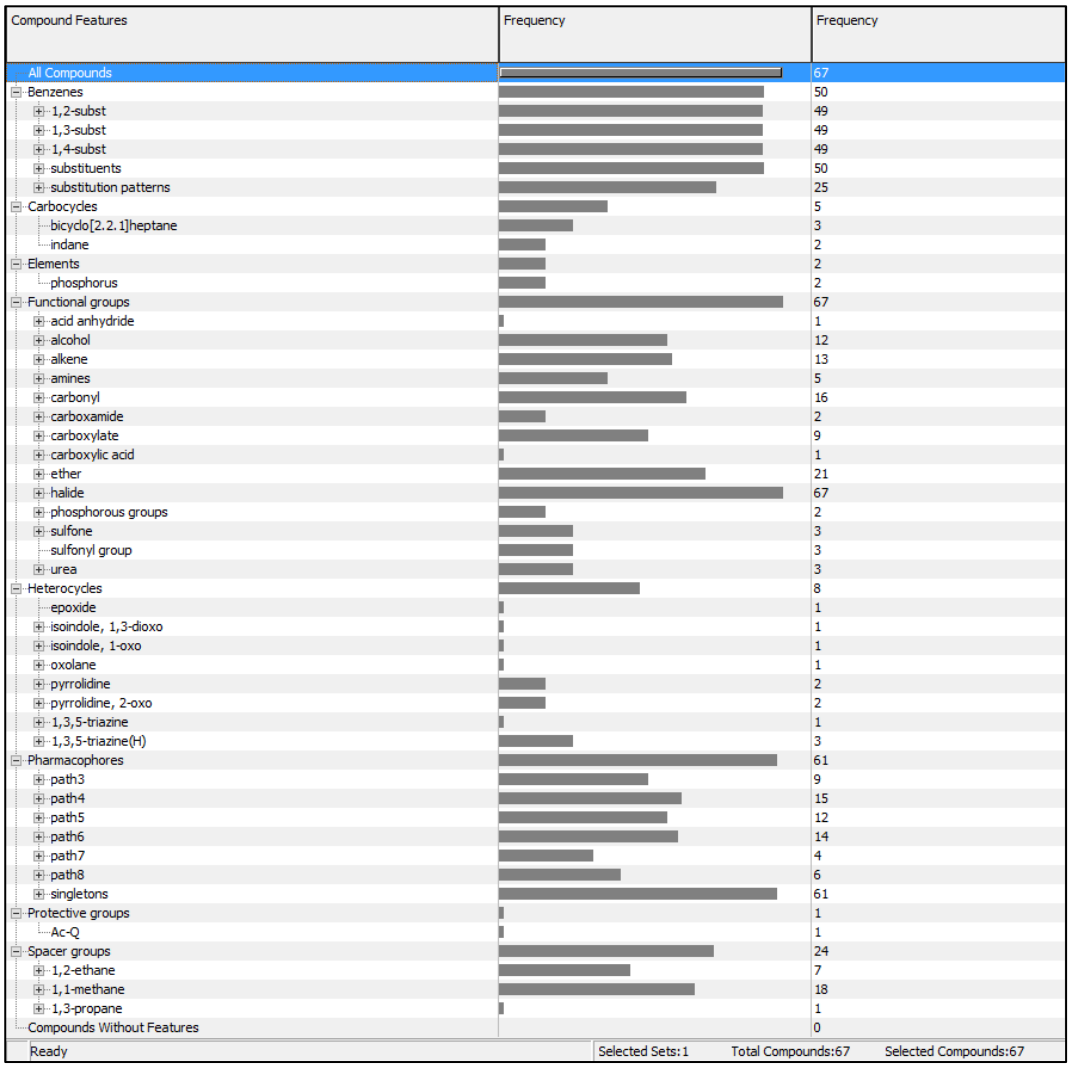


FIGURE 1 STRUCTURAL GROUPING OF THE 67 BFRs ON TOP LEVEL 1 AND 2 IN LEADSCOPE

The program was applied to analyse and attempt possible groupings, denoted as “clusterings” in Leadscope, based on structural similarity alone or including (Q)SAR predictions. Clusterings based on information from ToxCast/Tox21 experimental data did not result in meaningful groups, likely because of the vast amount of information for only a limited part of the 67 substances.

2.5 Identified groups from the preliminary structural grouping

Leadscope clustering based solely on chemical structure resulted in 15 groups, in Leadscope denoted as clusters, which covered 60 out of the 67 structures[2]. The last 7 structures are denoted ‘singletons’ in Leadscope, meaning that they did not cluster/group together with any of the other structures in the set (see section 2.5.1-2.5.16).

The ID numbers of the structures presented are the CAS numbers prepended by a short abbreviation which shows the origin of the substance. ‘Br1_’ denotes that the survey report Table 1 was the origin of the substance. ‘Br1R_’ likewise denotes that the survey report Table 1 was the origin of the substances and furthermore that it was marked as solely reactive flame retardant (‘R’).

[2] The settings applied for the structural clustering in Leadscope were as follows. Analysis type: Similarity of structural features, hierarchical (agglomerative nesting) cluster method, cluster described by signature (substructure representative of the cluster), average linkage mechanism, cluster threshold distance: 0.5.

'Br2_' denotes that the survey report Table 2 was the origin of the substance. 'Br3_' denotes the 6 additional substances as marked in Appendix 1 with source "3".

The structure signatures are the substructures that Leadscope chose to build the group around. These signatures, or representative substructures, are marked with purple in the members of the group. In some cases a member did not contain the substructure but was allocated to the group because of high structural similarity.

All the substances included in initial structural groups were checked to see if they were among the ToxCast/Tox21 substances and thereby had experimental in vitro data. Six substances were found to be in ToxCast and 20 substances were found to be in Tox21 (overlapping the 6 ToxCast substances). The ToxCast/Tox21 results were used in the considerations of the choice of a group for further work into a possible category.

To give a quick indication of whether further experimental information might be available for the members of the clusters it was noted if they were registered under REACH. In the selection of a group for a possible category approach, preference was given to groups where REACH registrations were made for at least two members, as this indicated possible experimental information, which is important to have for a representative number of the members to be able to find possible trends in the critical effect and to perform read-across for the full group. For groups without at least two REACH registered members there may possibly be experimental information in the scientific literature, but a literature search was not made at this stage of the project.

To give rough indications of the possible relevance of the different groups in relation to a number of effect areas, (Q)SAR predictions-based clusterings were performed in Leadscope[3]. The individual clusterings were in each case performed for the full set of 67 substances based on (Q)SAR predictions for carcinogenicity, genotoxicity, reproductive toxicity effects, endocrine activity and skin sensitization. Each (Q)SAR predictions-based clustering produced its own set of clusters. The resulting (Q)SAR-based clusterings for the different effect areas are given in Appendix 4, and referred to in the tables in section 2.5.1-2.5.16.

A number of models were included within each effect area. For example for carcinogenicity, the predictions included were from seven commercial CASE Ultra FDA models (male rat, female rat, male mouse, female mouse, rat, mouse, and rodent) and OECD (Q)SAR Application Toolbox carcinogenicity relevant profilings (Oncologic primary classification and ISS carcinogenicity alerts).

The Toolbox profilers were run on the parent compounds (i.e. known and simulated metabolites were not included because of feasibility) and in order to be able to perform clustering on the results, the identified alerts were "binarized", meaning that every possible positive alert outcome was split out as a separate yes/no prediction. The derived Toolbox (TB) fields with individual numbers are given in Appendix 4.

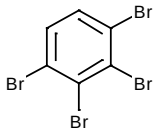
For each structural group it was investigated if the members of the group belong to the same (Q)SAR predictions-based clusters. Some of the resulting (Q)SAR predictions-based clusters within the different effect areas are chemically quite big and broad. This may not be surprising, since different molecular structures may for example have similarities in their predicted genotoxicity profiles because of common or similar alerts in the applied models even though they have chemical differences.

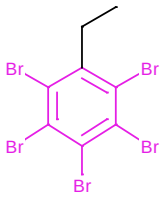
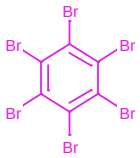
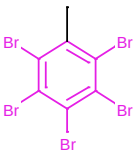
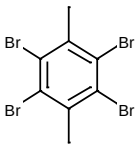
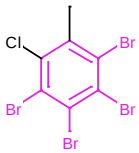
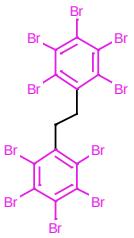
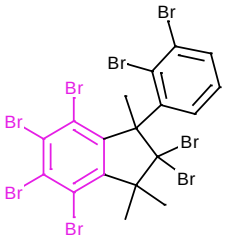
[3] The settings applied for the (Q)SAR-based clusterings in Leadscope were as follows. Analysis type: Similarity of data, hierarchical (agglomerative nesting) cluster method, cluster described by signature (substructure representative of the cluster), average linkage mechanism, cluster threshold distance: 0.5.

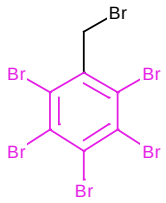
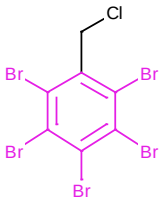
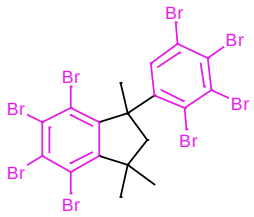
For each group presented in section 2.5.1-2.5.16, results in relation to the different (Q)SAR-based clusters are presented. Furthermore, possible positive (Q)SAR indications for the group as a whole are given in brief. More detailed inspections of the (Q)SAR predictions and the ToxCast/Tox21 results for the individual substances were done for a number of groups, which were considered as candidates for a possible category approach, but this is not presented here.

It should be bared in mind that a category hypothesis is endpoint specific, and in theory a given category may be relevant for read-across in relation to one effect and not others. When looking at how the chemical structures group in the rough (Q)SAR predictions-based cluster outputs, it is also very important to remember that not all 67 substances were within the applicability domain (AD) of all the applied (Q)SAR models. So the observed differences between the clusters within the different effect areas for a given structural group could either be due to differences in predictions (within AD), or differences in relation to whether the individual structures were within the ADs of the models, or both.

2.5.1 Group 1 (Aromatic substances)

Structure signature

Cluster 1

			
Br1_85-22-3	Br1_87-82-1	Br1_87-83-2	Br1_23488-38-2
			
Br1_39569-21-6	Br1_84852-53-9	Br1_155613-93-7	

 Br2_38521-51-6	 Br2_58495-09-3	 Br2_1084889-51-9

Abbreviated names for members	85-22-3: PBEB 87-82-1: HBB 87-83-2: PBT 23488-38-2: TBX 39569-21-6: TBCT 84852-53-9: DBDPE 155613-93-7: OBTMPI 38521-51-6: PBBB 58495-09-3: PBBC 1084889-51-9: OBTMPI
Members that are REACH registered	DBDPE
Members that are in ToxCast/Tox21	Tox21: HBB
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Neutral organics: PBEB, HBB, PBT, TBX, TBCT, PBBB, PBBC
Carcinogenicity (Q)SAR-based clustering	The two OBTMPI structures are in cluster 5, PBEB, HBB, PBT, TBX and TBCT are in cluster 6, and PBBB and PBBC are in cluster 12.
Genotoxicity (Q)SAR-based clustering	OBTMPIs, PBEB, HBB, PBT, TBX and TBCT are in cluster 3, and PBBB and PBBC are in cluster 1.
Reproductive toxicity (Q)SAR-based clustering	PBEB, HBB, PBT, TBCT, DBDPE, OBTMPI (CAS 155613-93-7), PBBB and PBBC are in cluster 2, and TBX and OBTMPI (CAS 1084889-51-9) are singletons.
Endocrine (Q)SAR-based clustering	PBEB, DBDPE, PBBB, PBBC and OBTMPI (CAS 1084889-51-9) are in cluster 1, HBB is in cluster 7, PBT, TBX and TBCT are in cluster 6, and OBTMPI (CAS 155613-93-7) is in cluster 3.
Skin sensitization	OBTMPIs, PBEB, HBB, PBT, TBX and TBCT are in cluster 3, and PBBB and PBBC are in cluster 5.

OBTMPI (CAS 155613-93-7) is an incompletely defined substance, and the molecular structure is a representative of the substance.

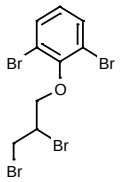
This is chemically a broad structural group, which may not as a whole be suited as a category for read-across, but which have common properties and may also have possible relevant subgroups. For example, PBBB and PBBC which are chemically quite similar, although one bromine in the first is replaced by chlorine in the second, seem according to the rough (Q)SAR-based clusterings to have similar predicted profiles for carcinogenicity, genotoxicity, reproductive toxicity effects, endocrine activity and skin sensitization. Likewise, the OBTMPIs have not surprisingly according to the

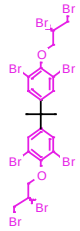
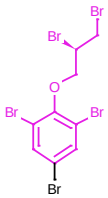
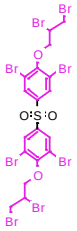
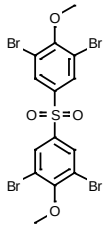
applied (Q)SAR models also similar profiles, at least for carcinogenicity, genotoxicity and skin sensitization.

Seven members were predicted to be persistent and bioaccumulative (based on bioconcentration predictions). Furthermore, all members have some positive indications for genotoxicity and carcinogenicity, and all have one or more positive indications for effects within reproductive toxicity. Two members have positive indications within the applicability domain for skin sensitization.

One member, DBDPE, is found to be REACH registered, and another member, HBB, is found to be in Tox21. The identified sources of experimental information are in itself not enough to give promise of possible successful read-across for the large group.

2.5.2 Group 2 (dibromo-(2,3- dibromopropoxy)benzene derivatives)

Structure signature

Cluster 2

			
Br1R_21850-44-2	Br1_35109-60-5	Br1_42757-55-1	Br2_70156-79-5

Abbreviated names for members	21850-44-2: TBBPA-BDBPE 35109-60-5: DPTE 42757-55-1: TBBPS-BDBPE 70156-79-5: TBPPS-BME
Members that are REACH registered	TBBPA-BDBPE
Members that are in ToxCast/Tox21	Tox21: TBBPA-BDBPE
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Neutral organics: DPTE, TBPPS-BME
Carcinogenicity (Q)SAR-based clustering	TBPPS-BME is in cluster 4, TBBPA-BDBPE and TBBPS-BDBPE are in cluster 7, and DPTE is a singleton
Genotoxicity (Q)SAR-based clustering	TBBPA-BDBPE, DPTE and TBBPS-BDBPE are in cluster 9, and

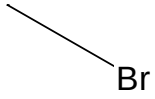
	TBPPS-BME is in cluster 3.
Reproductive toxicity (Q)SAR-based clustering	TBBPA-BDBPE and DPTE are in cluster 3, and TBBPS-BDBPE and TBPPS-BME are in cluster 2.
Endocrine (Q)SAR-based clustering	TBBPA-BDBPE is in cluster 4, and DPTE, TBBPS-BDBPE and TBPPS-BME are in cluster 1.
Skin sensitization	TBBPA-BDBPE, TBBPS-BDBPE and DPTE are in cluster 1, and TBPPS-BME is in cluster 2.

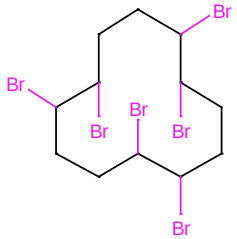
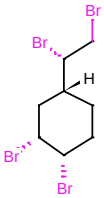
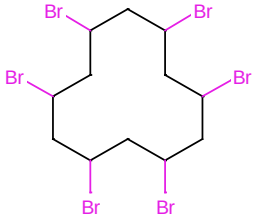
This group is centered around the cluster signature (dibromo-(2,3- dibromopropoxy)benzene). It is noted that TBPPS-BME does not contain the cluster signature. According to the rough (Q)SAR-based clusterings this substance (TBPPS_BME) falls in different clusterings than the other members for carcinogenicity, genotoxicity and skin sensitization. TBBPA-BDBPE, DPTE and TBBPS-BDBPE seem to have similar profiles for genotoxicity and skin sensitization. This could possibly be a relevant group for the three members containing the signature, if it is the same active metabolite, e.g. 2,3-dibromopropanol, which is responsible for the effect for which read-across is attempted.

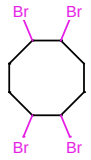
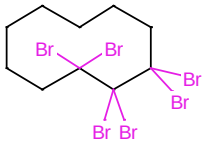
All members were predicted to be persistent, and DPTE and TBPPS-BME were predicted to be vPvB. Where the models could make robust predictions, TBBPA-BDBPE, DPTE and TBBPS-BDBPE have many positive indications for genotoxicity and carcinogenicity, however also with indication that carcinogenicity may possibly be specific to rodents. TBBPA-BDBPE and DPTE have a few positive hits for reproductive toxicity.

One member, TBPPA-BDBPE, is found to be both REACH registered and in Tox21. The identified sources of experimental information are in itself not enough to give promise of possible successful read-across for the group as it only covers one member.

2.5.3 Group 3 (Cycloalkanes)

Structure signature
 Cluster 3

 Br1_3194-55-6	 Br1_3322-93-8	 Br1_25637-99-4

 Br2_3194-57-8	 Br2_25495-98-1

Abbreviated names for members	3194-55-6: HBCDD 3322-93-8: DBE-DBCH 25637-99-4: HBCDD 3194-57-8: TBCO 25495-98-1: HBCYD
Members that are REACH registered	HBCDD (under CAS No 25637-99-4)
Members that are in ToxCast/Tox21	ToxCast: HBCDD (CAS 3194-55-6) Tox21: HBCDD (CAS 3194-55-6), DBE-DBCH
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	-
Carcinogenicity (Q)SAR-based clustering	All except DBE-DBCH (which is a singleton) are in cluster 3
Genotoxicity (Q)SAR-based clustering	HBCDD (CAS 3194-55-6) and TBCO are in cluster 5, DBE-DBCH is a singleton, HBCDD (CAS 25637-99-4) is in cluster 3, and HBCYD is in cluster 8.
Reproductive toxicity (Q)SAR-based clustering	HBCDD (CAS 3194-55-6), DBE-DBCH, TBCO and HBCYD are in cluster 3, and HBCDD (CAS 25637-99-4) is in cluster 2.
Endocrine (Q)SAR-based clustering	HBCDDs and HBCYD are in cluster 1, DBE-DBCH is a singleton, and TBCO is in cluster 6.
Skin sensitization	HBCDDs, DBE-DBCH and TBCO are in cluster 1, and HBCYD is in cluster 2.

HBCDD (CAS 25637-99-4) is an incompletely defined substance, and the molecular structure is a representative of the substance.

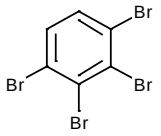
This is chemically a broad structural group, which need thorough consideration before possible read-across between members. The two HBCDDs are chemically the closest analogues, although the different positions (alpha/beta vs. alpha/gamma) of the bromine atoms may give different activities. According to the rough (Q)SAR-based clusterings the two HBCDDs have similar profiles for carcinogenicity, endocrine activity and skin sensitization. HBCDD (CAS 3194-55-6), DBE-DBCH, TBCO and HBCYD all have bromine in alpha/beta position (however with HBCYD having two bromines at the same carbon) and these substances have according to the rough (Q)SAR based clusterings similar profiles for reproductive toxicity effects. HBCDD (CAS 3194-55-9) and TBCO have similar (Q)SAR-based profiles for carcinogenicity, genotoxicity, reproductive toxicity and skin sensitization.

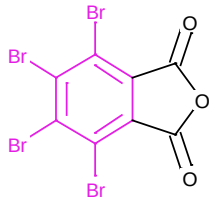
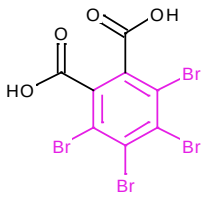
HBCDDs and HBCYD were predicted to be vPvB. HBCDD (CAS 3194-55-6) is included in training sets for DTU models with negative experimental results for Ames, AR antagonism and ER agonism.

Where the models could make robust predictions, all members had a number of positive indications for genotoxicity. Especially DBE-DBCH had many positive genotoxicity indications. HBCDDs, DBE-DBCH and TBCO were positive within AD in a number of cancer models. HBCDD (CAS 3194-55-6), DBE-DBCH, TBCO and HBCYD had a few positive hits for reproductive toxicity and cardio toxicity.

One member, HBCDD (CAS 3194-55-6), is found to be both REACH registered and in Tox21, and DBE-DBCH is found to be in Tox21. The identified sources of experimental information are in itself not enough to give promise of possible successful read-across.

2.5.4 Group 4 (Phthalate acid and its anhydride)

Structure signature
 Cluster 4

 Br1R_632-79-1	 Br3_75790-69-1

Abbreviated names for members	632-79-1: TEBP-Anh 75790-69-1: TBPA (CAS refers to mixed esters)
Members that are REACH registered	TEBP-Anh
Members that are in ToxCast/Tox21	Tox21: TEBP-Anh
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Anhydrides, Carboxylic acid: TEBP-Anh Neutral organics: TBPA
Carcinogenicity (Q)SAR-based clustering	Both are in cluster 11.
Genotoxicity (Q)SAR-based clustering	Both are in cluster 2.
Reproductive toxicity (Q)SAR-based clustering	Both are in cluster 4.
Endocrine (Q)SAR-based clustering	Both are in cluster 6.
Skin sensitization	TEBP-Anh is a singleton and TBPA is in cluster 3.

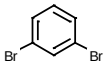
Please note that for TBPA the CAS refers to mixed esters with diethylene glycol and propylene glycol. However, in this exercise the phthalic acid without esters parts was applied in the (Q)SAR profiling.

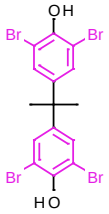
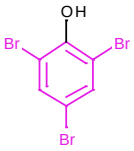
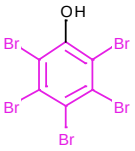
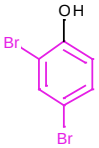
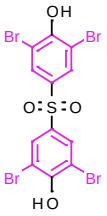
The two members are chemically very similar with TEBH-Anh being the anhydride of TBPA. According to the rough (Q)SAR-based clusterings they also have similar profiles for carcinogenicity, genotoxicity, reproductive toxicity and endocrine activity.

TEBP-Anh is predicted to be PBT and TBPA is predicted to be persistent. TEBP-Anh is included in the training set for the DTU Ames model with a negative experimental result. Both have some positive indications for carcinogenicity. TEBP-Anh has positive indications of sensitization. Both have a few positive indications for reproductive toxicity.

TEBP-Anh is found to be both REACH registered and in Tox21. The identified sources of experimental information could maybe be used in an analogue read-across for TBPA. However, as the CAS refers to mixed esters of TBPA in this case it would not be relevant.

2.5.5 Group 5 (Phenols and bisphenols)

Structure signature
 Cluster 5

 Br1_79-94-7	 Br1_118-79-6	 Br1_608-71-9
 Br1_615-58-7	 Br1_39635-79-5	

Abbreviated names for members	79-94-7: TBBPA 118-79-6: TBP 608-71-9: PBP 615-58-7: DBP 39635-79-5: TBBPS
Members that are REACH registered	TBBPA, TBP
Members that are in ToxCast/Tox21	ToxCast: TBBPA, TBP Tox21: TBBPA, TBP, PBP
Members of OECD HPV Chemical	-

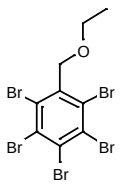
Categories	
Members of US-EPA New Chemical Categories	Phenols (Acute toxicity): TBBPA, TBP, PBP, DBP, TBBPS
Carcinogenicity (Q)SAR-based clustering	TBP and DBP are in cluster 13, TBBPA and TBBPS are in cluster 14, and PBP is a singleton.
Genotoxicity (Q)SAR-based clustering	TBBPA, TBP, DBP and TBBPS are in cluster 7, and PBP is in cluster 3.
Reproductive toxicity (Q)SAR-based clustering	All are in cluster 2.
Endocrine (Q)SAR-based clustering	TBBPA is in cluster 4, TBP and PBP are in cluster 10, DBP and TBBPS are singletons.
Skin sensitization	TBBPA, TBP, DBP and TBBPS are in cluster 2, and PBP is in cluster 3.

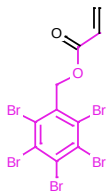
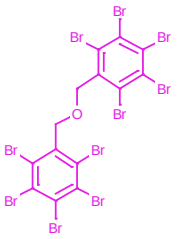
This group contains both phenols and bisphenols, and possible subgrouping may be relevant. According to the rough (Q)SAR-based clusterings they all have similar reproductive toxicity profiles. TBP and DBP have similar profiles for carcinogenicity, genotoxicity and skin sensitization. TBBPA and TBBPS likewise have similar profiles for carcinogenicity, genotoxicity and skin sensitization.

TBBPA, TBP, PBP and TBBPS are predicted to be persistent, TBBPA is predicted to be vPvB and PBP is predicted to be PBT. TBBPA, TBP and PBP are included in the training set for the DTU Ames model with negative experimental results. TBBPA is included with positive experimental results in the DTU model for AR antagonism. TBBPA, TBP, PBP and DBP are included with negative experimental results in the DTU model for ER binding, and TBBPA and TBP are included in the DTU model for ER agonism with negative experimental results. All five members are predicted negative for Ames but have some positive indications in other genotoxicity models. TBP, PBP and DBP have weak indications for carcinogenicity. All five are predicted positive for thyroperoxidase (TPO) inhibition, and DBP is predicted positive in two small DTU models for TR alpha and beta binding. TBP, PBP, DBP, TBBPS have positive indications for skin sensitization.

TBBPA and TBP are found to be both REACH registered and in Tox21, and PBP is found to be in Tox21. The identified sources of experimental information are in itself not enough to give promise of possible successful read-across.

2.5.6 Group 6 (benzyl ethyl oxygen bridge derivatives)

Structure signature
 Cluster 6

 Br1R_59447-55-1	 Br2_497107-13-8

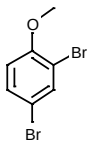
Abbreviated names for members	59447-55-1: PBB-Acr 497107-13-8: DBDBE
Members that are REACH registered	PBB-Acr
Members that are in ToxCast/Tox21	-
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Acrylates/Methacrylates (Chronic toxicity)|Esters (Chronic toxicity): PBB-Acr (NB)
Carcinogenicity (Q)SAR-based clustering	PBB-Acr is a singleton and DBDBE is in cluster 6.
Genotoxicity (Q)SAR-based clustering	PBB-Acr is in cluster 4 and DBDBE is in cluster 3.
Reproductive toxicity (Q)SAR-based clustering	Both are in cluster 2.
Endocrine (Q)SAR-based clustering	PBB-Acr is in cluster 1 and DBDBE is in cluster 3.
Skin sensitization	PBB-Acr is a singleton and DBDBE is in cluster 2.

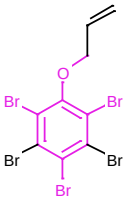
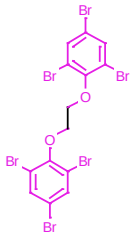
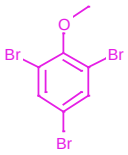
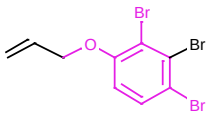
The two members are grouped around the common benzyl ethyl oxygen bridge. However, chemically the oxygen bridge is part of an acrylate group for PBB-Acr and for DBDBE it is a "simple" ether group. According to the rough (Q)SAR-based clusterings they also have dissimilar profiles for carcinogenicity, genotoxicity, endocrine activity and skin sensitization.

PBB-Acr is predicted to be vPvB and DBDBE is predicted to be persistent. PBB-Acr have positive indications for genotoxicity, carcinogenicity, reproductive toxicity and skin sensitization. DBDBE has positive indications for carcinogenicity and cardiotoxicity.

PBB-Acr is found to be REACH registered. Based on the chemical and (Q)SAR-based dissimilarities between the structures read-across between the two members does not seem relevant.

2.5.7 Group 7 (Methoxy dibromobenzene derivatives)

Structure signature
 Cluster 7

Structure signature		
 Br1_3278-89-5	 Br1_3555-11-1	 Br1_37853-59-1
 Br2_607-99-8	 Br3_26762-91-4	

Abbreviated names for members	3278-89-5: TBP-AE 3555-11-1: PBPAE 37853-59-1: BTBPE 607-99-8: TBA 26762-91-4: AO-TBB
Members that are REACH registered	-
Members that are in ToxCast/Tox21	Tox21: TBP-AE
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Neutral organics: TBP-AE, PBPAE, TBA, AO-TBB
Carcinogenicity (Q)SAR-based clustering	TBP-AE, TBA and AO-TBB are in cluster 1, BTBPE is in cluster 4 and PBPAE is in cluster 6.
Genotoxicity (Q)SAR-based clustering	TBP-AE, PBPAE, TBA and AO-TBB are in cluster 3, and BTBPE is in cluster 2.
Reproductive toxicity (Q)SAR-based clustering	All are in cluster 2.
Endocrine (Q)SAR-based clustering	TBP-AE, TBA and AO-TBB are in cluster 6, and PBPAE and BTBPE are in cluster 1.
Skin sensitization	TBP-AE, BTBPE, TBA and AO-TBB are in cluster 2, and PBPAE is in cluster 3.

AO-TBB is an incompletely defined substance, and the molecular structure is a representative of the substance.

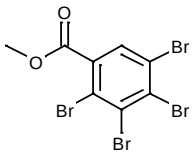
This group is centered around the cluster signature methoxy dibromobenzene. TBP-AE, PBPAE and AO-TBB have the same propene ether group. According to the rough (Q)SAR-based clusterings all the members have similar reproductive toxicity profiles. TBA-AE, TBA and AO-TBB furthermore

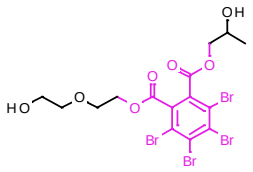
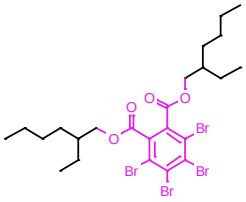
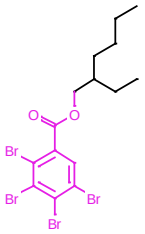
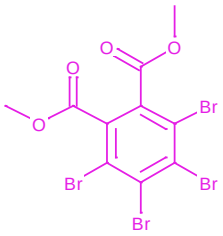
fall in the same (Q)SAR-based clusterings for carcinogenicity, genotoxicity, endocrine activity and skin sensitization.

All members were predicted to be persistent, PBPAE was furthermore predicted to be bioconcentrating, TBP-AE and AO-TBB were predicted to be PBT and PBPAE was predicted to be vPvB. BTBPE is included in the training set for the DTU Ames model with negative experimental result. All the members have some positive indications for genotoxicity, carcinogenicity and skin sensitization. PBPAE, BTBPE and AO-TBB have indications that carcinogenicity may be rodent specific. TBP-AE, PBPAE and AO-TBB have weak indications for reproductive toxicity.

None of the members are found to be REACH registered. TBP-AE is found to be in Tox21. The identified sources of experimental information are in itself not enough to give promise of possible successful read-across for the group or for the brominated benzene propene ethers.

2.5.8 Group 8 (Phthalates/Benzoate)

Structure signature
 Cluster 8

 Br1_20566-35-2	 Br1_26040-51-7
 Br1_183658-27-7	 Br3_55481-60-2

Abbreviated names for members	20566-35-2: HEEHP-TEBP 26040-51-7: BEH-TEBP 183658-27-7: EH-TBB 55481-60-2: Bis(methyl)tetrabromophtalate (BM-TEBP)
Members that are REACH registered	HEEHP-TEBP, BEH-TEBP
Members that are in ToxCast/Tox21	ToxCast: BEH-TEBP Tox21: BEH-TEBP

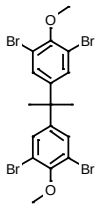
Members of OECD HPV Chemical Categories	High molecular weight phthalate esters: BEH-TEBP
Members of US-EPA New Chemical Categories	Esters (Acute toxicity)|Nonionic Surfactants: HEEHP-TEBP, Esters (Chronic toxicity): BM-TEBP
Carcinogenicity (Q)SAR-based clustering	BM-TEBP is in cluster 11, and BEH-TEBP, EH-TBB and BM-TEBP are singletons.
Genotoxicity (Q)SAR-based clustering	All are in cluster 2.
Reproductive toxicity (Q)SAR-based clustering	EH-TBB is in cluster 3, and HEEHP-TEBP, BEH-TEBP and BM-TEBP are singletons
Endocrine (Q)SAR-based clustering	All are in cluster 1.
Skin sensitizatin	All are in cluster 3.

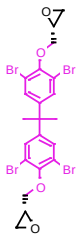
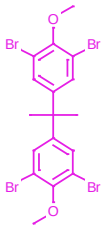
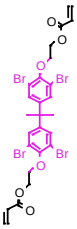
This group is centered around the benzoate cluster signature. However, HEEHP-TEBP, BEH-TEBP and BM-TEBP are phthalate esters and EH-TBB is a benzoate and would be expected to have a different toxicity profile. According to the rough (Q)SAR-based clusterings all the members have similar genotoxicity, endocrine activity and skin sensitization profiles. For carcinogenicity and reproductive toxicity profiles none of the members cluster together.

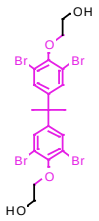
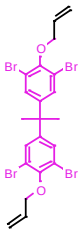
All members were predicted to be persistent but not to be bioconcentrating. All are furthermore predicted to have positive indications for carcinogenicity and weak indication for genoxiticy. All have positive indications for reproductive toxicity, the phthalate esters having most positive hits.

HEEHP-TEBP and BEH-TEBP are found to be REACH registered, and BEH-TEBP is also in ToxCast and Tox21.

2.5.9 Group 9 (TBBPA ethers)

Structure signature
 Cluster 9

 Br1R_3072-84-2	 Br1R_37853-61-5	 Br1R_66710-97-2

 Br1_4162-45-2	 Br1_25327-89-3

Abbreviated names for members	3072-84-2: TBBPA-BGE 37853-61-5: TBBPA-BME 66710-97-2: TBBPA-BHEEBA 4162-45-2: TBBPA-BHEE 25327-89-3: TBBPA-bAE
Members that are REACH registered	-
Members that are in ToxCast/Tox21	Tox21: TBBPA-BHEE, TBBPA-bAE
Members of OECD HPV Chemical Categories	Multifunctional acrylates: TBBPA-BHEEBA
Members of US-EPA New Chemical Categories	Epoxides: TBBPA-BGE, Acrylates/Methacrylates (Acute toxicity): TBBPA-BHEEBA Neutral Organics: TBBPA-BHEE
Carcinogenicity (Q)SAR-based clustering	TBBPA-BME, TBBPA-BHEE and TBBPA-bAE are in cluster 4, TBBPA-BHEEBA are in cluster 15 and TBBPA-BGE is a singleton.
Genotoxicity (Q)SAR-based clustering	TBBPA-BGE is a singleton, TBBPA-BME and TBBPA-bAE are in cluster 3, TBBPA-BHEEBA are in cluster 4, and TBBPA-BHEE is in cluster 2.
Reproductive toxicity (Q)SAR-based clustering	All are in cluster 2.
Endocrine (Q)SAR-based clustering	All are in cluster 4.
Skin sensitization	TBBPA-BME, TBBPA-BHEE and TBBPA-bAE are in cluster 2, TBBPA-BHEEBA and TBBPA-BGE are singletons.

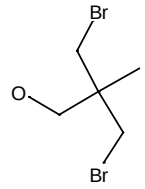
This group is centered around the TBBPA methyl ether signature. However, the group does not seem very chemically homogeneous since TBBPA-BGE contains an epoxy group, TBBPA-BHEEBA contains an acrylate group and TBBPA-bAE contains a propene ether group. So it is to be expected that some members may be more reactive. According to the rough (Q)SAR-based clusterings all the members have similar reproductive toxicity and endocrine activity profiles. For carcinogenicity TBBPA-BME, TBBPA-BHEE and TBBPA-bAE cluster together. For genotoxicity only TBBPA-BME and TBBPA-bAE cluster together. For skin sensitization TBBPA-BME, TBBPA-BHEE and TBBPA-bAE cluster together.

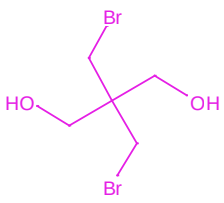
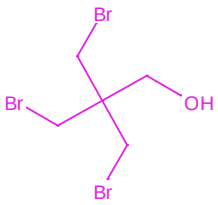
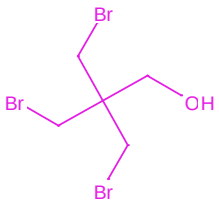
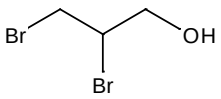
All members were predicted to be persistent, TBBPA-BME is bioconcentrating, TBBPA-BGE and TBBPA-BHEE are vPvB, and TBBPA-BHEE is also PBT. TBBPA-BHEE is included in the training set for the DTU AR antagonism model with negative experimental result. TBBPA-BGE, TBBPA-BHEEBA and TBBPA-bAE have positive indications for genotoxicity and carcinogenicity with TBBPA-BGE having the strongest profile. However, there is also indication that carcinogenicity may be rodent specific. All have a few positive reproductive toxicity indications. All have positive indications from pregnane X receptor (PXR) binding, ER binding and agonism, and TPO inhibition.

TBBPA-BHEE has positive indications from several cardiotoxicity models. TBBPA-BGE, TBBPA-BME and TBBPA-BHEEBA have positive indications for skin sensitization.

None of the members are found to be REACH registered. TBBPA-BHEE and TBBPA-bAE are found to be in Tox21. Apart from the differences in chemical structure between the group members, the identified sources of experimental information are also not sufficient to give promise of possible successful read-across.

2.5.10 Group 10 (small alkyl alkohols)

Structure signature
 Cluster 10

			
Br1R_3296-90-0	Br1R_36483-57-5	Br2_1522-92-5	Br3_96-13-9

Abbreviated names for members	3296-90-0: DBNPG 36483-57-5: TBNPA 1522-92-5: TBNPA 96-13-9: 2,3-DBPA
Members that are REACH registered	DBNPG, TBNPA (CAS 36483-57-5)
Members that are in ToxCast/Tox21	ToxCast: DBNPG Tox21: DBNPG, TBNPA, DBPA
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	-
Carcinogenicity (Q)SAR-based clustering	DBNPG and TBNPA are in cluster 10, and 2,3-DBPA is in cluster 7.
Genotoxicity (Q)SAR-based clustering	DBNPG and TBNPA are in cluster 1, and 2,3-DBPA is a singleton.
Reproductive toxicity (Q)SAR-based clustering	TBNPA is in cluster 2, 2,3-DBPA is in cluster 3, and DBNPG is a singleton.
Endocrine (Q)SAR-based clustering	All are in cluster 9.
Skin sensitization	All are in cluster 2.

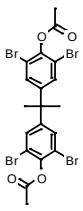
Note that CAS 36483-57-5 and CAS 1522-92-5 have identical molecular structures (TBNPA).

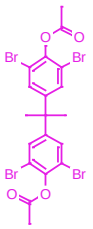
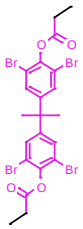
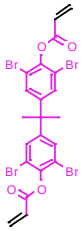
The group consists of three different molecular structures, all being small alkylalcohols, either branched or linear. According to the rough (Q)SAR-based clusterings all have similar profiles for endocrine activity and skin sensitization. DBNPG and TBNPA are in the same (Q)SAR-based clusters for carcinogenicity and genotoxicity. For reproductive toxicity the three structures are in separate clusters.

None of the members are predicted to be persistent or bioconcentrating. All three members are included in the training set for the DTU Ames model with positive experimental results and 2,3-DBPA is included with positive experimental results in the DTU models for chromosomal aberrations in CHL cells and SHE cell transformation *in vitro*. All three have positive predicted indications in all included models for carcinogenicity. DBNPG and TBNPA have positive predicted indications in few models for reproductive toxicity, and 2,3-DBPA have positive predicted indications in more reproductive toxicity models. All have positive predicted indications for airway allergy.

DBNPG and TBNPA (CAS 36483-57-5) are found to be REACH registered. DBNPG is found to be in both ToxCast and Tox21, and TBNPA and 2,3-DBPA are found to be in Tox21. The identified sources of experimental information could give promise of possible successful read-across and although all three substances do not cluster together in the (Q)SAR based clusterings for genotoxicity, carcinogenicity and reproductive toxicity, manual inspection of the predictions showed good consistency in the (Q)SAR predictions between the members, especially for genotoxicity and carcinogenicity.

2.5.11 Group 11 (TBBPA esters/acrylate)

Structure signature
 Cluster 11

 Br1_33798-02-6	 Br2_37419-42-4	 Br2_55205-38-4

Abbreviated names for members	33798-02-6: TBBPA-bOAc 37419-42-4: TBBPA-BP 55205-38-4: TBBPA-BA
Members that are REACH registered	-

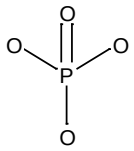
Members that are in ToxCast/Tox21	-
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Esters (Chronic toxicity): TBBPA-bOAc
Carcinogenicity (Q)SAR-based clustering	TBBPA-bOAc and TBBPA-BP are in cluster 4 and TBBPA-BA is in cluster 15.
Genotoxicity (Q)SAR-based clustering	TBBPA-bOAc and TBBPA-BP are in cluster 2. and TBBPA-BA is in cluster 4.
Reproductive toxicity (Q)SAR-based clustering	All three are in cluster 2.
Endocrine (Q)SAR-based clustering	TBBPA-bOAc is in cluster 2, and TBBPA-BP and TBBPA-BA are in cluster 4.
Skin sensitization	TBBPA-bOAc and TBBPA-BP are in cluster 6 and TBBPA-BA is a singleton.

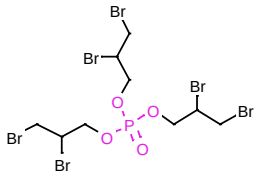
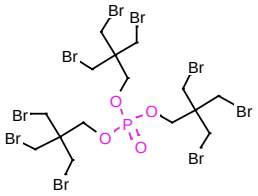
This group is centered around the TBBPA di-acetate signature, although for TBBPA-BA the ester group is part of an acrylate group, which may be expected to be more chemically reactive. TBBPA-bOAc and TBBPA-BP are structurally very similar being TBBPA di-acetate and di-propionate esters, respectively. According to the rough (Q)SAR-based clusterings TBBPA-bOAc and TBBPA-BP have similar profiles for carcinogenicity, genotoxicity and skin sensitization. For reproductive toxicity the rough (Q)SAR-based clusterings indicated that all three substances have similar profiles.

All members were predicted to be persistent and bioconcentrating, and TBBPA-bOAc is predicted to be vPvB. TBBPA-BA has positive indications for genotoxicity, carcinogenicity and reproductive toxicity. TBBPA-bOAc and TBBPA-BP have weak indications for genotoxicity. All three have positive indications for ER binding, AR antagonism and TPO inhibition. TBBPA-bOAc has furthermore positive indications for PXR binding and cardiotoxicity.

None of the members are found to be REACH registered or in ToxCast/Tox21, and there is thereby not identified any sources of experimental information for possible read-across.

2.5.12 Group 12 (Phosphates)

Structure signature

Cluster 12

 Br1_126-72-7	 Br1_19186-97-1

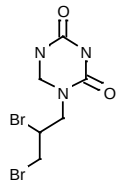
Abbreviated names for members	126-72-7: TDBPP 19186-97-1: TTBNPP
Members that are REACH registered	TTBNPP
Members that are in ToxCast/Tox21	ToxCast: TDBPP Tox21: TDBPP
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	-
Carcinogenicity (Q)SAR-based clustering	Both are singletons.
Genotoxicity (Q)SAR-based clustering	Both are singletons.
Reproductive toxicity (Q)SAR-based clustering	Both are singletons.
Endocrine (Q)SAR-based clustering	Both are in cluster 7.
Skin sensitization	Both are singletons.

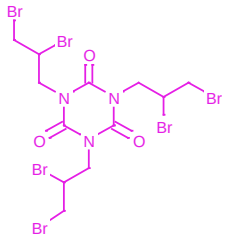
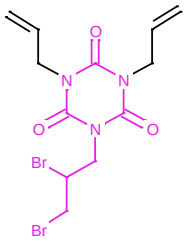
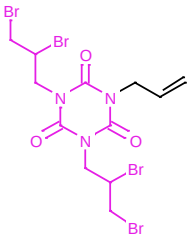
This group is centered around the phosphate cluster signature. According to the rough (Q)SAR-based clusterings the two members have similar endocrine activity profiles, and in all the other (Q)SAR-based clusterings they both come out as singletons.

Both members were predicted to be persistent but not to be bioconcentrating. TDBPP is included in the training sets for the DTU models for Ames, Ames sub-model for base-pair mechanism, chromosomal aberrations in CHL cells, unscheduled DNA synthesis (UDS, *in vitro*), SHE cell transformation (*in vitro*), Drosophila SLRL (*in vivo*), sister chromatid exchange (*in vivo*), strong skin irritation with positive experimental results and in DTU models for Ames sub-model for direct acting mutagens and possible rodent-specific carcinogenicity with negative experimental results. Both members have positive predicted indications in all included models for carcinogenicity, in many models for *in vitro* and in *vivo* genotoxicity, for PXR binding and TPO inhibition, in many models for reproductive toxicity, for severe skin irritation and for skin sensitization. TDBPP has positive predicted indications in a few models for cardiotoxicity.

TTBNPP is found to be REACH registered and TDBPP is found to be in ToxCast and Tox21.

2.5.13 Group 13 (Triazines)

Structure signature
 Cluster 13

 Br1_52434-90-9	 Br2_57829-89-7	 Br2_75795-16-3

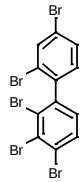
Abbreviated names for members	52434-90-9: TDBP-TAZTO 57829-89-7: DBP-TAZTO 75795-16-3: BDBP-TAZTO
Members that are REACH registered	-
Members that are in ToxCast/Tox21	-
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	-
Carcinogenicity (Q)SAR-based clustering	All three are in cluster 9.
Genotoxicity (Q)SAR-based clustering	All three are in cluster 10.
Reproductive toxicity (Q)SAR-based clustering	TDBP-TAZTO is in cluster 3, and DBP-TAZTO and BDBP-TAZTO are in cluster 7.
Endocrine (Q)SAR-based clustering	TDBP-TAZTO and BDBP-TAZTO are in cluster 7, and DBP-TAZTO is in cluster 6.
Skin sensitization	All three are in cluster 4.

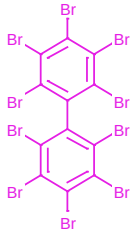
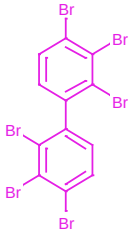
This group is centered around the (2,3- dibromopropyl)-1,3,5- triazine-2,4,6-trione cluster signature, however DBP-TAZTO and BDBP-TAZTO also contain two and one allyl groups, respectively. According to the rough (Q)SAR-based clusterings the three members have similar profiles for carcinogenicity, genotoxicity and skin sensitization. For reproductive toxicity DBP-TAZTO and BDBP-TAZTO have according to the rough (Q)SAR based clusterings similar profiles, and TDBP-TAZTO and BDBP-TAZTO have similar profiles for endocrine activity.

All members were predicted to be bioconcentrating, and TDBP-TAZTO and BDBP-TAZTO were predicted to be vPvB. All three members have positive predicted indications in many models for genotoxicity, carcinogenicity and reproductive toxicity. However, there are indications that carcinogenicity may be rodent specific. TDBP-TAZTO has positive indications for PXR binding, TPO inhibition and skin sensitization, and BDBP-TAZTO has positive indications for PXR binding.

None of the members were found to be REACH registered or in ToxCast/Tox21.

2.5.14 Group 14 (Biphenyles)

Structure signature

Cluster 14

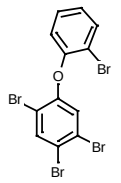
	
Br1_13654-09-6	Br1_36355-01-8

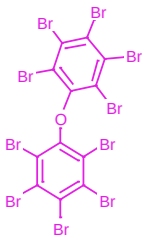
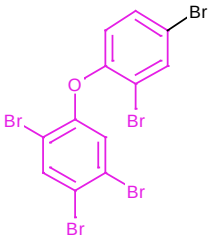
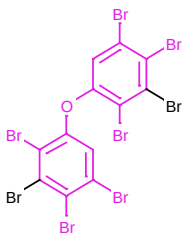
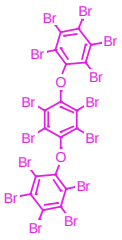
Abbreviated names for members	13654-09-6: DecaBB 36355-01-8: HexaBB
Members that are REACH registered	-
Members that are in ToxCast/Tox21	-
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	-
Carcinogenicity (Q)SAR-based clustering	DecaBB is in cluster 8 and HexaBB is in cluster 2.
Genotoxicity (Q)SAR-based clustering	Both are in cluster 3.
Reproductive toxicity (Q)SAR-based clustering	Both are in cluster 6.
Endocrine (Q)SAR-based clustering	DecaBB is a singleton and HexaBB is in cluster 2.
Skin sensitization	DecaBB is in cluster 3 and HexaBB is in cluster 2.

HexaBB is an incompletely defined substance, and the molecular structure is a representative of the substance.

This group contains two brominated biphenyls. These substances are already assessed and treated as a group in the RoHS directive, and since the cluster does not contain any other types of chemical structures this group would not bring new knowledge applicable in the regulation of groups of substances in the RoHS directive.

2.5.15 Group 15 (Diphenyl ethers)

Structure signature

Cluster 15

			
Br1_1163-19-5	Br1_32534-81-9	Br1_32536-52-0	Br1_58965-66-5

Abbreviated names for members	1163-19-5: decaBDE 32534-81-9: pentaBDE 32536-52-0: octaBDE 58965-66-5: 4′-PeBPOB-DE208
Members that are REACH registered	decaBDE
Members that are in ToxCast/Tox21	Tox21: pentaBDE, octaBDE
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Neutral Organics: pentaBDE,
Carcinogenicity (Q)SAR-based clustering	DecaBDE is in cluster 8, pentaBDE is a singleton, octaBDE is in cluster 2 and 4′-PeBPOB-DE208 is in cluster 6.
Genotoxicity (Q)SAR-based clustering	All are in cluster 6.
Reproductive toxicity (Q)SAR-based clustering	DecaBDE is in cluster 2, and pentaBDE, octaBDE and 4′-PeBPOB-DE208 are in cluster 5.
Endocrine (Q)SAR-based clustering	DecaBDE, octaBDE and 4′-PeBPOB-DE208 are in cluster 5, and pentaBDE is in cluster 2.
Skin sensitization	All are in cluster 3.

PentaBDE and octaBDE are incompletely defined substances, and the molecular structures are representatives of the substances.

This group contains three brominated diphenyl ethers and one with an extra brominated p-phenyl ether fragment. The polybrominated diphenyl ethers are already assessed and treated as a group and restricted in the RoHS directive. According to the rough (Q)SAR-based clusterings all the

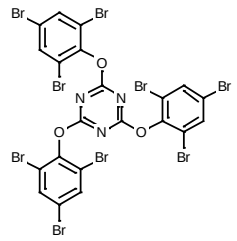
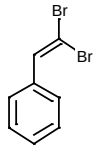
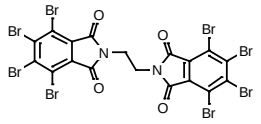
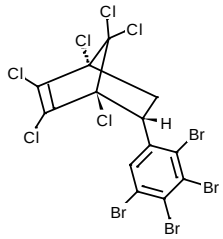
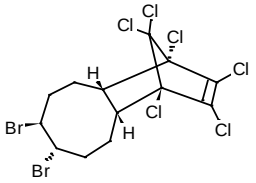
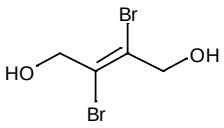
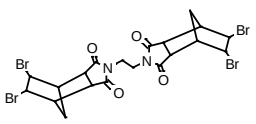
members have similar profiles for genotoxicity and skin sensitization. For reproductive toxicity 4′-PeBPOB-DE208 clusters together with pentaBDE and octaBDE, and for and for endocrine activity it clusters together with DecaBDE and octaBDE.

All members were predicted to be persistent, and pentaBDE was predicted to be PBT and vPvB. DecaBDE is included in the training sets for the DTU models for Ames, HGPRT, SHE cell transformation and AR antagonism with negative experimental results, and in DTU model for mouse micronucleus (bone marrow, *in vivo*) and rodent specific carcinogenicity with positive experimental results. PentaBDE is included in the training set for the DTU model for AR antagonism with positive experimental result. All four members have positive predicted indications in a few models for genotoxicity and carcinogenicity. However, with indications for all four that carcinogenicity may be rodent specific. PentaBDE and octaBDE have positive indications in one model for human teratogenicity. All four have positive indications for AR antagonism and TPO inhibition, and decaBDE, pentaBDE and 4′-PeBPOB-DE208 have positive indications for ER binding.

DecaBDE was found to be REACH registered. PentaBDE and octaBDE were found to be in Tox21.

The polybrominated diphenyl ethers (PBDE) are already assessed and regulated as a group in the RoHS directive.

2.5.16 Singletons

Structure signature		
 Br1_25713-60-4	 Br1_31780-26-4	 Br1_32588-76-4
 Br1_34571-16-9	 Br1_51936-55-1	
 Br3_3234-02-4	 Br3_52907-07-0	

Abbreviated names for members	25713-60-4: TTBP-TAZ 31780-26-4: DBS 32588-76-4: EBTEBPI 34571-16-9: HCTBPH 51936-55-1: DBHCTD 3234-02-4: 2,3-Dibromo-2-butene-1,4-diol 52907-07-0: Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide)
Members that are REACH registered	TTBP-TAZ and EBTEBPI
Members that are in ToxCast/Tox21	Tox21: 2,3-Dibromo-2-butene-1,4-diol
Members of OECD HPV Chemical Categories	-
Members of US-EPA New Chemical Categories	Imides (Acute toxicity): Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide)
Carcinogenicity (Q)SAR-based clustering	DBHCTD and Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide) are in cluster 3, TTBP-TAZ is in cluster 4, DBS and EBTEBPI are in cluster 5, and HCTBPH and 2,3-Dibromo-2-butene-1,4-diol are singletons.
Genotoxicity (Q)SAR-based clustering	TTBP-TAZ is in cluster 3, HCTBPH is in cluster 8, DBHCTD are in cluster 5, and DBS, EBTEBPI, 2,3-Dibromo-2-butene-1,4-diol and Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide) are singletons.
Reproductive toxicity (Q)SAR-based clustering	DBS and 2,3-Dibromo-2-butene-1,4-diol are in cluster 1, and TTBP-TAZ, EBTEBPI, HCTBPH, DBHCTD and Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide) are singletons.
Endocrine (Q)SAR-based clustering	TTBP-TAZ is a singleton, DBS is in cluster 6, EBTEBPI and Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide) are in cluster 8, HCTBPH and DBHCTD are in cluster 3, and 2,3-Dibromo-2-butene-1,4-diol is in cluster 9.
Skin sensitization	DBS is in cluster 2, Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide) is in cluster 4, and TTBP-TAZ, EBTEBPI, HCTBPH, DBHCTD and 2,3-Dibromo-2-butene-1,4-diol are singletons.

These substances are structurally clustered as “singletons” meaning that they did not cluster together with any of the other substances included.

3. (Q)SAR predictions

The group of small linear and branched brominated alkyl alcohols was selected for a possible category approach. This decision was based on (Q)SAR predicted positive profiles for genotoxicity and cancer with positive experimental information in the training sets for a number of the applied (Q)SAR models for two of the substances, DBNPG (CAS RN 3296-90-0) and DBPA (CAS RN 96-13-9).

These two substances were furthermore REACH registered and also for that reason expected possibly to have experimental information for a number of endpoints. To enable read-across by the category approach for a given effect it is necessary to have experimental information for this effect for a representative number of the category members, as a minimum and depending on the size of the category, for at least two of the members.

DBNPG and DBPA have been identified by the EU Commission (Oeko-Institut 2014) to have highest priority in relation to undergoing further evaluation leading to possible restriction under the RoHS directive. In the evaluation by the EU Commission emphasis have been laid on the application in electric and electronic equipment. This has been extracted from the REACH dossiers.

3.1 Definition of the category

The working definition of the category was made on the basis of the span of the four members of the structural group identified in the preliminary structural grouping exercise. In the identified group the smallest member (DBPA) has 3 carbons, two bromine atoms and one alcohol group, and the two biggest members (TBNPA and DPNPA) have 5 carbons, 2-3 bromine atoms and 1-2 alcohol groups. The category was therefore defined as small brominated linear and branched alkyl alcohols with 3-5 carbons, 1-2 alcohol groups, and 2-4 bromine atoms. Four bromine atoms were allowed although this was not seen in the members in the preliminary structural group. In correspondence with the category members from the preliminary structural group a maximum of one bromine atom or one alcohol group was allowed on a carbon, bromine being allowed only on primary or secondary carbons. However, chains were allowed to end with a carbon atom without a bromine or alcohol group even though this is not seen in the members from the preliminary structural group. The purpose of “relaxing” on the chemical specifications in the working definition of the category compared to the members in the preliminary structural group was to possibly identify more structural analogs for which experimental data might be available. Depending on the findings in a later stage it can be decided to narrow down the category.

3.2 Identification of category members

The chemical structures of the theoretical members of the category were identified and SMILES codes were generated. There were 62 in total including the four from the preliminary structural group. Of these, 2 were structurally identical but with different CAS RNs, and as the number of unique structures is important in this exercise (and as some of the other members also have more than one CAS RN) in the following text the category is referred to as containing 61 members. Possible CAS RNs for the 58 newly generated members were identified by SMILES lookup in SciFinder. For 22 of these one or more CAS RNs could be found in SciFinder. Only CAS RNs for isomers not including mixtures or substances with deuterium (^{2}H) or carbon-14 (^{14}C) were retrieved. For the remaining members no CAS RN was located. However, these substances were still

kept in the category as theoretical members, as they may potentially be used in the future. For the members with no CAS RNs no experimental data is expected to be available.
The full list with the 61 (62) members is presented in Appendix 5 with 2D structures and a few physical-chemical descriptors. All the ID numbers, which are given as part of the images, have the prefix "BFR_", which were used in Leadscope to give the structures unique identifiers so that they did not merge with other projects stored in the Leadscope server. The ID number follows the prefix. The first three digits of the ID numbers encode the number of carbon atoms, bromine atoms and alcohol groups in the molecule, followed by a further numbering as there was in many cases more than one member with the same first three digits. If a CAS RN was found it is given as the last part of the ID number.

3.3 ToxCast/Tox21

All the identified CAS RNs for the theoretical members of the category were searched to see if they were among the substances included in ToxCast or Tox21. However, only the three substances from the preliminary structural group which were already identified ToxCast/Tox21 members were found.

TBNPA (CAS RN 1522-92-5):	65 assays, 2 hits (AhR, NRF2)
DBPA (CAS RN 96-13-9):	65 assays, 0 hits
DBNPG (CAS RN 3296-90-0):	384 assays, 1 hit (COX-2)

3.4 (Q)SAR models applied

Although the category was chosen based on the genotoxicity and carcinogenicity profiles for the members from the preliminary structural group, the 61 category members were predicted in all the human health related (Q)SAR models and in the OECD (Q)SAR Application Toolbox profilers (see Appendix 3). PBT in relation to environmental endpoints were not predicted as PBT properties for the category members were not identified in the preliminary structural group.

3.5 Results from the (Q)SAR predictions

3.5.1 Membership of existing OECD HPV Chemical Categories or US-EPA New Chemical Categories

The OECD (Q)SAR Application Toolbox was used to check whether any of the 61 category members were associated with an existing category assessed within the OECD HPV Chemicals Programme or a category defined within the new chemical notification scheme and the HPVC challenge programme of the US-EPA. None of the category members were associated with either the OECD HPV or the US EPA New Chemical Categories.

3.5.2 Bioavailability

All the 61 category members are bioavailable according to estimated Lipinski's rule-of-five as predicted both in Leadscope and the OECD (Q)SAR Application Toolbox. Human intestinal absorption was predicted to be between 90% and 96% for the individual members.

3.5.3 Metabolism / transformation

The following metabolism profilers in the OECD (Q)SAR Application Toolbox was run: Observed Mammalian metabolism, observed microbial metabolism, observed rat in vivo metabolism, observed rat liver S9 metabolism, simulated microbial metabolism, and simulated rat liver metabolism. One metabolite was identified in the observed rat in vivo metabolism profiler for DBNPG (glucuronide metabolite), but none for any of the other members. I.e. this means that for DBNPG the Toolbox contains information about a metabolite and it does not contain information about metabolites for the remaining members. However, this does not mean that they do not possibly have any metabolites. Multiple metabolites were generated for all of the 61 category members in both of the two metabolism simulators.

3.5.4 Endocrine endpoints

All 61 category members were predicted to be non-binders in the OECD profiler for Estrogen Receptor (ER) binding. All members were furthermore negative or outside AD for *in vitro* ER binding in the DTU model, and in further DTU models all members were predicted negative for *in vitro* ER agonism, Androgen Receptor (AR) antagonism, Pregnane X Receptor (PXR) binding, PXR activation and thyroperoxidase (TPO) inhibition.

3.5.5 Repro-developmental toxicity

The 61 category members were predicted negative or were outside AD in many of the commercial suites (Leadscope FDA) for non-human reproductive toxicity (9 models for: Rodent male composite, Rat male, Mouse male, Rodent male sperm composite, Rat male sperm, Mouse male sperm, Rodent female composite, Rat female and Mouse female). However, in the model for Rat male 34 members were predicted positive and the remaining were outside AD, in the model for Rodent male sperm composite 33 members had positive predictions, 3 were negative and the remaining were outside AD, and in the model for Rodent female composite 9 members had positive predictions, 1 was negative and the remaining were outside AD.

The category members were predicted negative or were outside AD in all of the commercial suites (Leadscope FDA) for non-human developmental toxicity (27 models within Structural dysmorphogenesis, Visceral dysmorphogenesis, Fetal growth retardation, Fetal weight decrease, Fetal survival: fetal death, Fetal survival: post-implantation loss and Fetal survival: pre-implantation loss). However, DBNPG (CAS RN 3296-90-0) had positive experimental results in the training sets of many of the models (Growth Retard Mouse, retard rodent (AH1), Wt Dec Mouse, wt dec rodent (AI1), Fetal Death Mouse, Fetal Death Rodent, post impl mouse (AG6), post impl rodent (AG1), Pre Impl Loss Mouse, Pre Impl Loss Rodent, Repro Mouse Male, Repro Rodent Male, sperm mouse(AP5), sperm Eff Rodent) and negative results in others (struct mouse (AL6), struct rodent (AL1), Visc Org Mouse, Visc Org Rodent, Repro Mouse Female, Repro Rodent Female).

Commercial CASE Ultra model A49 for human teratogenicity potential: 4 positive predictions and many negative.

3.5.6 Organ toxicity

The category members were outside the AD in commercial suite models for renal toxicity (CASE Ultra), hepatotoxicity (Leadscope) and cardiotoxicity (CASE Ultra).

3.5.7 Repeated dose toxicity

All the category members were identified to belong to Cramer classification scheme for oral systemic toxicity (with extensions) class III.

None of the members were categorized in the OECD (Q)SAR Application Toolbox profiler for repeated dose toxicity (Hazard Evaluation Support System, HESS).

In a DTU model for human maximum recommended daily dose (in pharmaceutical clinical trials employing primarily oral route of exposure and daily treatments, usually for 3-12 months) based on US FDA data, and found by the US FDA to be directly related to NOEL in humans, the majority of the members were outside the AD and the remaining (9) were predicted negative (positive meaning side effects at daily dose below 2.69 mg/kg-bw).

3.5.8 Genotoxicity and carcinogenicity

All category members were predicted using a number of (Q)SAR models for genotoxicity and carcinogenicity. This section presents the predictions from DTU models and commercial models implemented in MultiCASE and Leadscope first, preseded by predictions from OECD (Q)SAR

Application Toolbox profilers. Heat maps over the (Q)SAR predictions and of the OECD (Q)SAR Application Toolbox profilers alerts are included in Appendix 6.

DTU models and commercial models implemented in MultiCASE and Leadscope

In the commercial MultiCASE CASE Ultra model for Ashby structural alerts for DNA reactivity (NTP data) all members were predicted positive, except 1 substance (DBNPG). In a commercial CASE Ultra model for Ames mutagenicity (*Salmonella typhimurium*) all members were predicted positive. In a DTU model for Ames mutagenicity predictions within the defined AD could be obtained for 47 of the members and they were all predicted positive, and the 3 members (i.e. including DBNPG) from the preliminary structural group had positive experimental data. In Appendix 6 the predictions for Ashby alerts, and the CASE Ultra commercial and DTU models for Ames are included in columns a, b and c, respectively.

The two identified alerts in the DTU Ames model for the different substances were one bromine on a primary carbon or one bromine on a secondary carbon. To check the specificity of the models it was investigated if two or three bromines on the same carbon would also be alerted, and these checked substances (which were not part of the category) were predicted negative with no alert found.

In a DTU Ames sub-model for direct acting Ames mutagens (not requiring liver S9) DBNPG and DBPA are included in the training set as experimental positives and TBNPA is included as an experimental negative. In another DTU Ames sub-model for frameshift mutagenicity DBPA is included in the training set as an experimental positive and DBNPG and TBNPA are included as experimental negatives. In a third DTU Ames sub-model for base-pair mutagenicity DBPA is included in the training set as experimental positive and DBNPG and TBNPA are not included. For the DTU Ames sub-model for Base-pair all 48 members within the AD of the model are predicted positive. This is illustrated in the heat map for the (Q)SAR predictions in Appendix 6 column f.

None of the 61 members were predicted to be positive for chromosomal aberrations *in vitro* in Chinese hamster ovary cells (1/61 was out of the AD for this model), see column h in the (Q)SAR predictions heat map in Appendix 6. One substance (2,3-DBPA) was predicted to be positive for chromosomal aberrations *in vitro* in Chinese hamster lung cells, 8 substances were predicted to be negative and the remaining 52 substances were out of the applicability domain (column i in the (Q)SAR predictions heat map in Appendix 6).

Regarding the mouse lymphoma assay, all substances were out of the AD in this model.

In the model for the HGPRT test, 5 substances were predicted to be positive and the remaining 56 substances were out of the AD (column k in the (Q)SAR predictions heat map in Appendix 6). 24 substances were predicted to be positive for unscheduled DNA synthesis *in vitro*, while the remaining 37 substances were out of the AD (column j in the (Q)SAR predictions heat map in Appendix 6). In the *in vivo* sister chromatid exchange assay, 51 substances were predicted to be positive and the remaining 10 substances were out of the AD (column m in the (Q)SAR predictions heat map in Appendix 6). In the model for the *in vivo* bone marrow micronucleus test, 23 substances were predicted to be negative and the remaining 38 substances were out of the AD for this test (column n in the (Q)SAR predictions heat map in Appendix 6). In the *in vivo* dominant lethal test in rodents, 41 substances were predicted to be negative and the remaining 20 substances were out of the applicability domain for this test (column o in the (Q)SAR predictions heat map in Appendix 6). Increases in sex-linked recessive lethal mutations in male germ cells of *Drosophila melanogaster* were predicted for 44 substances, but not for 13 substances; the remaining 4 substances were out of the applicability domain for this test (column p in the (Q)SAR predictions heat map in Appendix 6). In the model for *in vivo* Comet assay, there were positive predictions for

14 substances, whereas the remaining 47 substances were out of the applicability domain (column q in the (Q)SAR predictions heat map in Appendix 6).

In a commercial CASE Ultra FDA cancer suite with 7 models (male rat, female rat, male mouse, female mouse, rat, mouse, rodent (columns r-x in the (Q)SAR predictions heat map in Appendix 6)) all members had at least four positive predictions within the AD. All members were predicted positive in the CASE Ultra FDA RCA algorithm for overall cancer call, which requires at least two positive predictions in the four male/female mouse/rat models and overlapping alerts (column y in the (Q)SAR predictions heat map in Appendix 6). In a Leadscope DTU model which predicts whether carcinogenicity is specific to rodent liver 34 members were predicted negative and 27 were outside the AD. DBNPG (CAS RN 3296-90-0) is included as an experimental negative in the training set of the model. I.e. according to this model there were no indications that carcinogenicity is rodent specific.

All the 61 category members were predicted positive in the MultiCASE commercial model for Ames mutagenicity, and all except one (DBNPG) were predicted positive for Ashby structural alerts. In further DTU or commercial models for genotoxicity, positive or out-of-AD predictions were obtained in the models for *in vitro* chromosomal aberrations in Chinese hamster ovary cells, HGPRT, unscheduled DNA synthesis and *in vivo* sister chromatid exchange. Negative or out-of-AD predictions were obtained in the models for *in vivo* bone marrow micronucleus in mice and dominant lethal test in rodents.

Both positive and negative predictions were obtained in models for *in vitro* chromosomal aberrations in Chinese hamster lung cells and *in vivo* increases in sex-linked recessive lethal mutations in male germ cells of *Drosophila melanogaster*, so for these particular endpoints the models indicate possible differences between the category members.

The fact that robust predictions within the defined ADs of the models could not be obtained for all the category members (i.e. some of the chemical structures were out of the AD) does not in itself indicate anything about the relevance of the defined category in relation to genotoxicity and cancer. It merely means that not all the chemical structures were sufficiently known by the models. It is also important to treat the negative predictions with particular care, as the applied systems require more statistical significance for “alerts” than for “non-alerts”. All the 61 category members were predicted positive in the MultiCASE CASE Ultra commercial FDA suite for carcinogenicity, and no indications were found that carcinogenicity may be rodent specific by a Leadscope DTU model.

In other words, the following models gave positive predictions within AD for all category members: MultiCASE commercial models for Ames and FDA cancer suites. As these models are commercial the identified alerts cannot be presented here due to license restrictions. The outputs from commercial (Q)SAR programmes are related to the mode of action.

The overall accuracies of the models in terms of sensitivity and specificity estimated by cross-validation are presented in Table 2 for the Ashby structural alerts, Salmonella mutagenicity (Ames) and cancer models. For the Salmonella mutagenicity model the results were extracted from the MultiCASE CASE Ultra model information where it was presented as the result of a ten-fold (i.e. 10%) cross-validation. For the Ashby structural alerts and cancer models the cross-validation was performed by DTU as a 5 times random (but keeping the balance between positives and negatives) two-fold (i.e. 50%) cross-validation. Documentation in (Q)SAR Model Reporting Format (QMRF) is available for the Ashby structural alerts and cancer models, as well as for the other genotoxicity models presented in the heat map in Appendix 6, in the online Danish (Q)SAR database (DTU 2016). The estimated specificities of the models are between 85.9% and 95.1%, i.e. the overall false positive rates of the models are around 5%- 14%.

Endpoint	N	Cross validation result (%)[a]
CU Ashby structural alerts	782	Sens=89.7, Spec=95.1, Conc=91.9
CU SALM, Salmonella mutagenicity (TA97,98,100,1535-1538))	10479	Sens=91 ±0.7, Spec=89 ±1.2
CU FDA RCA cancer male rat	1324	Sens=34.2, Spec=95.0, Conc=63.9
CU FDA RCA cancer female rat	1321	Sens=44.4, Spec=93.3, Conc=71.6
CU FDA RCA cancer rat	1379	Sens=41.7, Spec=94.0, Conc=66.9
CU FDA RCA cancer male mouse	1197	Sens=38.4, Spec=86.1, Conc=66.1
CU FDA RCA cancer female mouse	1208	Sens=41.5, Spec=85.9, Conc=65.6
CU FDA RCA cancer mouse	1221	Sens=43.1, Spec=86.9, Conc=66.9
CU FDA RCA cancer rodent	1530	Sens=51.4, Spec=88.3, Conc=68.2

TABLE 2. TRAINING SET NUMBERS (N) AND RESULTS FROM CROSS VALIDATIONS.
SENS: SENSITIVITY; SPEC: SPECIFICITY; CONC: CONCORDANCE.

OECD (Q)SAR Application Toolbox profilers

In its workflow for read across, the OECD (Q)SAR Toolbox aims to group chemicals into categories on the basis of a common molecular initiating event. One common molecular initiating event for genetic toxicity and genotoxic carcinogenicity is often the ability of a chemical to bind covalently to DNA (OECD 2011). A number of profilers in the OECD (Q)SAR Application Toolbox were applied to identify alerts and point to possible mechanisms.

All the category members as well as their observed (Mammalian/Rat S9/*in vivo*) and simulated metabolites (Rat S9/*in vivo*) were profiled in a number of profilers of relevance for genotoxicity and carcinogenicity:

- DNA binding by OECD
- DNA binding by OASIS v.1.3
- Protein binding alerts for Chromosomal aberration by OASIS v1.1
- In vitro mutagenicity (Ames test) alerts by ISS
- DNA alerts for AMES, MN and CA by OASIS v.1.3
- In vivo mutagenicity (Micronucleus) alerts by ISS
- Oncologic Primary Classification
- Carcinogenicity (genotox and nongenotox) alerts by ISS

The structural alerts-output from the profilers for all the category members is included as a heat map in Appendix 6. The rows of the matrix represent structural alerts identified either in the parent compound or in one or more metabolites. Each structural alert is associated with one or more possible mechanisms in the OECD (Q)SAR Application Toolbox alerts explanations provided in Appendix 7 .

In the DNA binding OECD profiler, one or more of 5 alerts were identified in each of the 61 category members, either in the parent compound or in one or more metabolites (columns A1-A5 in the profiler alerts heat map in Appendix 6):

- Aliphatic halides (identified in 52 members)
- 1,2-Dihaloalkanes (identified in 34 members)
- Mono aldehydes (identified in 59 members)
- Epoxides (identified in 51 members)
- Mustards (identified in 3 members)

For DNA binding by OASIS v.1.3 one or more of 5 alerts were identified in each of the 61 category members, either in the parent compound or in one or more metabolites (columns B1-B5 in the profiler alerts heat map in Appendix 6):

- Haloalkanes Containing Heteroatom (identified in 57 members)
- Haloalkane Derivatives with Labile Halogen (identified in 23 members)
- Vicinal Dihaloalkanes (identified in 34 members)
- Epoxides and Aziridines (identified in 51 members)
- Haloalcohols (identified in 52 members)

For Protein binding alerts for Chromosomal aberration by OASIS v1.1 one or more of the following 2 alerts were identified in each of 41 members either in the parent compound and/or in one or more metabolites (columns C1-C2 in the profiler alerts heat map in Appendix 6):

- Halogenated Vicinal Hydrocarbons (identified in 27 members)
- Alpha-Activated Haloalkanes (identified in 41 members)

In the profiler for *in vitro* mutagenicity (Ames test) alerts by ISS one or more of the following 3 alerts were identified in each of the 61 category members either in the parent compound and/or in one or more metabolites (columns D1-D3 in the profiler alerts heat map in Appendix 6):

- Aliphatic halogens (identified in 61 members)
- Simple aldehyde (identified in 59 members)
- Epoxides and aziridines (identified in 51 members)

For DNA alerts for Ames, Mouse micronucleus (MN) and chromosomal aberration (CA) by OASIS v.1.3 no alert was identified in 7 category members, and in each of the remaining 55 members one or more of the following 4 structural alerts were identified either in the parent compound and/or in one or more metabolites (columns E1-E4 in the profiler alerts heat map in Appendix 6):

- Haloalkane Derivatives with Labile Halogen (identified in 20 members)
- Vicinal Dihaloalkanes (identified in 10 members)
- Haloalcohols (identified in 44 members)
- Epoxides and Aziridines (identified in 45 members)

In the profiler for in vivo mutagenicity (Micronucleus) alerts by ISS one or more of the following 4 alerts were identified in each of the 61 category members either in the parent compound and/or in one or more metabolites (columns F1-F4 in the profiler alerts heat map in Appendix 6):

- Aliphatic halogen (identified in 61 members)
- Epoxides and aziridines (identified in 51 members)
- H-acceptor-path3-H-acceptor (identified in 60 members)
- Simple aldehyde (identified in 59 members)

In the Oncologic Primary Classification profiler one or more of the following 4 alerts were identified in 61 category members either in the parent compound and/or in one or more metabolites (columns G1-G4 in the profiler alerts heat map in Appendix 6):

- Aldehyde Type Compounds (identified in 59 members)
- Alpha, beta-Haloether Reactive Functional Groups (identified in 52 members)

- Reactive Ketone Reactive Functional Groups (identified in 25 members)
- Epoxide Reactive Functional Groups (identified in 51 members)

In the profiler for carcinogenicity (genotox and nongenotox) alerts by ISS one or more of the following 5 alerts were identified in each of 61 category members either in the parent compound and/or in one or more metabolites (columns H1-H5 in the profiler alerts heat map in Appendix 6):

- (Poly) Halogenated Cycloalkanes (Nongenotox) (identified in 34 members)
- Aliphatic halogens (Genotox) (identified in 60 members)
- Epoxides and aziridines (Genotox) (identified in 51 members)
- Simple aldehyde (Genotox) (identified in 59 members)
- Substituted n-alkylcarboxylic acids (Nongenotox) (identified in 6 members)

The OECD (Q)SAR Application Toolbox profilers are expert rule-based systems with defined positive alerts ("positive list"). The profilers are not associated with a defined AD. They can be applied for positive identification but if no alert is identified it is not a "negative" prediction, but absence of identification of a positive alert in that particular profiler. For some but not all of the applied profilers the identified structural alerts are associated with mechanistic information.

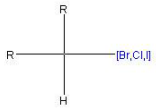
The OECD (Q)SAR Application Toolbox profilers for genotoxicity identified alerts in all category parent members and/or their metabolites in the profilers for DNA binding by OECD, DNA binding by OASIS, *in vitro* mutagenicity (Ames test) alerts by ISS and *in vivo* mutagenicity (Micronucleus, in rats and mice) alerts by ISS. The profilers for DNA alerts for AMES, MN and CA and for protein binding for chromosomal aberration, both by OASIS, identified alerts in the majority of the category members (55 and 41, respectively). Oncologic Primary Classification identified alerts in all but one member (ID number 52125_3). Carcinogenicity (genotox and nongenotox) alerts by ISS identified alerts in all parent members and/or their metabolites.

As it appears from the listing above, a number of alerts in the different profilers were identified indicating multiple possible genotoxic mechanisms. However, there was overlap in some of the identified alerts from the different profilers. Some alerts were identified in fewer members and/or their metabolites and some were identified in many or all of the members and/or their metabolites.

One alert was identified in all 61 category members, namely the "Aliphatic halogen" alert in the three ISS profilers for *in vitro* mutagenicity (Ames test), *in vivo* mutagenicity (Micronucleus, in rats and mice) and carcinogenicity (genotox and nongenotox). Furthermore, there was one alert which was identified in all but one (ID number 52125_3) category members, namely the "H-acceptor-path3-H-acceptor" alert in the ISS profiler for *in vivo* mutagenicity (Micronucleus). The alerts "Mono aldehydes" from DNA binding by OECD, "Simple aldehyde" from the three ISS profilers and "Aldehyde Type Compounds" from Oncologic Primary Classifications profilers were identified in all but two members (ID numbers 52123_213821-20-6 and 52125_3). The explanations for these alerts as provided by the Toolbox are shown in Figures 2-6 (see also Appendix 7).

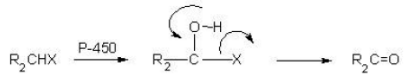
As described above, the "Aliphatic halogen" alert from the three ISS profilers for *in vitro* mutagenicity (Ames test), *in vivo* mutagenicity (Micronucleus, in rats and mice) and carcinogenicity (genotox and nongenotox) was identified in all 61 category members. The explanation for the "Aliphatic halogen" alert as provided by the Toolbox for all three ISS profilers is shown in Figure 2.

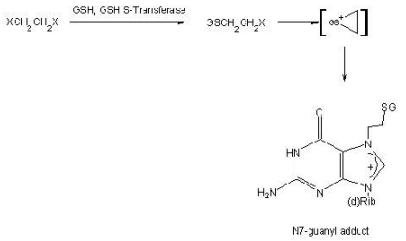
Aliphatic halogens



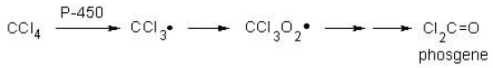
R = any atom/group

Numerous haloalkanes have been tested for carcinogenic and mutagenic activities. In general, the genotoxic potential is dependent on the nature, number, and position of halogen(s) and the molecular size of the compound (Woo et al. 2002). Although some aliphatic halogens have been shown to directly alkylate macromolecules (Bolt and Gansewendt 1993), biotransformation may also play an important role in their toxicity. Cytochrome P450 oxidation may produce gem-halohydrins that spontaneously dehydrohalogenate to reactive carbonyl compounds (Guengerich 1991), (see reaction 1). Alternatively, glutathione (GSH) conjugation via GSH transferases, has been proposed as an activation mechanism for several halogenated alkanes (Guengerich 2003b); (Guengerich 2003a) (as an example for dihaloethanes see Reaction 2).

1. 

2. 

Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms (Woo *et al.* 2002). In the case of CCl4 (see reaction 3.), P450 reduces CCl4 to the trichloromethyl radical which can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes.

3. 

Adduct formation between CCl3* and DNA is thought to function as initiator in the case of hepatic cancer. This radical can also react with oxygen to form highly reactive species, the trichloromethylperoxy radical CCl3OO*, that may initiate the chain reaction of lipid peroxidation, and ultimately generate phosgene (Guengerich 1991).

References Cited

Bolt, H. M. and Gansewendt, B. (1993). Mechanisms of Carcinogenicity of Methyl Halides. *Crit.Rev.Toxicol.* 23, 237-253.

Guengerich, F. P. (1991). Oxidation of Toxic and Carcinogenic Chemicals by Human Cytochrome P-450 Enzymes. *Chem.Res.Toxicol.* 4, 391-407.

Guengerich, F. P. (2003a). Activation of Dihaloalkanes by Thiol-dependent Mechanisms. *J.Biochem.Mol.Biol.* 36, 20-27.

Guengerich, F. P. (2003b). Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. Arch.Biochem.Biophys. 409, 59-71.

Woo, Y. T., Lai, D. Y., McLain, J. L., Ko Manibusan, M., and Dellarco, V. (2002). Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ.Health Perspect. 110, 75-87.

FIGURE 2 OECD (Q)SAR APPLICATION TOOLBOX EXPLANATION OF THE "ALIPHATIC HALOGEN" STRUCTURAL ALERT FROM THE THREE ISS PROFILERS

According to the alert explanation some aliphatic halogens may be direct alkylating agents while metabolic activation may play a role for others. Cytochrome P450 oxidation may as shown in reaction 1 lead to reactive carbonyl (aldehyde or ketone) compounds. Reaction 2 gives an example of another proposed activation mechanism by glutathione (GSH) conjugation. Reaction 3 in Figure 2 does not seem relevant for this category of small linear and branched brominated alkyl alcohols, since the members were required to have maximum one bromine atom per carbon atom.

As described above, the "H-acceptor-path3-H-acceptor" alert from the ISS profiler for *in vivo* mutagenicity (Micronucleus) was identified in all but one (ID number 52125_3) category members and/or their metabolites. The explanation for the "H-acceptor-path3-H-acceptor" alert is shown in Figure 3.

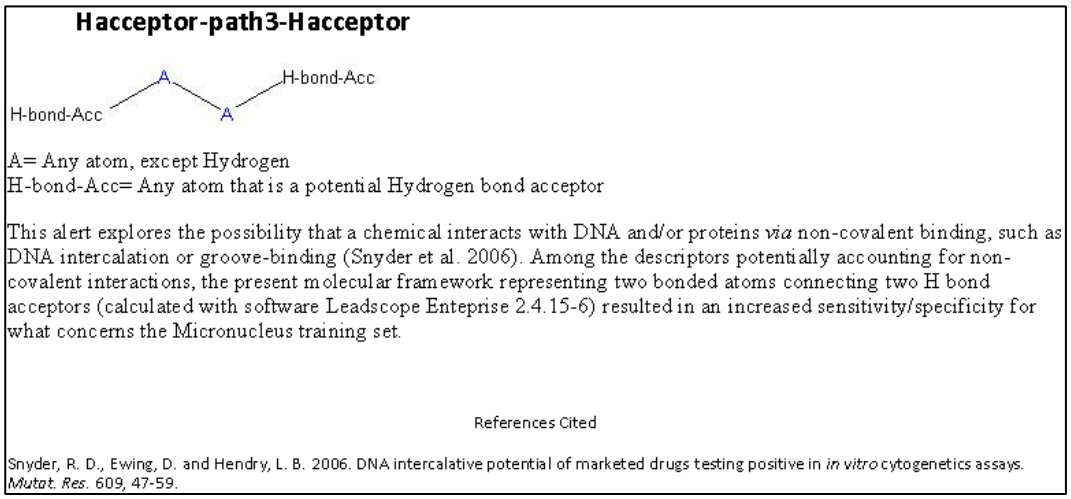
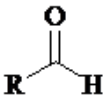


FIGURE 3 OECD (Q)SAR APPLICATION TOOLBOX EXPLANATION OF THE "H-ACCEPTOR-PATH3-H-ACCEPTOR" STRUCTURAL ALERT IN THE ISS PROFILER FOR *IN VIVO* MUTAGENICITY (MICRONUCLEUS)

According to the explanation in Figure 3 this alert explores the possibility that a chemical interact with DNA and/or protein via non-covalent binding.

As described above, the alerts "Mono aldehydes" from DNA binding by OECD, "Simple aldehyde" from the three ISS profilers and "Aldehyde Type Compounds" from Oncologic Primary Classifications profilers were identified in metabolites from all but two members (ID numbers 52123_213821-20-6 and 52125_3). The explanations for the alerts are shown in Figure 4, 5 and 6.

Structural alert: Mono-aldehydes

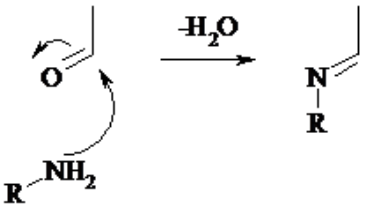


R = sp3 carbon, hydrogen

-

Mechanism

Mono aldehydes undergo Schiff base formation (Garcia et al 2009, Hecht et al 2001).



R = DNA chain

Sructural alert mitigating factors

- No mitigating factors have been reported for the chemicals in this mechanistic alert.

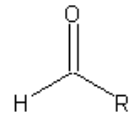
-

References

Garcia CL et al (2009) Mutation Research, 662, 3-9

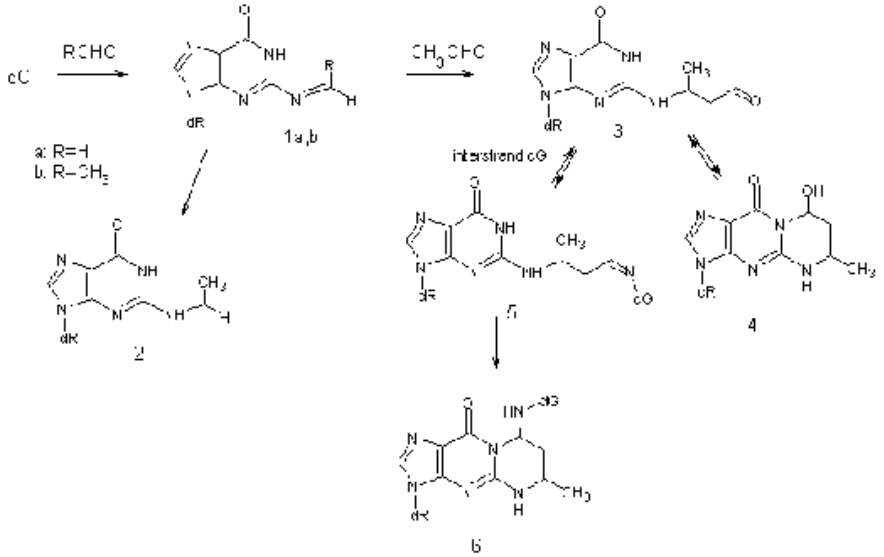
FIGURE 4 OECD (Q)SAR APPLICATION TOOLBOX EXPLANATION OF THE "MONO ALDEHYDES" STRUCTURAL ALERT FROM THE DNA BINDING BY OECD PROFILER

Simple Aldehydes



R= aliphatic or aromatic carbon
alpha, beta-unsaturated aldehydes are excluded

All compounds carrying an aldehydic group can potentially undergo Schiff base formation with a primary amine. They are to be considered potentially genotoxic, as demonstrated *in vivo* ability to react with nucleobases, without metabolic activation, forming adducts, interbase cross-links (both intra and inter-strand), and DNA-protein crosslinks The length of carbon chain for aliphatic aldehydes, and in general molecular size, can strongly modulate the formation of every type of cross-link and even the accessibility of the DNA nucleobases (Romano Zito, personal communication). DNA-protein crosslinks have been reported as the primary DNA damage induced by formaldehyde (Speit *et al.* 2007). The initial step of the reaction probably involves formation of an unstable Schiff base with the exocyclic amino group of deoxyguanosine dG (1a). In the case of acetaldehyde, this intermediate (1b) could be stabilized by reduction, producing *N*2-ethyl-dG (2), or alternatively may react with a second molecule of acetaldehyde forming a new aldehyde adduct (3) that ultimately cyclize in an 8-hydroxypropano adduct (4). The latter exists in equilibrium with its ring-opened aldehyde form, and may undergo condensation with another guanine to form imine-linked bis-nucleoside (5) which in turn cyclizes to pyrimidopurinone (6) (Wang *et al.* 2000).



Some aldehydes may also induce hydroxyalkyl adducts in DNA, but the relevance of these DNA modifications for mutagenicity is unclear (Speit *et al.* 2007).

References Cited

Speit, G., Schütz, P., Högel, J., and Schmid, O. (2007). Characterization of the genotoxic potential of formaldehyde in V79 cells. *Mutagenesis* 22, 387-394.

Wang, M., McIntee, E. J., Cheng, G., Shi, Y., Villalta, P. W., and Hecht, S. S. (2000). Identification of DNA Adducts of Acetaldehyde. *Chem.Res.Toxicol.* 13, 1149-1157.

FIGURE 5 OECD (Q)SAR APPLICATION TOOLBOX EXPLANATION OF THE "SIMPLE ALDEHYDE" STRUCTURAL ALERT FROM THE THREE ISS PROFILERS

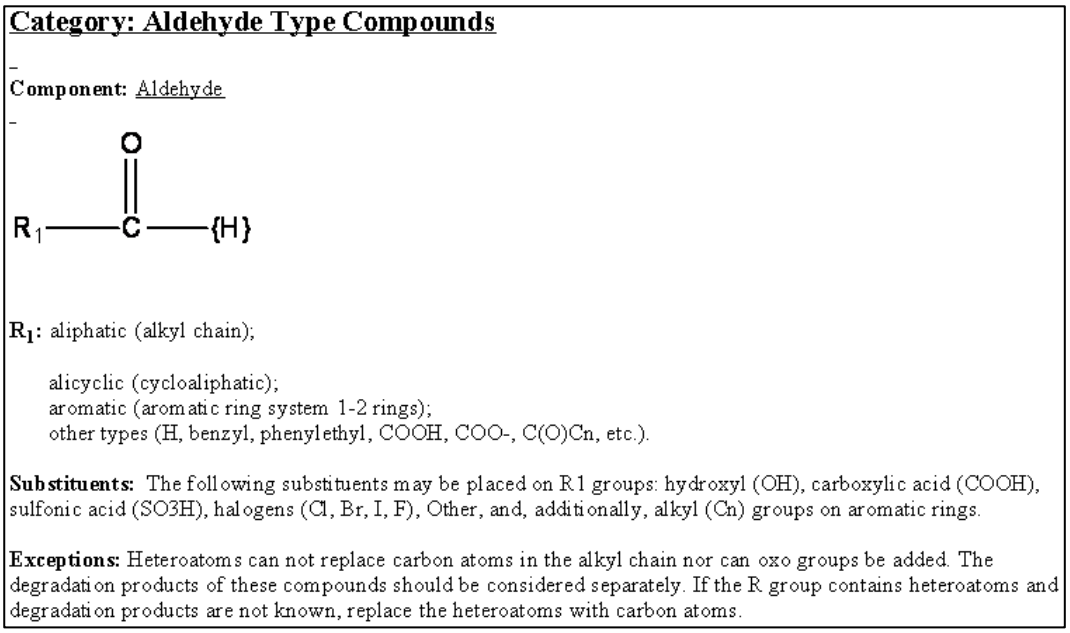


FIGURE 6 OECD (Q)SAR APPLICATION TOOLBOX EXPLANATION OF THE "ALDEHYDE TYPE COMPOUNDS" STRUCTURAL ALERT FROM THE ONCOLOGIC PRIMARY CLASSIFICATIONS PROFILER

According to the explanations in Figure 4 and 5 aldehyde compounds can potentially undergo Schiff base formation with a primary amine to form DNA adducts and cross-links.

The OECD (Q)SAR Application Toolbox does not contain information about the accuracy of the profilers or the individual alerts. However, it has been assessed by some studies as described below.

In 1988 Ashby and Tennant (Ashby et al. 1988) analysed 222 chemicals evaluated for carcinogenicity in mice and rats by the United States NCI/NTP. The structure of each chemical was assessed for potential electrophilic (DNA-reactive) sites, its mutagenicity to Salmonella, and the level of its carcinogenicity to rodents. A strong correlation of 90% was found between the chemical structure and Salmonella mutagenicity across 115 carcinogens, 24 equivocal carcinogens and 83 non-carcinogens. As part of their well-known poly-carcinogen, aromatic and aliphatic substituted primary alkyl halides were identified by Ashby and Tennant to be a structural alert for genotoxic carcinogenicity. Tertiary aliphatic halogen substituents and geminal tri- or di-halogen substituents, as well as primary aliphatic halocarbons adjoined to a sterically crowded atom (e.g. to a $CHCl_2$ or CCl_3 substructure) were classified as negative. Such types of compounds are not included in this category of small linear and branched brominated alkyl alcohols.

Kazius et al. (2005) analysed a dataset of 4337 molecular structures with corresponding Ames test data (2401 mutagens and 1936 nonmutagens). Specific toxicophores were derived and approved by employing chemical and mechanistic knowledge in combination with statistical criteria. Toxicophores were defined as substructures that indicate an increased potential for mutagenicity, whether this is caused by DNA reactivity or not. I.e. a toxicophore represents a reactive substructure or a substructure that is prone to either metabolic activation or intercalation. A final set of 29 toxicophores containing new substructures was assembled that could classify the mutagenicity of the investigated dataset with a total classification error of 18%. Furthermore, mutagenicity predictions of an independent validation set of 535 compounds were performed with an error percentage of 15%. Since these error percentages approach the average interlaboratory reproducibility error of Ames tests, which is 15%, it was concluded that these toxicophores can be applied to risk assessment processes and can guide the design of chemical libraries for hit and lead optimization.

As part of this work Kazius et al. identified the aliphatic halide (excluding the fluorine atom) substructure to be an alert for mutagens. Out of 416 aliphatic halide compounds, 297 were mutagens, corresponding to 71% (p-value <<0.05). If compounds containing other toxicophores were excluded, there were 330 aliphatic halide compounds, of which 217 were mutagens, corresponding to 66% (p-value <<0.05). I.e. this alert does not discriminate very precisely between positives and negatives and identifies 34% false positives among the training set chemicals.

According to Benigni et al. (2008 and 2010) the aliphatic halogen alert from the three ISS profilers has a positive predictivity for carcinogenicity of 74% (49 out of 66 substances experimentally tested for carcinogenicity and containing the alert fragment had positive results from the carcinogenicity studies).

According to Benigni et al. (2010) the hacceptor-path3-hacceptor alert from the ISS *in vivo* mutagenicity (Micronucleus) profiler has a positive predictivity for *in vivo* micronucleus of 34% (55 out of 163 substances experimentally tested for in vivo micronucleus and containing the alert fragment had positive results from the micronucleus studies). No statistics for positive predictivity for carcinogenicity was found.

According to Benigni et al. (2008) the simple aldehyde alert from the three ISS profilers has a positive predictivity for carcinogenicity of 88% (7 out of 8 substances experimentally tested for carcinogenicity and containing the alert fragment had positive results from the carcinogenicity studies).

Additional Toolbox category investigation exercises

The OECD (Q)SAR Application Toolbox v 3.3.2 was used in a preliminary exercise to investigate further sub-category formation possibilities and to find possible analogs with experimental data. The chemical structures of all 61 category members were imported into the Toolbox. No experimental results in any of the Toolbox inventories were found for the category members.

Mutagenicity and carcinogenicity properties were profiled using the following profilers: DNA alerts for Ames, MN and CA by OASIS v.1.3, in vitro mutagenicity (Ames test) alerts by ISS, in vivo mutagenicity (Micronucleus) alerts by ISS, Carcinogenicity (genotox and non-genotox) by ISS and Oncologic Primary Classification.

It was discovered in the process that the (Q)SAR Application Toolbox does not suggest possible sub-categorizations when multiple “targets” are processed as a category, in this case the 61 potential category members.

As a next step, the category definition function of the (Q)SAR Application Toolbox was used to find analogues among chemicals in the contained inventories (analogues chemicals are chemicals that are similar to other chemical). The above profilers were selected as filters one by one to retrieve possible analogs with high chemical similarity to the category members. The selection has different modes of operation, among others, two logical modes (AND and OR) are available, and the OR was selected as the AND mode is too restrictive for this size of category and therefore not expected to return analogs (confirmed for selected profilers).

This exercise returned thousands of possible analogs, for example:

- the *in vitro* mutagenicity (Ames test) alerts by ISS based retrieval of analogs returned 3,570 analogs, of which 1,108 contained bromine,
- the Oncologic Primary Classification profiler based retrieval of analogs returned 65,998 analogs, of which 1,697 contained bromine, and
- the Carcinogenicity (genotox and nongenotox) alerts by ISS profiler returned 32,474 analogs of which 1,878 contained bromine.

Some of these analogs have experimental information for mutagenicity and cancer in the (Q)SAR Application Toolbox, but it requires further analysis to filter the data and retrieve experimental information only for the chemically closest analogs, and this was not possible within the scope of this project.

Overall (Q)SAR results for genotoxicity and carcinogenicity

The 61 members in the category of small linear and branched brominated alkyl alcohols were predicted by a number of (Q)SAR models to be positive for carcinogenic and genotoxic properties indicating that they have a carcinogenic potential with a possible mutagenic/genotoxic mode of action. The estimated specificities of the models as established by leave-many-out cross-validations are between 85.9% and 95.1%, i.e. the overall false positive rates of the models are around 5%- 14%.

From the identified alerts in a number of OECD (Q)SAR Application Toolbox profilers, there was one alert which was identified in all 61 category members, namely the “Aliphatic halogen” alert identified in the three ISS profilers for *in vitro* mutagenicity (Ames test), *in vivo* mutagenicity (Micronucleus, in rats and mice) and carcinogenicity (genotox and nongenotox). This alert identified 34% false positives among the mutagenicity training set chemicals (Kazius et al. 2005). According to Benigni et al. (2008 and 2010) this alert has a positive predictivity for carcinogenicity of 74%.

From the explanation of the alert contained in the OECD (Q)SAR Application Toolbox it does not seem that there is one single mechanistic interpretation of the ISS aliphatic halogen alert in relation to mutagenicity and cancer. Specific read-across hypotheses on the molecular level for different sub-categories within the category may possibly be based on in-depth analysis of the mechanistic interpretations of the remaining identified alerts in the OECD (Q)SAR Application Toolbox profilers. However, this was outside the scope of this project.

4. Experimental data

This phase of the project consisted of a literature search to collect experimental data on human health effects and an evaluation of the retrieved data with focus on identification of a critical human health effect for the members of the category of brominated flame retardants selected for this phase, i.e. the category of small linear and branched alkyl alcohols.

4.1 Data collection and evaluation of data

The literature search was performed for the three members of the category identified in the preliminary structural grouping, i.e. 2,3-DBPA (CAS RN 96-13-9, 83165-36-0, 83165-35-9), DBNPG (CAS RN 3296-90-0) and TBNPA (CAS RN 1522-92-5, 36483-57-5), as well as for the 22 new category members identified in the definition of the category, which have a CAS RN assigned.

The literature search was performed in the databases SciFinder, PubMed and Scopus with selected search terms, e.g. substance name, CAS RN and combinations (e.g. 'propanol AND dibromo'; 'hydroxypropane AND dibromo') etc. as documented in the literature search document, see Appendix 8.

All the retrieved data from the literature search were checked in order to identify relevant data on human health effects. For the purpose of this project, i.e. to perform a category approach and read across for the critical effect across all or some of the theoretical members of the category, the relevant data on human health effects are: human data and animal data on repeated dose toxicity, genotoxicity, carcinogenicity and reproductive toxicity, as well as *in vitro* data on genotoxicity, endocrine activity and other mode/mechanisms of action.

Data were retrieved for 24 of the 25 category members with a CAS RN assigned. Relevant data on human health effects were only retrieved for two of these members, i.e. for two of the three members of the category identified in the preliminary structural grouping, 2,3-DBPA (CAS RN 96-13-9) and DBNPG (CAS RN 3296-90-0), see Table 3.

Two of the three members of the category identified in the preliminary structural grouping are registered in REACH, i.e. DBNPG (CAS RN 3296-90-0) and TBNPA (CAS RN 36483-57-5). The third member of the category identified in the preliminary structural grouping is pre-registered in REACH, i.e. 2,3-DBPA (CAS RN 96-13-9). Two of the 22 category members with a CAS RN assigned identified in the preliminary structural grouping are also pre-registered in REACH, i.e. 1,3-dibromo-2-propanol (CAS RN 96-21-9) and 1,4-dibromo-2-butanol (CAS RN 19398-47-1).

For the two substances registered under REACH, the REACH registrations in the publicly accessible part of the REACH Registration Dossier Database, hosted on the ECHA website, were checked in order to identify eventual additional relevant information. For TBNPA (CAS RN 36483-57-5), the REACH registration was the only source of information. For DBNPG (CAS RN 3296-90-0), no additional relevant information was identified in the REACH registration.

Substance name	CAS RN	Data found	Relevant data	REACH
Members of the category identified in the preliminary structural grouping				
2,3-Dibromo-1-propanol (2,3-DBPA)	96-13-9 83165-36-0 83165-35-9	Yes Yes Yes	Yes No No	Pre-registered No No
2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG)	3296-90-0	Yes	Yes	Registered
2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA)	1522-92-5 36483-57-5	Yes Yes	No No	No Registered
New members identified in the definition of the category				
1,3-Dibromo-2-propanol	96-21-9	Yes	No	Pre-registered
2,3,4-Tribromo-1-butanol	855236-37-2	Yes	No	No
1,2,4-Tribromo-3-butanol	87018-38-0	Yes	No	No
3,4-Dibromo-1,2-butanediol	35330-59-7	Yes	No	No
1,4-Dibromo-2,3-butanediol	14396-65-7 299-70-7 1947-59-7 15410-44-3	Yes Yes Yes Yes	No No No No	No No No No
3-Bromo-2-(bromomethyl)-1-propanol	106023-63-6	Yes	No	No
1,4-Dibromo-2-butanol	19398-47-1 64028-90-6 1360729-08-3	Yes Yes Yes	No No No	Yes No No
3,4-Dibromo-2-butanol	79033-40-2	Yes	No	No
2,3-Dibromo-1-butanol	4021-75-4 54899-03-5 70528-70-0	Yes Yes Yes	No No No	No No No
3,4- Dibromo-1-butanol	87018-30-2	Yes	No	No
2,2-Bis(bromomethyl)-1-propanol	105100-80-9	Yes	No	No
4,5-Dibromo-2-pentanol	213821-22-8	Yes	No	No
1,2-Dibromo-3-pentanol	408319-76-6	Yes	No	No
1,4-dibromo-(R*,R*)-(9CI)-3-pentanol	159475-15-7 159475-16-8	Yes Yes	No No	No No
2,4-Dibromo-3-pentanol	343268-04-2 72770-99-1	Yes Yes	No No	No No
3,4-Dibromo-(2R*,3S*,4S*)- (9CI)-2-pentanol	76377-07-6 76420-11-6	Yes Yes	No No	No No
4,5-Dibromo-1-pentanol	59287-66-0	Yes	No	No
2,5-Dibromo-1-pentanol	856991-78-1	No	No	No
1,5-Dibromo-2-pentanol	100606-66-4 1092554-97-6	Yes Yes	No No	No No
2,5-Dibromo-2-pentanol	213821-20-6 159475-17-9 159475-18-0	Yes Yes Yes	No No No	Yes No No
4-Bromo-2-(bromomethyl)- 1-butanol	98069-26-2	Yes	No	No
4-Bromo-2-(bromomethyl)- 1,3-butanediol	44804-46-8	Yes	No	No

TABLE 3 SUMMARY OF THE RESULTS OF THE DATA COLLECTION AND EVALUATION FOR THE 25 CATEGORY MEMBERS WITH A CAS RN ASSIGNED

For one of the three members of the category identified in the preliminary structural grouping, there is a harmonised classification. For the two other members of the category identified in the preliminary structural grouping, there are notified classifications. Also for two of the new members of the category identified in the definition of the category, there are notified classifications. The classifications for these substances are summarised in Table 4.

Substance name	CAS RN	C&L Inventory
Members of the category identified in the preliminary structural grouping		
2,3-Dibromo-1-propanol (2,3-DBPA)	96-13-9	Harmonised classification: Acute Tox. 4 H302 Acute Tox. 3 H311 Acute Tox. 4 H332 Carc. 1B H350 Repr. 2 H361f *** Aquatic Chronic 3 H412
2,2-bis(bromomethyl)-1,3-propanediol (DBNPG)	3296-90-0	Notified classification: Acute Tox. 4 H302 Skin Irrit. 2 H315 Eye Irrit 2 H319 STOT SE 3 H335 (respiratory system) STOT RE 2 H373 (kidney, bladder - oral) Muta. 1B H340 / Muta. 2 H341 Carc. 1B H350 / Carc. 2 H351 Aquatic Chronic 4 H413
2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA)	1522-92-5	Notified classification: Acute Tox. 4 H302 Acute Tox. 4 H312 Acute Tox. 4 H332 Skin Irrit. 2 H315 Eye Irrit 2 H319
	36483-57-5	Notified classification: Acute Tox. 4 H302 Eye Irrit 2 H319 Muta. 1B H340 / Muta. 2 H341 Carc. 1B H350 Aquatic Chronic 3 H412

New members identified in the definition of the category		
1,3-Dibromo-2-propanol	96-21-9	Notified classification: Flam. Liq. 3 H226 Acute Tox. 3 H301 Skin Irrit. 2 H315 Eye Irrit 2 H319 STOT SE 3 H335 Carc. 2 H351
1,4-Dibromo-2,3-butanediol	14396-65-7	Notified classification: Skin Irrit. 2 H315 Eye Dam 1 H318 STOT SE 3 H335

TABLE 4 HARMONISED / NOTIFIED CLASSIFICATION AVAILABLE FOR THE MEMBERS OF THE CATEGORY

4.2 Experimental studies on repeated dose toxicity and carcinogenicity

Experimental studies examining repeated dose toxicity and carcinogenicity are summarised in Table 5.

Method	Results	Reference
2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)		
Test substance: FR-1138® technical grade (with a composition of 78.6% 2,2-bis(bromomethyl)- propane-1,3-diol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol and 7.7% dimers and structural isomers) Sub-chronic repeated dose toxicity study Test substance administered in the feed for 13 weeks F344/N rats (10 per sex per group): 0, 1,250, 2,500, 5,000, 10,000, 20,000 ppm (corresponding to approximately 0, 100, 200, 400, 800, 1700 mg/kg bw/day for males and 0, 100, 200, 400, 800, 1600 mg/kg bw/day for females)	Rats: No rats died during the study. Final mean body weights and weight gains of 5,000, 10,000, and 20,000 ppm males and females were significantly lower than those of the controls. Chemical-related differences in clinical pathology parameters included increased urine volumes accompanied by decreased urine specific gravity and minimally increased protein excretion in 10,000 and 20,000 ppm males; in females, urine parameters were less affected than males. Serum protein and albumin concentrations in female rats exposed to 2,500 ppm and higher were slightly lower than those of the controls. Renal papillary degeneration was present in 5,000 and 10,000 ppm males, and in 20,000 ppm males and females. Hyperplasia of the urinary bladder was present in 20,000 ppm males. Mice: One control female, two males and one female receiving 625 ppm, one female receiving 1,250 ppm, one female receiving 2,500 ppm, one female receiving 5,000 ppm, and three males receiving 10,000 ppm died during the study.	NTP (1996)

B6C3F1 mice (10 per sex per group): 0, 625, 1,250, 2,500, 5,000, 10,000 ppm (corresponding to approximately 0, 100, 200, 500, 1300, 3000 mg/kg bw/day for males and 0, 140, 300, 600, 1200, 2900 mg/kg bw/day for females)	Final mean body weights and body weight gains of males and females receiving 1,250, 2,500, 5,000, or 10,000 ppm and of females receiving 625 ppm were significantly lower than those of the controls. Feed consumption by exposed mice was generally higher than that by controls throughout the study. Clinical findings included abnormal posture and hypoactivity in 10,000 ppm male and female mice. Blood urea nitrogen concentrations of 5,000 ppm females and 10,000 ppm males and females were greater than those of controls. Also, urine specific gravity was lower in 10,000 ppm females. Differences in organ weights generally followed those in body weights. Papillary necrosis, renal tubule regeneration, and fibrosis were observed in the kidneys of 2,500 and 5,000 ppm males and 10,000 ppm males and females. Urinary bladder hyperplasia was observed in 5,000 and 10,000 ppm males and females.	
Test substance: FR-1138® technical grade (78.6% pure according to IARC (2000); no information in the article). As the feed study is included in the NTP (1996) report, the composition of the test substance for gavage administration is probably the same as for the feed study Sub-chronic repeated dose toxicity study Test substance administered in the feed or by gavage (in corn oil, 5 days a week) for 13 weeks F344/N rats (10 per sex per group) Gavage: 0, 50, 100, 200, 400, 800 mg/kg bw Feed: 0, 1,250, 2,500, 5,000, 10,000, 20,000 ppm (corresponding to approximately 0, 100, 200, 400, 800, 1700 mg/kg bw/day for males and 0, 100, 200, 400, 800, 1600 mg/kg bw/day for females) B6C3F1 mice (10 per sex per group) Gavage: 0, 25, 50, 100, 200, 400 mg/kg bw Feed: 0, 625, 1,250, 2,500, 5,000, 10,000 ppm (corresponding to	Rats – test substance administered by gavage: 2/10 high-dose males died during the study. Dose-related clinical signs of toxicity included inactivity or lethargy after dosing at 400 and 800 mg/kg in males and females. Final mean body weights of 800 mg/kg bw males and females were significantly lower than those of the controls. Renal papillary degeneration was present in 800 mg/kg bw males. Hyperplasia of the urinary bladder was present in 40 and 800 mg/kg bw males. Mice – test substance administered by gavage: 3/10 high-dose males died during the study. Dose-related clinical signs of toxicity included inactivity or lethargy after dosing at 400 mg/kg in males and females. Final mean body weights of 200 and 400 mg/kg bw males and of 400 mg/kg bw females were significantly lower than those of the controls. Papillary necrosis and renal tubule regeneration were observed in the kidneys of 200 (renal tubule regeneration) and 400 mg/kg bw males, and renal tubule regeneration in the kidneys of 400 mg/kg bw females. Urinary bladder hyperplasia was observed in 200 and 400 mg/kg bw males and in 400 mg/kg bw females. Rats and mice - test substance administered in the feed: See previous reference as the feed study is included in the NTP (1996) report.	Elwell et al. (1989)

approximately 0, 100, 200, 500, 1300, 3000 mg/kg bw/day for males and 0, 140, 300, 600, 1200, 2900 mg/kg bw/day for females)		
Test substance: FR-1138® (with a composition of 80% 2,2-bis(bromomethyl)propane-1,3-diol, 8% 2,2′-bis(bromomethyl)-1-bromo-3-hydroxypropane and 6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane) Combined repeated dose and carcinogenicity study Test substance in the feed for 2 years Sprague-Dawley rats (49-50 per sex per dose): 0, 5, 100 mg/kg bw/day	Survival was not significantly different among the groups; most rats died or were killed at between 17 and 24 months. 100 mg/kg bw/day: Slight reduction in body weight was noted in males. Slight increase in bromide content was noted in the tissues. Degenerative changes were noted in the liver, eye and possibly thyroid gland. No treatment-related effects on tumour incidence were noted. 5 mg/kg bw/day: Marginal increase in bromide content of some tissues was noted, with most values in the same range as the controls.	Keyes et al. (1980)
Test substance: FR-1138® technical grade (with a composition of 78.6% 2,2-bis(bromomethyl)- propane-1,3-diol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol and 7.7% dimers and structural isomers) Combined repeated dose and carcinogenicity study Test substance administered in the feed for 2 years (104-105 weeks) F344/N rats (60 per sex per group): 0, 2,500, 5,000, 10,000 ppm - 'continuous exposure' (corresponding to approximately 0, 100, 200, 430 mg/kg bw/day for males and 0, 115, 230, 460 mg/kg bw/day for females) An additional group of 70 male rats received 20,000 ppm (corresponding to approximately 800 mg/kg bw/day) for three months and which animals received un-dosed feed for the remainder of	Rats: Survival of 5,000 and 10,000 ppm 'continuous-exposure' males and females and 20,000 ppm 'stop-exposure' males was significantly lower than that of the controls. Mean body weights of exposed male and female rats receiving 10,000 ppm and 'stop-exposure' males receiving 20,000 ppm were lower than those of the controls throughout most of the study. In the 'continuous-exposure' study, feed consumption by exposed rats was generally similar to that by controls throughout the study; in 20,000 ppm 'stop-exposure' males, the feed consumption was lower than that by controls. Clinical findings included skin and/or subcutaneous masses on the face, tail, and the ventral and dorsal surfaces of exposed rats. In males, neoplastic effects were observed in the skin, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, hematopoietic system, and seminal vesicle. Non-neoplastic effects in the kidney, lung, thyroid gland, seminal vesicle, pancreas, urinary bladder, and forestomach were also observed. In females, neoplastic effects were observed in the oral cavity, esophagus, mammary gland, and thyroid gland. Non-neoplastic effects in the kidney were also observed. Mice:	NTP (1996), Dunnick et al. (1997)

the 2-year study period (21 months) - 'stop-exposure' Ten animals from the male control group and the 20,000 ppm 'stop-exposure' were evaluated at 3 months; nine or 10 control animals and five to nine animals from each of the 'continuous-exposure' were evaluated at 15 months. B6C3F1 mice (60 per sex per group): 0, 312, 625, 1,250 ppm (corresponding to approximately 0, 35, 70, 140 mg/kg bw/day for males and 0, 40, 80, 170 mg/kg bw/day for females) Eight to 10 animals from each group were evaluated at 15 months	Survival of 1,250 ppm males and females was significantly lower than that of the controls. Clinical findings included tissue masses involving the eye in exposed mice. In males, neoplastic effects were observed in the Harderian gland, lung, and kidney. In females, neoplastic effects were observed in the Harderian gland, lung, and skin. Non-neoplastic effects in the lung were also observed.	
2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)		
Test substance: 2,3-DBPA (98% pure) Sub-acute repeated dose toxicity study Test substance administered by dermal application (in 95% ethanol, 5 days a week) for 16 days F344/N rats, B6C3F1 mice, (5 per sex per group): 0, 44, 88, 177, 375, 750 mg/kg bw	Rats: One male and one female receiving 750 mg/kg bw died before the end of the study. The mean body weight gains and final mean body weights of dosed rats were similar to those of the controls. There were no clinical findings or gross lesions associated with chemical application. Mice: Four males and one female receiving 750 mg/kg bw died before the end of the study. The mean body weight gains and final mean body weights of dosed mice were similar to those of the controls. There were no clinical findings or gross lesions associated with chemical application.	NTP (1993)
Test substance: 2,3-DBPA (98% pure) Sub-chronic repeated dose toxicity study Test substance administered by dermal application (in 95% ethanol, 5 days a week) for 13 weeks F344/N rats, B6C3F1 mice, (10 per sex per group): 0, 44, 88, 177, 375, 750 mg/kg bw	Rats: All rats survived until the end of the study. The mean body weight gain for rats in the 750 mg/kg bw group was lower than that of the controls. The mean absolute and relative liver weights were increased in males receiving 375 or 750 mg/kg bw and of females receiving 750 mg/kg bw. Chemical-related lesions occurred in the kidney of male rats and in the liver of female rats. The average severity of nephropathy was slightly increased in males receiving 750 mg/kg bw, and individual cell necrosis was observed in the liver of all female rats in the 750 mg/kg bw group. Mice:	NTP (1993)

	Eight male mice receiving 750 mg/kg bw died during the study, while all female mice survived. The final mean body weights of dosed and control mice were similar. The mean absolute and relative liver weights were increased in males receiving 375 or 750 mg/kg bw and of females receiving 750 mg/kg bw. Chemical-related lesions occurred in the liver and lung of mice. Centrilobular hepatocellular necrosis occurred in all males in the 750 mg/kg bw group that died during the study, while individual cell necrosis was observed in the liver of females receiving 177, 375, or 750 mg/kg bw. Pleomorphism of the epithelium in pulmonary bronchioles occurred with a dose-related increased incidence in males and females. Necrosis of the bronchiolar epithelium was observed in males receiving 750 mg/kg bw.	
Test substance: 2,3-DBPA (98% pure) Combined repeated dose and carcinogenicity study Test substance administered by dermal application (in 95% ethanol, 5 days a week) for 48-51 weeks (male rats), 52-55 weeks (female rats), 36-39 weeks (male mice), and 39-42 weeks (female mice) F344/N rats (50 per sex per group): 0, 188, 375 mg/kg bw B6C3F1 mice (50 per sex per group): 0, 88, 177 mg/kg bw	Rats: The survival of 375 mg/kg bw male and female rats was significantly lower than that of the controls (males: 50/50, 41/50, 16/50; females: 48/50, 38/50, 24/50). The final mean body weight was lower than that of the controls in the 375 mg/kg bw group. In male rats, the incidences of neoplasms of the skin, nose, Zymbal gland, oral cavity, esophagus, and small and large intestines were significantly increased in the low- and high-dose groups, while the incidences of neoplasms of the forestomach and liver were significantly increased only in the high-dose group. Neoplasms of the kidney, vascular neoplasms of the spleen, and mesotheliomas in males occurred with a significant positive trend. In female rats, the incidences of benign or malignant neoplasms of the nose, Zymbal gland, oral cavity, esophagus, large intestine, and liver were significantly increased in the low- and high-dose groups, while the incidences of neoplasms of the skin, forestomach, small intestine, mammary gland, and clitoral gland were significantly increased in the high-dose group only. Neoplasms of the kidney in females occurred with a significant positive trend. Non-neoplastic lesions included increased incidences of hyperkeratosis in the skin, forestomach, and esophagus, epithelial dysplasia in the nose, pleomorphism and basophilic and clear cell changes in the liver, and nuclear enlargement in the kidney. There were also chemical-related increases in the incidences of forestomach ulcers and acanthosis, angiectasis in the liver, and renal hyperplasia in male rats and epithelial dysplasia of the forestomach and bileduct hyperplasia in the liver in female rats. Mice:	NTP (1993)

	All mice (except two low-dose females) survived until study termination. In male and female mice, the incidences of benign or malignant neoplasms of the forestomach were significantly increased in the low- and high-dose groups, while the incidences of neoplasms of the skin were significantly increased only in the high-dose groups. The incidences of liver and lung neoplasms were increased in high-dose males. Non-neoplastic lesions included increased incidences of hyperplasia in the skin, epithelial dysplasia of the forestomach, and bronchiolar epithelial pleomorphism and hyperplasia in male and female mice and in the incidence of eosinophilic cytoplasmic change in the liver in males.	
2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA) (CAS RN 36483-57-5)		
Test substance: 2,2-Dimethylpropan-1-ol, tribromo derivative (98.4% pure) Sub-acute repeated dose toxicity study Test substance administered by gavage (in corn oil) daily for 14 days (except high-dose males, 4 consecutive days) Crl:CD(SD) rats (5 per sex per group): 0, 100, 300, 1000 mg/kg bw/day	All males receiving 1000 mg/kg bw/day were killed for welfare reasons on day 4 of treatment. Clinical signs included abnormal gait, unresponsive, underactive, flat posture, prostrate posture and high levels of urine staining. Macroscopic examination revealed abnormal contents and pallor of the jejunum in 3/5 animals. Clinical signs in females revealed urine staining during the treatment period in 3/5 animals at 1000 mg/kg bw/day. No other treatment-related findings were noted.	Study report – cited from REACH Registration Dossier Database
Test substance: 2,2-Dimethylpropan-1-ol, tribromo derivative (98.4% pure) Sub-acute repeated dose toxicity study Test substance administered in feed for 30 days Sprague-Dawley rats (5 per sex per group): 0, 10, 30, 100, 300 mg/kg bw/day	A decrease in serum glutamic pyruvic transaminase (SPGT) was noted at 100 and 300 mg/kg bw/day, and an increase in blood urea nitrogen (BUN) was noted in males at 300 mg/kg bw/day. Renal tubular damage and generalized hyperplasia of the mucosal lining of the urinary bladder were observed in males at 100 and 300 mg/kg bw/day.	Study report – cited from REACH Registration Dossier Database

TABLE 5. STUDIES ON REPEATED DOSE TOXICITY AND CARCINOGENICITY

In the repeated dose toxicity and carcinogenicity studies summarised in Table 5, several possible target organs and tissues were identified. The findings are summarised in Table 6 and further detailed in the following sections.

Target organ	96-13-9	3296-90-0	36483-57-5
Liver	Lesions / necrosis / neoplasms	Degeneration / neoplasms	-
Kidney	Necrosis / fibrosis/ neoplasms	Hyperplasia/ degeneration / necrosis / neoplasms	Tubular damage
Urinary bladder	Hyperplasia	Hyperplasia / neoplasms	Hyperplasia
Oral cavity	Neoplasms	Neoplasms	-
Esophagus	Neoplasms / non-neoplastic changes	Neoplasms	-
Forestomach	Neoplasms/ non-neoplastic changes	Neoplasms / non-neoplastic changes	-
Intestines, small and large	Neoplasms	Neoplasms	-
Hematopoietic system	-	Neoplasms	-
Spleen	Neoplasms	-	-
Thyroid gland	-	Degeneration/ neoplasms / non-neoplastic changes	-
Pancreas	-	Neoplasms / non-neoplastic changes	-
Mammary gland	Neoplasms	Neoplasms	-
Clitoral gland	Neoplasms	-	-
Seminal vesicle	-	Neoplasms / non-neoplastic changes	-
Nose	Neoplasms / non-neoplastic changes	-	-
Lung	Necrosis / neoplasms	Neoplasms / non-neoplastic changes	-
Skin	Neoplasms / non-neoplastic changes	Neoplasms	-
Zymbal gland	Neoplasms	Neoplasms	-
Harderian gland	-	Neoplasms	-
Eye	-	Degeneration	-
Mesothelium	Increased incidence	Increased incidence	-

TABLE 6. POSSIBLE TARGET ORGANS IN RATS AND MICE

4.2.1 Liver

4.2.1.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), there were treatment-related increased incidences of neoplasms in the liver.

In the two-year study with administration of technical grade FR-1138® containing 80% of DBNPG (CAS RN 3296-90-0) in the diet to rats (Keyes et al. 1980), degenerative changes in the liver (increased centrilobular homogeneity of the hepatocellular cytoplasm) were observed.

4.2.1.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 13-week studies with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), liver lesions were observed in female rats and male and female mice.
Male mice were more sensitive to the acute toxic effects of this chemical than were rats or female mice. Eight of 10 male mice receiving dermal applications of 750 mg/kg bw died during the 13-week study, but there were no deaths in rats or female mice receiving up to 750 mg/kg bw 2,3-dibromo-l-propanol for 13weeks. Male mice dying as a result of treatment with 2,3-dibromo-l-propanol had generalized centrilobular necrosis of the liver.
In contrast to male mice, female mice and female rats receiving dermal applications of 750 mg/kg 2,3- dibromo-1-propanol exhibited slight individual cell necrosis in the liver.

In the 2-year studies with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the liver of male and female rats and in the liver of male mice.

4.2.2 Kidney and urinary bladder

4.2.2.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In both 13-week studies with administration of technical grade FR-1138® containing 78.6% of DBNPG to rats and mice by gavage or in the diet (NTP 1996, Elwell et al. 1989), treatment-related lesions were limited to the kidney (papillary degeneration and necrosis) and urinary bladder (hyperplasia of the transitional-cell epithelium) of treated rats and mice.
Mice were more sensitive than rats. Male rats and mice were more sensitive than females to the development of renal papillary degeneration or necrosis. At similar dose levels, on a mg/kg bw basis, treatment-related lesions in rats were similar in the gavage and feed studies. Lesions developed at a slightly lower dose level in mice treated by gavage than in those given the test substance in the diet.

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats (NTP 1996), non-neoplastic effects observed in the kidney of rats included papillary degeneration, increases in the incidences of hyperplasia of the renal papilla epithelium, hyperplasia of the transitional epithelium lining the renal pelvis and focal renal tubule atrophy in male rats. In male rats, transitional-cell hyperplasia of the urinary bladder was also present.
Neoplastic effects observed in the kidney of male rats included four renal tubule adenomas (one in the 5,000 ppm group and three in the 10,000 ppm group). These neoplasms are uncommon in males (mean: 2%) and may have been related to chemical administration. There was no evidence for a carcinogenic response in the kidney of the female rat. (NTP 1996).
There were increased incidences of urinary bladder transitional cell neoplasms in male rats. These incidences were low, but these neoplasms rarely occur in untreated animals (mean: 0.2%), and these neoplasms were considered to be related to treatment. Only 10 chemicals studied by the NTP have caused treatment-related urinary bladder neoplasms in male rats. It has been suggested that

some of these chemicals caused the urinary bladder neoplasms by formation of calculi, subsequent irritation, and tumour formation, but this does not appear to be the mechanism for the development of urinary bladder neoplasms observed in the present study. The early occurrence of transitional cell hyperplasia suggests that 2,2-bis(bromomethyl)-l,3-propanediol or its metabolites have a direct toxic effect on the urinary bladder in male rats. (NTP 1996).

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to mice (NTP 1996), the toxicity observed in the urinary bladder and kidney of mice in the 13-week study was not seen in this 2-year study. However, the highest dose in the 2-year study (1,250 ppm) was below the level at which these lesions were seen in the 13-week study where there was renal toxicity characterized by papillary necrosis and increased tubule regeneration. Although the highest dose in the 2-year study was half the dose causing these lesions in the 13-week study, there was a small increase in the incidence of renal tubule adenoma in male mice. In NTP studies of approximately 450 chemicals, only seven other chemicals have been identified as causing kidney neoplasms in the male mouse. Two of these were brominated chemicals (bromodichloromethane and tris(2,3-dibromopropyl)phosphate). (NTP 1996).

In another two-year study with administration of technical grade FR-1138® containing 80% of DBNPG in the diet to rats (Keyes et al. 1980), no effects in the kidney or urinary bladder were reported.

4.2.2.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 13-week studies with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a slight increase in the severity of nephropathy, primarily in the 750 mg/kg bw group.
Although it is apparent that 2,3-DBPA has some effect on the kidneys, these findings confirm previous studies indicating that 2,3-dibromo-l-propanol is not the primary metabolite responsible for the acute renal tubule necrosis associated with the administration of tris(2,3-dibromopropyl) phosphate to rats. Chemical-induced nephrotoxicity in rats and mice in NTP studies has been associated with exposure to many short-chain halogenated hydrocarbons, but no consistent sex- or species-related differences in response were found. In general, however, rats seem to be more susceptible to the nephrotoxic effects of these compounds than mice, and male rats appear to be more susceptible than female rats. (NTP 1993).

In the 2-year study with administration of 2,3-DBPA to rats by dermal application (NTP 1993), there was a marginally increased incidence of neoplasms in the kidney of male and female rats.

4.2.2.3 2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA) (CAS RN 36483-57-5)

Non-neoplastic changes were observed in the kidney and urinary bladder

In the 30-day study with administration of TBNPA to rats by gavage (study report, cited from the REACH Registration Dossier Database), renal tubular damage and generalized hyperplasia of the mucosal lining of the urinary bladder were observed in male rats.

4.2.3 Oral cavity, esophagus, forestomach, and small and large intestines

4.2.3.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), there were treatment-related increased incidences of squamous cell neoplasms in the oral cavity (tongue and pharynx) and esophagus in male and female rats. In addition, there were treatment-related squamous cell neoplasms of the forestomach and adenoma and carcinoma of the small and large intestine in male rats.

Minimal increases in the incidences of neoplasms of the forestomach were seen in male and female mice. There was no treatment-related increase in the incidence of hyperplasia of the forestomach squamous epithelium. Because the number of forestomach neoplasms was within or just above the historical control range, it was uncertain if this increase was related to treatment. (NTP 1996).

The presence of neoplasms in the gastrointestinal tract of exposed rats suggests that the chemical may interact directly with the mucosal epithelium. Although the increased incidence in intestinal neoplasms was limited to male rats, this effect was seen primarily in the 'stop-exposure' group, which did not include females. Other brominated chemicals also cause intestinal neoplasms in rats suggesting that these brominated chemicals may be acting by a similar mechanism. (NTP 1996).

Other chemicals which have been found to cause oral cavity neoplasms in rats are also genotoxic chemicals. Rats are more susceptible than mice to the formation of oral cavity neoplasms, and oral cavity neoplasms have previously been reported only in one NTP mouse study. Chemical-related esophageal neoplasms have previously been observed in rats in only two other NTP studies. (NTP 1996).

4.2.3.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year studies with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the oral mucosa, esophagus, forestomach, and small and large intestine of male and female rats and in the forestomach of male and female mice.

The pattern of neoplasm response in the stratified squamous epithelium of the upper gastrointestinal tract of rats suggests that the chemical induction of neoplasms in the oral mucosa, esophagus, and forestomach may be related to oral exposure through grooming behavior rather than from dermal absorption. The incidences of squamous cell neoplasms and the proportion of malignant to benign neoplasms decreased as the distance from the oral cavity increased. Of these three sites, the incidence of squamous cell neoplasms and the proportion of carcinomas was highest in the oral mucosa. The incidence of squamous cell neoplasms in the esophagus was intermediate between those of the oral cavity and forestomach, and few carcinomas were observed. The lowest incidence of neoplasms occurred in the forestomach, and no carcinomas were observed. (NTP 1993).

4.2.4 Hematopoietic system

4.2.4.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), the incidences of mononuclear cell leukemia in treated male rats were significantly greater than that in the control group.

4.2.5 Spleen

4.2.5.1 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year study with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a marginally increased incidence of neoplasms in the spleen of male rats.

4.2.6 Thyroid gland

4.2.6.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

No histopathological changes in the thyroid gland were reported at any dose level in either rats or mice following administration of FR-1138® containing 78.6% of DBNPG in the diet (NTP 1996, Elwell et al. 1989) or by gavage (Elwell et al. 1989) for 13 weeks.

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), the incidence of follicular cell adenoma or carcinoma were significantly greater in the male and female rats.
The occurrence of these neoplasms in the absence of diffuse thyroid gland hyperplasia supports the hypothesis that 2,2-bis(bromomethyl)-1,3- propanediol causes a direct thyroid response that is not likely secondary to sustained high concentrations of thyroid stimulating hormone. (NTP 1996).

A statistically significant increase in the incidence of thyroid retention cyst formation was seen in male rats administered 100 mg/kg bw/day FR-1138® containing 80% of DBNPG for 2 years (Keyes et al. 1980). According to the authors, this effect may or may not have been treated-related as there was no increase in follicular hypertrophy or hyperplasia.

The carcinogenic effect DBNPG was not thyroid specific, as the compound was also a clear carcinogen in many other tissues in both rats and mice. Overall, it is concluded that DBNPG is not a thyroid gland toxicant in mice and rats.

4.2.7 Pancreas

4.2.7.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG (CAS RN 3296-90-0) in the diet to rats and mice (NTP 1996), a marginal increase in the incidence of acinar cell adenoma of the pancreas was observed in exposed groups of male rats. Focal acinar cell hyperplasia was significantly increased in all exposure groups.
Because there was no dose-related increase in the incidence of adenomas, and all incidences were within the NTP historical control range, it was uncertain if these neoplasms were related to treatment (NTP 1996).

4.2.8 Mammary gland

4.2.8.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG (CAS RN 3296-90-0) in the diet to rats and mice (NTP 1996), the incidence of mammary gland neoplasms was increased in rats. The treatment-related increase in mammary gland fibroadenoma was greater in female than in male rats. However, there was a significant increase in subcutaneous fibroma in exposed groups of male rats.
In exposed groups of female mice there were only four mammary gland carcinomas (one in the 625 ppm group and three in the 1,250 ppm group). Because the incidences for these neoplasms were within the historical range, it was uncertain if the increase was related to chemical administration. (NTP 1996).

Other chemicals which have caused an increase in the incidences of mammary gland neoplasms in female rats have also been associated with an increased incidence in fibroma, or a combination of fibroma and fibroadenoma in male rats. The chemicals that cause mammary gland neoplasms in rats are frequently genotoxic chemicals suggesting that genetic damage may contribute to this neoplastic response. (NTP 1996).

4.2.8.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year study with administration of 2,3-DBPA rats and mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the mammary gland of female rats.

4.2.9 Clitoral gland

4.2.9.1 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year study with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the clitoral gland of female rats.

4.2.10 Seminal vesicle

4.2.10.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), there was an adenoma and a carcinoma of the seminal vesicle in the male 'stop-exposure' group.
The spontaneous development of these neoplasms is extremely rare in control rats, but treatment-related increases in hyperplasia and neoplasms have been reported in other strains of rats. Because of the rarity of these neoplasms in control rats and the presence of a dose-related increase in hyperplasia, the neoplasms in the 'stop-exposure' group were considered to be related to treatment. (NTP 1996).

4.2.11 Nose

4.2.11.1 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year study with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the nose of male and female rats.
Exposure by inhalation may have contributed to the induction of neoplasms of the nasal mucosa.

4.2.12 Lung

4.2.12.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), the incidences of lung neoplasms were increased in exposed in both species.

Lung neoplasms have been observed in mice (but not in rats) in several NTP studies with halogenated hydrocarbons. It is not known why the mouse lung is particularly responsive to the effects from these halogenated hydrocarbons, but this response could be due to differences in metabolism between species. (NTP 1996).

4.2.12.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 13-week study with administration of 2,3-DBPA to mice by dermal application (NTP 1993), lung lesions were observed in both male and female mice.

Five of the eight male mice receiving 750 mg/kg bw that died during the 13-week study had necrosis of the bronchial and bronchiolar epithelium, while males and females exhibited cytologic alterations (pleomorphism) in the distal airway epithelium. Because there may have been some volatilization of 2,3-dibromo-l-propanol after dermal application, inhalation exposure in the group-housed mice may have contributed to the lesions in the pulmonary airways. It is unknown why the intrapulmonary airways were more sensitive to 2,3-DBPA than the nasal and tracheal epithelium, but the secondary bronchi and bronchioles contain fewer goblet cells and a higher proportion of Clara cells, which are known to contain microsomal cytochrome P-450. The differences in cell population and in airflow pattern and velocity are thought to contribute to the regional specificity of airway lesions caused by chemicals. (NTP 1993).

In the 2-year study with administration of 2,3-DBPA to mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the lung of male mice. A slight increase in lung neoplasms in female mice may also have been chemical induced.

4.2.13 Skin and Zymbal gland

4.2.13.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), the incidences of skin tumors in male rats were significantly greater than those in the control group, and included increased incidences of squamous cell papilloma, keratoacanthoma, basal cell adenoma, sebaceous gland adenoma, and trichoepithelioma. There was also an increased incidence of neoplasms in the Zymbal gland (a modified sebaceous gland) in male rats.

The Zymbal gland and skin are related epithelial tissues. Most of the chemicals inducing Zymbal gland and skin neoplasms also caused neoplasms at other sites. These chemicals are generally genotoxic in the *Salmonella* assay system, and chemically induced genetic damage is thought to be the underlying mechanism for development of skin and Zymbal gland neoplasms. (NTP 1996).

Another study with 2,3-dibromo-l-propanol (CAS RN 96-13-9) has shown that genotoxic chemicals administered orally can cause skin tumors in rats, and the incidence for these tumors is generally greater in male rats than in female rats. The mechanism for this sex difference could not be determined from this study but may be due, in part, to metabolic differences between the sexes.

4.2.13.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year studies with administration of 2,3-DBPA to rats and mice by dermal application (NTP 1993), there was a significantly increased incidence of neoplasms in the skin and Zymbal gland of male and female rats and in the skin of male and female mice.

4.2.14 Harderian gland

4.2.14.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), the incidences of Harderian gland neoplasms were increased in male and female mice.
Other chemicals causing these neoplasms are usually multispecies/site carcinogens (NTP 1996).

4.2.15 Eye

4.2.15.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year study with administration of technical grade FR-1138® containing 80% of DBNPG in the diet to rats (Keyes et al. 1980), degenerative changes in the eye (bilateral diffuse opacity of the lenses) were observed.

4.2.16 Mesothelioma

4.2.16.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats (NTP 1996), there was a treatment-related increased incidence of mesothelioma in male rats.

Mesothelioma typically arises in the abdominal peritoneal cavity of F344 rats and is seen almost exclusively in males. Treatment-related increases of mesothelioma observed in previous NTP studies have also been in male rats. Other chemicals which have caused a marked increase in the incidence of mesotheliomas in male rats have also caused increases in mammary gland neoplasms in females, as is also the case for DBNPG (NTP 1996).

4.2.16.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the 2-year study with administration of 2,3-DBPA to rats by dermal application (NTP 1993), there was a marginally increased incidence of mesothelioma in male rats.

4.2.17 Other tumors

4.2.17.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP study with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to mice (NTP 1996), there was a significant increase in hemangiosarcoma and hemangioma (combined) in the female 1,250 ppm group. Two of the hemangiosarcomas were in the subcutis, which was also a site for treatment-related sarcomas in female mice. Since the combined total number of neoplasms marginally exceeded the historical control range, it is uncertain if the increase in the incidence of these neoplasms was related to treatment.

4.3 Experimental studies on reproductive toxicity

Only one study examining reproductive toxicity was retrieved. This study was performed with the substance, 2,2-bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0), see Table 7.

Method	Results	Reference
Continuous breeding RACB, Swiss CD-1 mice **0.1, 0.2, 0.4% in feed (approximately 141, 274, 589 mg/kg bw)**	At high dose: F0 and F1: Reduced litter size, reduced dam and pup body weight (also seen in crossover mating trial indicating a maternal effect) F0: Reduced number of small follicles (primordial and primary). No effect on sperm end points or vaginal cyclicity. Reduced body weight and absolute weight of seminal vesicle and epididymis F1: reduced pup body weight, relative liver weight, absolute testis weight. Increased prostate weight at middle dose Reduced sperm density. No change in estrous cyclicity At middle dose: : F1: Reduced number of small follicles (primordial and primary)	Gulati 1986. Treinen 1989. Bolon 1997. Lamb 1997. (Reviewed by Moorman 2000, Beranger et al., 2012, Morrissey 1988a, 1988b, 1989)

TABLE 7 STUDY ON REPRODUCTIVE TOXICITY

Overall, DBNPG is considered a reproductive toxicant with effects on the female reproductive system (reduced number of small follicles (primordial and primary) in F0 and F1 generation mice and fewer and lighter offspring). Additionally, DBNPG may be a developmental toxicant with effects on reproductive organs of male offspring.

As only data on one category member was retrieved, category members cannot be compared with respect to this endpoint. Therefore, reproductive and developmental effects are not selected as a critical health effect for the purpose of this project.

4.4 Experimental studies on toxicokinetics

Data on absorption, distribution, metabolism and excreting are available for one of the three members of the category identified in the preliminary structural grouping, 2,2-bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0).

After a single oral administration of 10 or 100 mg/kg bw, >80% of the low dose and 48% of the high dose were excreted within 12 hours in the urine predominantly as a glucuronide metabolite. After repeated daily oral doses for 5 or 10 days, route and rate of elimination were similar to those obtained after single administrations of the substance. In all studies, the recovery in faeces was low (<15%). The total amount of the substance remaining in tissues at 72 hours after a single oral administration of 100 mg/kg bw was less than 1% of the dose, and repeated daily dosing did not lead to retention in tissues. After iv administration, the amount of test substance found in blood decreased rapidly; excretion profiles were similar to those after oral administration. The parent compound and the glucuronide of the parent compound were present in blood plasma after oral or iv dosing. After an iv dose of 15 mg/kg bw the hepatic glucuronide of the parent compound was

primarily excreted into the bile (>50% within 6 hours), but it underwent enterohepatic recycling with subsequent elimination in the urine. (Hoehle et al. 2009).

4.5 Studies of mode/mechanisms of action

4.5.1 Genotoxicity

4.5.1.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

A positive result was obtained in *Salmonella typhimurium* TA100 only when S9 from the liver of Aroclor-induced male Syrian hamsters was used for metabolic activation at a concentration of 30%; negative results were observed when rat liver S9 was used for metabolic activation, as well as with a lover concentration of hamster liver S9 (10%). In other strains of *Salomonella typhimurium*, no mutagenic activity was detected. (NTP 1996, IARC 2000b).

In cytogenetic tests with cultured Chinese hamster ovary cells, DBNPG did not induce sister chromatid exchanges, with or without rat liver S9 (NTP 1996; the result was judged to be equivocal by IARC 2000b), but a dose-related increase in chromosomal aberrations was observed in cultured Chinese hamster ovary cells in the presence of rat liver S9 (NTP 1996; only at doses that caused significant cytotoxicity according to IARC 2000b). Both tests were conducted up to doses which induced marked cytotoxicity. A majority of the breaks were located in the heterochromatic region of the long arm of chromosome X, but the reasons for this are unclear (NTP 1996, IARC 2000b). Also, the type of damage pattern seen with DBNPG (induction of chromosomal aberrations but not sister chromatid exchanges) is unusual; most chemicals which induce chromosomal aberrations also induce sister chromatid exchanges (NTP 1996).

DBNPG was also shown to be genotoxic *in vivo*. Significant increases in micronucleated normochromatic erythrocytes were observed in peripheral blood samples obtained from male and female mice exposed for 13 weeks via the diet (NTP 1996, IARC 2000b). However, in tests for micronucleus formation in mouse bone marrow, results were positive for females but inconsistent for males (routes of administration were different) (NTP 1996, IARC 2000b) and therefore, the results were concluded to be equivocal (NTP 1996).

In conclusion, DBNPG was shown to be mutagenic/genotoxic *in vitro* and *in vivo*, inducing gene mutations in *Salmonella typhimurium* strain TA100, chromosomal aberrations in cultured Chinese hamster ovary cells, and micronuclei in peripheral erythrocytes of male and female mice. The *in vitro* responses required the presence of a metabolic activation system.

4.5.1.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

2,3-DBPA was mutagenic in several strains of *Salmonella typhimurium* both in the presence and in the absence of exogenous metabolic systems. It also gave positive results in the mouse lymphoma assay in the absence of S9 activation; it was not tested with S9. Increases in sister chromatid exchanges and chromosomal aberrations were induced in cultured Chinese hamster ovary cells both with and without rat liver S9. 2,3-DBPA induced significant increases in sex-linked recessive lethal mutations and reciprocal translocations in male germ cells of *Drosophila melanogaster*. However, the substance did not increase the frequency of micronucleated polychromatic erythrocytes in the bone marrow in male mice. (NTP 1993, IARC 2000a).

4.5.1.3 2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA) (CAS RN 36483-57-5)

TBNPA showed no evidence of mutagenic activity in the absence or presence of rat liver S9, but showed a clear evidence of mutagenic activity in strains Ta100 and TA1535 in the presence of hamster liver S9. It also gave positive results in the mouse lymphoma assay in the presence of rat liver S9. Increases in chromosomal aberrations were induced in cultured peripheral human lymphocytes in the presence of metabolic activation, and at the highest test substance concentration

in the absence of metabolic activation. The substance did not induce any marked or significant increases in the incidence of cells undergoing unscheduled DNA synthesis in isolated rat liver cells following in vivo exposure and therefore, the substance was considered to be non-genotoxic in this study. Furthermore, the substance did not increase the frequency of micronucleated polychromatic erythrocytes in the bone marrow in mice. (Study reports, cited from the REACH Registration Dossier Database).

4.5.2 Endocrine activity

No data regarding an endocrine activity of the members of the category of brominated flame retardants selected for this phase have been located in the literature search.

Several brominated flame retardants have been shown to cause adverse effects in the thyroid gland secondary to interference with the thyroid hormones. Exposure to 2,2-bis(bromomethyl)-1,3-propanediol (DBNPG) for 2 years caused neoplasms of the thyroid gland in male and female rats. The occurrence of these neoplasms in the absence of diffuse thyroid gland hyperplasia supports the hypothesis that 2,2-bis(bromomethyl)-1,3- propanediol causes a direct thyroid response that is not likely secondary to sustained high concentrations of thyroid stimulating hormone (NTP 1996).

4.6 Identification of critical effect(s)

Relevant experimental data on human health effects were retrieved for three of the 25 members of the category of brominated flame retardants selected for this phase, i.e. for the three members of the category identified in the preliminary structural grouping, 2,3-DBPA (CAS RN 96-13-9), DBNPG (CAS RN 3296-90-0) and TBNPA (CAS RN 36483-57-5). No relevant experimental data were retrieved for the 22 new members of the category with a CAS RN assigned and identified in the definition of the category.

4.6.1 2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG) (CAS RN 3296-90-0)

In the two-year NTP studies with administration of technical grade FR-1138® containing 78.6% of DBNPG in the diet to rats and mice (NTP 1996), significant dose-related increases in the incidences of neoplasms were observed at numerous sites in male and female rats and, to a lesser extent, in mice. Thus, these studies show that this flame retardant is a multi-site, multispecies carcinogen (NTP 1996).

DBNPG, as well as other brominated chemicals have been shown to be genotoxic in a spectrum of tests. It is hypothesized that the carcinogenic activity of brominated chemicals is due to genotoxic mechanisms (NTP 1996).
Based on the findings from the stop-exposure study with DBNPG in male rats, genetic damage appears to occur within the first few months of exposure. This genetic damage is irreversible, and neoplasms develop in the absence of a toxic response. (NTP 1996)

Of the 11 aliphatic and three aromatic brominated chemicals studied by the NTP in 2-year rodent studies, 13 of 14 chemicals were carcinogenic (NTP 1996).
It would be expected that C-Br bonds in DBNPG would be cleaved more readily than C-Cl bonds in halogenated compounds because of a lower bond energy (bond strengths: C-Cl, 95 kCal; C-Br, 67 kCal). Once the C-Br bond is broken, a free radical is available that can participate in various chemical reactions. It has been shown that eosinophils contain a lysosomal peroxidase that oxidizes halides to highly reactive and toxic hypohalous acids. Even though chloride is found at 1,000 times the concentration of bromide, the eosinophils used bromide preferentially to form the hypobromous acid. Bromide was shown to bind more readily to cellular proteins and macromolecules than other halide ions. (NTP 1996).

Two hypotheses for the carcinogenic activity of brominated chemicals are: 1) bromine causes oxidative damage to DNA and other cellular constituents and 2) the C-Br bond is broken and the

remaining carbon-containing electrophilic group forms DNA adducts with subsequent DNA damage (NTP 1996).
Studies with potassium bromate have shown that this chemical administered in drinking water at 250 or 500 ppm to F344 rats caused renal and intestinal neoplasms in male and female rats and mesotheliomas of the peritoneum in male rats. Following oral administration of potassium bromate a significant increase of 8-hydroxydeoxyguanosine was observed in DNA. 8-Hydroxydeoxyguanosine is one of the DNA-damage products formed by oxygen radicals, and this is thought to be one of the DNA lesions involved in potassium bromate carcinogenesis. (NTP 1996).

Common sites for carcinogenic activity from the brominated chemicals studied by the NTP include oral cavity, forestomach, intestine, lung, and kidney. Treatment-related lesions are generally not seen at these sites early in the study, but develop with time. In the DBNPG 'stop-exposure' study, neoplasm development in the male rat required only 3 months of exposure, and while lesions were not seen in the target organ at the end of this 3-month exposure period, the essential damage to the cell had been done, and carcinogenic lesions developed with time. (NTP 1996).
Non-neoplastic lesions were observed in the pancreas, seminal vesicles, thyroid gland, lung, kidney, and urinary bladder in male rats; in the kidney of female rats; and in the lung of female mice. A carcinogenic response was observed in some of these organs; however, there were many sites where a carcinogenic response was observed in the absence of non-neoplastic lesions. (NTP 1996).

DBNPG is classified (notified classification): Muta. 1B H340 / Muta. 2 H341; Carc. 1B H350 / Carc. 2 H351.

In conclusion, the critical effect of DBNPG is considered to be the multi-site, multispecies carcinogenic effect, most probably caused by a genotoxic metabolite of the parent compound, as the *in vitro* mutagenic/genotoxic responses were shown to require the presence of a metabolic activation system.

4.6.2 2,3-Dibromo-1-propanol (2,3-DBPA) (CAS RN 96-13-9)

In the two-year NTP studies with administration of 2,3-DBPA by dermal application to rats and mice (NTP 1993), caused significant dose-related increases in the incidences of neoplasms at numerous sites in male and female rats and, to a lesser extent, in mice.

The results of these studies showed that 2,3-DBPA is a multiple-organ carcinogen in rats and mice, as are its parent compound tris(2,3-dibromo-propyl)phosphate and the structurally related halogenated three-carbon 1,2-dibromo-3-chloropropane compounds, 1,2-dibromo-3-chloropropane, 1,2-dichloropropane and 1,3-dichloropropene. However, the number of sites affected by the dermal application of 2,3-DBPA was greater than the number of sites affected by the dosed feed or gavage administration of tris(2,3-dibromopropyl)phosphate and other three-carbon halogenated compounds. Although differences in dose level, strains of animals, route of administration, and duration of dose employed in the various studies could have contributed to the variation in response to these chemicals, the results suggest that 2,3-DBPA is the most potent carcinogen of these chemicals. (NTP 1993).

Among the short-chain hydrocarbons, including the halogenated hydrocarbons, are chemicals that are direct-acting carcinogens, such as epoxides and halo ethers, and others that are considered indirect-acting carcinogens, which require metabolic activation to the ultimate carcinogen in tissues such as the liver, stomach, lung, or kidney.
Epoxide intermediates are demonstrated metabolites of trichloroethylene (epoxy-1,1,2-trichloroethane), allyl chloride (epichlorohydrin and glycidaldehyde), and 1,2-dibromo-3-chloro-propane (1,1-epoxypropane). 2,3-Dibromo-l-propanol is a direct-acting mutagen, producing gene mutations in *Salmonella typhimurium* and gene mutation and chromosomal damage in cultured mammalian cells. It also produced sex-linked recessive lethal mutations and reciprocal

translocations in germ cells of *Drosophila melanogaster*. Moreover, the metabolism of 2,3-DBPA also appears to involve the formation of reactive intermediates including 2-bromoacrolein, 2,3-dibromopropanal, and 3-bromo-1,2-propaneepoxide. The first two inter-mediates are direct mutagens in *Salmonella typhimurium* and are potent inducers of DNA single-strand breaks in rat hepatoma cells. This mutagenic and chemical profile is consistent with the pattern of carcinogenic activity observed in these studies, that is, the induction of an early onset of neoplasms at multiple sites. (NTP 1993).

2,3-DBPA is classified (harmonised classification): Carc. 1B H350.

In conclusion, the critical effect of 2,3-DBPA is considered to be the multi-site, multispecies carcinogenic effect, most probably caused by a direct genotoxic action of the parent compound, as the *in vitro* mutagenic/genotoxic responses were shown both in the absence and the presence of a metabolic activation system.

4.6.3 2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA) (CAS RN 36483-57-5)

The available repeated dose toxicity studies on TBNPA include a 14-day and a 30-day study. No target organs were identified in the 14-day study. In the 30-day study, kidney and urinary bladder were identified as target organs with renal tubular damage observed in the kidney and generalized hyperplasia in the urinary bladder.
Whether TBNPA has carcinogenic properties cannot be evaluated based on the available data as no long-term studies have been located.

TBNPA showed mutagenic/genotoxic activity *in vitro* in the presence of a metabolic activation system.

TBNPA is classified (notified classification): Muta. 1B H340 / Muta. 2 H341; Carc. 1B H350.

In conclusion, the critical effect of TBNPA is considered to be a possible carcinogenic effect, most probably caused by a genotoxic metabolite of the parent compound, as the *in vitro* mutagenic/genotoxic responses were shown to require the presence of a metabolic activation system.

4.6.4 Conclusion on the critical effect, experimental studies

Based on the findings in the two 2-year NTP studies with 2,3-dibromo-l-propanol (2,3-DBPA) (NTP 1993) and 2,2-bis(bromomethyl)-1,3-propanediol (DBNPG) (NTP 1996), as well as the discussion on the underlying mode / mechanism(s) of action for the carcinogenic effect of these two brominated flame retardants provided in the NTP reports (NTP 1996, 1993), and the harmonized/notified classification(s), the critical effect of these two brominated flame retardants is the multiple-organ carcinogenic effect, most probably exerted by a genotoxic mode of action either by the parent compound itself (2,3-DBPA) or by a metabolite of the parent compound (DBNPG).

Supportive evidence for the critical effect of the brominated flame retardants in the category of small linear and branched alkyl alcohols being the carcinogenic effect (most probably exerted by a genotoxic action) comes from the limited available data, as well as the classification (notified) on the third member of the category identified in the preliminary structural grouping, 2,2-bis-(bromomethyl)-3-bromo-1-propanol (TBNPA).

Furthermore, the notified classification (Carc. 2 H351) for one of the new members identified in the definition of the category, 1,3-dibromo-2-propanol also supports that the critical effect of the brominated flame retardants in the category of small linear and branched alkyl alcohols is the carcinogenic effect.

5. Category approach for read-across

The category of small brominated linear and branched alkyl alcohols was a priori defined as chemical structures very similar to the three members identified in the preliminary structural grouping. The category was thus limited to 61 substances with 3-5 carbons, 2-3 bromine atoms and 1-2 alcohol groups.

A literature search was performed for the 25 category members with a CAS RN assigned. Relevant experimental data on human health effects were only retrieved for two of the members (2,3-DBPA and DBNPG). For a third member (TBNPA), relevant data were retrieved from the REACH registration dossier. No relevant experimental data were retrieved for the remaining members. The 61 category members were predicted in a number of human health related (Q)SAR models and in a number of OECD (Q)SAR Application Toolbox profilers.

An experimental study on toxicokinetics is available for DBNPG. After a single oral administration of 10 or 100 mg/kg bw, more than 80% of the low dose and 48% of the high dose were excreted within 12 hours in the urine predominantly as a glucuronide metabolite. This indicates that the substance is moderately to highly bioavailable following oral administration depending on the dose level. Toxicokinetic studies were not retrieved for the remaining category members. However, all category members were predicted to be bioavailable according to Lipinski's rule-of-five and were predicted by (Q)SAR to have high human intestinal absorption.

Based on the findings in the 2-year NTP studies with 2,3-DBPA (NTP 1993) and DBNPG (NTP 1996), as well as the discussion on the underlying mode/mechanisms of action for the carcinogenic effect of these two brominated flame retardants provided in the NTP reports (NTP 1996, 1993), and harmonised/notified classification(s), the critical effect of these two brominated flame retardants is the multiple-organ carcinogenic effect, most probably exerted by a genotoxic mode of action either by the parent compound itself (2,3-DBPA) or by a metabolite of the parent compound (DBNPG).

A comparison of the potency between the two substances could not be performed based on the available data. One reason is that tumours were observed at all dose levels for both substances. Another reason is that two different administration routes were used in the two studies, i.e., oral administration for DBNPG and dermal administration for 2,3-DBPA, and toxicokinetic information was only available for one of the substances (DBNPG).

The available experimental data and a notified classification (Muta. 1B H340 / Muta. 2 H341, Carc. 1B H350) for TBNPA, as well as a notified classification (Carc. 2 H351) for 1,3-DBPA indicate that the critical effect for these two substances may also be the carcinogenic effect.

An information matrix for the four source substances DBNPG, TBNPA, 2,3-DBPA and 1,3-DBPA covering the experimental data, harmonised/notified classifications, the (Q)SAR predictions and the identified OECD (Q)SAR Application Toolbox profiler alerts is presented in Appendix 9.

Experimental data on genotoxicity for DBNPG, TBNPA and 2,3-DBPA indicate both similarities and differences between these three substances. All three substances were positive in the Ames test in some *Salmonella typhimurium* strains. Two of the substances (2,3-DBPA and TBNPA) gave positive results in the *in vitro* mouse lymphoma assay. Two of the substances (DBNPG and 2,3-DBPA) increased chromosomal aberrations in cultured CHO cells, and one substance (TBNPA) increased chromosomal aberrations in cultured peripheral human lymphocytes. One substance (2,3-DBPA) increased sister chromatid exchanges in cultured CHO cells. Two substances (2,3-DBPA and TBNPA) were negative in the *in vivo* mouse micronucleus bone marrow study while DBNPG gave equivocal results for induction of micronuclei in the bone marrow of mice, but positive results in the peripheral blood samples. In general, DBNPG and TBNPA required metabolic activation in the *in vitro* assays whereas 2,3-DBPA was positive also in the absence of metabolic activation.

The (Q)SAR predictions for carcinogenic and mutagenic/genotoxic properties for the category of small linear and branched brominated alkyl alcohols indicate that all the 61 members have a carcinogenic potential with a possible mutagenic/genotoxic mode of action with all members being predicted positive in the CU FDA RCA overall cancer call and in the CU Ames model. The estimated specificities of the applied (Q)SAR models as established by leave-many-out cross-validations are between 85.9% and 95.1%, i.e. the overall false positive rates of the models are around 5%- 14%.

A number of profilers of relevance for mutagenicity/genotoxicity and cancer were applied using the OECD (Q)SAR Application Toolbox. The profilers identified a number of structural alerts in the parent compound and/or in metabolites. This could indicate that all members share the same mutagenic/genotoxic mode of action, but also that there may be variations in their possible mechanisms of action. Some alerts were identified in fewer members and/or their metabolites while others were identified in many or all of the members and/or their metabolites.

One alert was identified in all 61 members of the category, namely the “aliphatic halogen” in the ISS profilers for *in vitro* mutagenicity (Ames test), *in vivo* mutagenicity (micronucleus) and carcinogenicity (genotoxic and non-genotoxic mode of action). According to the Toolbox alert explanation some aliphatic halogens may be direct alkylating agents while metabolic activation may play a role for others, where one proposed mechanism is that cytochrome P450 oxidation leads to reactive carbonyl (aldehyde or ketone) compounds. Aldehyde alerts from five profilers (DNA binding by OECD, the three ISS profilers and Oncologic Primary Classifications) were identified in the metabolites of all but two members (ID numbers 52123_213821-20-6 and 52125_3). According to the Toolbox explanation, aldehyde compounds can potentially undergo Schiff base formation with a primary amine to form DNA adducts and cross-links. Furthermore, the alert H-acceptor-path3-H-acceptor from the ISS profiler for *in vivo* mutagenicity (Micronucleus) was identified in all but one member and/or their metabolites (ID number 52125_3). According to the Toolbox explanation compounds containing the alert may interact with DNA and/or protein via non-covalent binding.

According to Benigni et al. (2011), ‘*Numerous haloalkanes have been tested for carcinogenic and mutagenic activities. In general, the genotoxic potential is dependent on the nature, number, and position of halogen(s) and the molecular size of the compound*’. Kazius et al. (2005) identified the aliphatic halide (excluding the fluorine atom) substructure to be an alert for Ames mutagens. Out of 416 aliphatic halide compounds, 297 were mutagens, corresponding to 71% (p-value <<0.05). If compounds containing other toxicophores were excluded, there were 330 aliphatic halide compounds, of which 217 were mutagens, corresponding to 66% (p-value <<0.05). I.e. this alert identified 34% false positives among the mutagenicity training set chemicals (Kazius et al. 2005). According to Benigni et al. (2010) this alert has a positive predictivity for carcinogenicity of 74%, i.e. 74% of the substances experimentally tested for carcinogenicity and containing the alert fragment had positive results from the carcinogenicity studies.

The aldehyde structural alert, which was identified in the metabolites of 60 out of the 61 members in five profilers (DNA binding by OECD, the three ISS profilers for *in vitro* and *in vivo* mutagenicity and carcinogenicity, and the Oncologic primary classification) has according to Benigni et al. (2008) a positive predictivity for carcinogenicity of 88% (7 out of 8 substances experimentally tested for carcinogenicity and containing the alert had positive results from the carcinogenicity studies).

5.1 Future perspectives

One way to establish an even more robust basis for read-across across the category could be to search the literature for information on carcinogenicity and mutagenicity/genotoxicity for structural analogues outside, but close to the category. Information on carcinogenic and mutagenic/genotoxic effects of other substances very close to, but outside the defined category were noticed in the literature search for the 25 category members and might be useful in relation to an evaluation of possible similarities or dissimilarities with the category members.

Other ways to further strengthen the read-across basis could be to perform experimental testing for mutagenicity/genotoxicity on selected representative category members, as well as further analysisof the underlying mechanisms of action.

Another brominated flame retardant identified in the preliminary structural grouping, tris(2,3-dibromopropyl)phosphate (group 12, phosphates) is also a multiple-organ carcinogen in rats and mice (NTP 1993). The carcinogenic effect of tris(2,3-dibromopropyl)phosphate is exerted by its metabolite, 2,3-DBPA – one of three members in the category of small linear and branched alkyl alcohols for which experimental data are available (described in chapter 4). Other brominated flame retardants identified in the preliminary structural grouping, e.g. three of the members of group 2 (dibromo-(2,3-dibromopropoxy)benzene derivatives), may possibly also be metabolised to 2,3-DBPA. Therefore, brominated flame retardants that may possibly be metabolised to 2,3-DBPA or one of the other members in the category of small linear and branched alkyl alcohols may equally likely as these members themselves possess the critical effect, i.e. the carcinogenic effect, most probably exerted by a mutagenic/genotoxic mode of action.

5.2 Conclusion

The category of small brominated linear and branched alkyl alcohols was defined as chemical structures very similar to the members identified in the preliminary structural group. The category was thus limited to 61 substances with 3-5 carbons, 2-3 bromine atoms and 1-2 alcohol groups.

Relevant experimental data on human health effects were retrieved for three of the category members, i.e. for the three members of the group identified in the preliminary structural group, 2,3-DBPA, DBNPG and TBNPA. No relevant experimental data were retrieved for the remaining 22 category members with CAS RN. Furthermore, 1,3-DBPA, a REACH pre-registered compound for which no experimental data on human health effects were retrieved, has a notified classification for a possible carcinogenic potential (Carc. 2 H351).

The critical effect of the three members with relevant experimental data is the multiple-organ carcinogenic effect, most probably exerted by a genotoxic mode of action either by the parent compound itself (2,3-DBPA) or by a metabolite of the parent compound (DBNPG and TBNPA).

Possible read-across for the critical effect from the three category members with experimental data and the one member with a classification for the identified critical effect to the remaining 57 structurally similar target analogues in the category is supported by the following observations:

a) The experimental data show comparable toxicological effects for the three members of the category identified in the preliminary structural grouping (2,3-DBPA, DBNPG and TBNPA), i.e. carcinogenic and mutagenic/genotoxic effects.
b) The classifications (harmonized or notified) as Muta. 1B H340 / Muta. 2 H341 and/or Carc. 1B H350 / Carc. 2 H351 for these three members and for 1,3-DBPA.
c) The (Q)SAR predictions for carcinogenic and mutagenic/genotoxic properties indicate that the 61 category members have a carcinogenic potential with a possible mutagenic/genotoxic mode of action. The structural alerts identified in the OECD (Q)SAR Application Toolbox indicate that all members share the same genotoxic/mutagenic mode of action with some variations in their possible mechanisms of action. Some alerts were identified in many or all of the members and/or their metabolites pointing to possible common mechanism(s) of action (e.g. metabolic activation to reactive carbonyl compounds and aldehyde Schiff base formation of DNA adducts and cross-links).

An even more robust basis for assessing the accuracy of the read across for the category could be pursued by searching the literature for information on carcinogenicity and mutagenicity/genotoxicity on structural analogues outside, but close to the category, by experimental testing for mutagenicity/genotoxicity on representative members across the category, as well as further analysis of the underlying mechanisms of action.

Other brominated flame retardants that may possibly be metabolised to one of the 61 brominated flame retardants in the category of small linear and branched alkyl alcohols may equally likely as these members themselves possess the critical effect, i.e. the carcinogenic effect, most probably exerted by a mutagenic/genotoxic mode of action.

References

Ashby et al. 1988. Ashby J.and Tennant R.W. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. Mutation Research, 204 (1988) 17-115.

Benigni et al. 2008. Benigni R., Bossa C., Jeliazkova N., Netzeva T. and Worth A. The Benigni / Bossa rulebase for mutagenicity and carcinogenicity – a module of ToxTree. JRC Scientific and Technical Reports: EUR 23241 EN – 2008.

Benigni et al. 2010. Benigni R., Bossa C. and Worth A. Structural analysis and predictive value of the rodent in vivo micronucleus assay results. Mutagenesis vol. 25:4 pp. 335–341.

Benigni et al. 2011. Benigni R and Bossa C, Mechanisms of Chemical Carcinogenicity and Mutagenicity: A Review with Implications for Predictive Toxicology, Chem Rev. 2011 Apr 13;111(4):2507-36.

Beranger et al. 2012. Béranger R, Hoffmann P, Christin-Maitre S and Bonneterre V. Occupation exposures to chemicals as a possible etiology in premature ovarian failure: A critical analysis of the literature. Reproductive Toxicology 2012; vol. 33: 269-279.

Bolon et al. 1997. Bolon B, Bucci TJ, Warbritton AR, Chen JJ, Mattison DR and Heindel JJ. Differential Follicle Counts as a Screen for Chemically Induced Ovarian Toxicity in Mice: Results from Continuous Breeding Bioassays. Fundamental and Applied Toxicology 1997; vol. 39: 1-10.

Bossa rulebase for mutagenicity and carcinogenicity – a module of ToxTree. JRC Scientific and Technical Reports: EUR 23241 EN – 2008.

Danish EPA 2014. Danish Environmental Protection Agency, Environmental Project No. 1536, Survey of brominated flame retardants

DTU 2016. Danish (Q)SAR Database , http://qsar.food.dtu.dk. Accessed 22nd January 2016.

Dunnick et al. 1997. Dunnick JK, Heath JE, Farnell DR, Prejean JD, Haseman JK and Elwell MR. Carcinogenic Activity of the Flame Retardant, 2,2-Bis(bromomethyl)-1,3-Propanediol in Rodents, and Comparison with the Carcinogenicity of Other
NTP Brominated Chemicals. Toxicologic Pathology 1997; vol. 25: 541-548.

Elwell et al. 1989. Elwell MR, Dunnick JK, Brown HR and Montgomery CA. Kidney and Urinary Bladder Lesions in F344/N
Rats and B6C3F1 Mice after 13 Weeks of 2,2-Bis(bromomethyl)-1,3-propanediol Administration. Fundamental and Applied Toxicology 1989; vol. 12: 480-490.

EU 2011. Directive 2011/65/EU of the European Parliament and of the council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment.

European Chemicals Agency 2008. REACH implementation guidance on information requirements and chemical safety assessment Chapter R.6: (Q)SARs and grouping of chemicals. European Chemicals Agency. May 2008.

European Chemicals Agency 2014. REACH Guidance on Information Requirements and Chemical Safety Assessment R.11 PBT_vPvB assessment vers 2.0 November 2014.

Fujitsu 2015. Fujitsu Group specified Control Substances, http://www.fujitsu.com/ar/Images/chemical-031.pdf, accessed 18 November 2015.

Gulati 1986. Environmental Health Research & Testing, Inc., 2,2-bis(bromomethyl)-1,3-propanediol: Reproduction and fertility assessment in CD-1 mice when administered in feed, NTP Contract # N01-ES-2-5013, Report NO. NTP-86-063, January 1986.

Hoehle et al. 2009. Hoehle SI, Knudsen GA, Sanders JM and Sipes IG. Absorption, Distribution, Metabolism, and Excretion of 2,2-Bis(bromomethyl)-1,3-propandediol in Male Fischer-344 Rats. Drug Metabolism and Disposition 2009; vol. 37: 408-416.

IARC 2000a. 2,3-dibromopropan-1-ol. IARC Monographs Volume 77: 439-453.

IARC 2000b. 2,2-Bis(bromomethyl)propane-1,3-diol. IARC Monographs Volume 77: 455-468.

Kazius et al. 2005. Kazius J., McGuire R., and Bursi R. Derivation and validation of toxicophores for mutagenicity prediction. J. Med. Chem. 48 1 312-320.

Keyes et al. 1980. Keyes DG, Kociba RJ, Schwetz RW, Wade CE, Dittenber DA, Quinn T, Gorzinski SJ, Hermann EA, Momany JJ and Schwetz BA. Results of a two-year toxicity and oncongenic study of rats ingesting diets containing dibromoneopentyl glycol (FR-1138). Journal of Combustion Toxicology 1980; vol. 7: 77-98.

Lamb et al. 1997. Lamb J, Gulati D, Mounce R, Shaver S, Russel S and Poonacha K. 2,2-bis(Bromomethyl)-1,3-propanediol CAS #3296-90-0 Swiss CD-1 mice, at 0.0, 0.1, 0.2, and 0.4% in feed. Environmental Health Research and Testing, Started 10/7/84; Completed 2/10/86, NTIS #PB86168341/AS.

Moorman et al. 2000. Moorman WJ, Ahlers HW, Chapin RE, Daston GP, Foster PMD, Kavlock RJ, Morawetz JS, Schnorr TM, Schrader SM. Prioritization of NTP reproductive toxicants for field studies. Reproductive Toxicology 2000; vol. 14: 293-301.

Morrissey 1989. Morrissey RE, Lamb JC 4th, Morris RW, Chapin RE, Gulati DK, Heindel JJ. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundam Appl Toxicol. 1989 Nov;13(4):747-77.

Morrissey et al. 1988a. Morrissey RE, Lamb JC, Schwetz BA, Teague JL and Morris RW. Association of Sperm, Vaginal Cytology, and Reproductive Organ Weight Data with Results of Continuous Breeding Reproduction Studies in Swiss (CD-1) Mice. Fundamental and Applied Toxicology 1988; vol. 11: 359-371.

Morrissey et al. 1988b. Morrissey RE, Schwetz BA, Lamb JC, Ross MD, Teague JL and Morris RW. Evaluation of Rodent Sperm, Vaginal Cytology, and Reproductive Organ Weight Data from National Toxicology Program 13-week. Fundamental and Applied Toxicology 1988; vol. 11: 343-358.

NTP 1993. Toxicology and carcinogenesis studies of 2,3-dibromo-1-propanol (CAS No. 96-13-9) in F344/N rats and B6C3F$_1$ mice (dermal studies). National Toxicology Program, Technical Report Series No. 400.
NTP 1996. Toxicology and carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol (FR-1138®) (CAS No. 3296-90-0) in F344/N rats and B6C3F$_1$ mice (feed studies). National Toxicology Program, Technical Report Series No. 452.

OECD 2011. OECD QSAR Toolbox User Manual, Strategies for grouping chemicals to fill data gaps to assess genetic toxicity and genotoxic carcinogenicity.

Oeko-Institut 2014. Study for the Review of the List of Restricted Substances under RoHS 2 Analysis of Impacts from a Possible Restriction of Several New Substances under RoHS 2. https://circabc.europa.eu/sd/a/0ee53cdd-5807-4679-a88c-3cabe30162f6/20140806_Substance_Review_revised_version_final_plus_Dossier.pdf. Accessed 15th January 2016.

ToxCast 2015. ToxCastTM Data 2015, United States Environmental Protection Agency, Computational Toxicology Research, http://epa.gov/ncct/toxcast/data.html, accessed 29 May 2015.

Treinen et al. 1989. Treinen KA, Chapin RE, Gulati DK, Mounce R, Morris LZ and Lamb JC. Reproductive Toxicity of 2,2-Bis(bromomethyl-1,3-propanediol in a Continuous Breeding Protocol in Swiss (CD-1) Mice. Fundamental and Applied Toxicology 1989; vol 13: 245-255.

Tyle et al. 2002. Tyle H., Larsen H.S., Wedebye E.B., Sijm D., Pedersen Krog T., Niemela J., 2002. Identification of potential PBTs and vPvBs by use of QSARs. Danish EPA, SHC/TS 2-3/029.
http://eng.mst.dk/media/mst/69087/QSAR%20PBT%20final%20clean.pdf

Appendix 1: Start list with 67 substances

CAS No	EC No	Substance name	Abbreviated name	R/A[1]	Tox Cast	Tox21	REACH registered	SMILES applied for (Q)SAR prediction	Notes on CAS and SMILES[2]	Source[3]
1163-19-5	214-604-9	Bis(pentabromophenyl) ether	decaBDE	A			*	c1(c(c(c(Br)c(c1Br)Br)Br)Br)Oc1c(c(c(Br)c(c1Br)Br)Br)Br		1
118-79-6	204-278-6	2,4,6-Tribromophenol	TBP	A/R	*	*	*	c1(c(cc(Br)cc1Br)Br)O		1
126-72-7	204-799-9	tris(2,3-dibromopropyl) phosphate	TDBPP	A	*	*	-	P(=O)(OCC(CBr)Br)(OCC(CBr)Br)OCC(CBr)Br		1
13654-09-6	237-137-2	Decabromo-1,1'-biphenyl	DecaBB	A			-	c1(c2c(c(c(Br)c(c2Br)Br)Br)Br)c(c(c(Br)c(c1Br)Br)Br)Br		1
155613-93-7	605-018-8	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-, octabromo deriv.	OBTMPI	A			-	CC1(c2c(c(c(c(c2Br)Br)Br)Br)C(C1(Br)Br)(C)c3cccc(c3Br)Br)C	SciFinder: incompletely defined substance. The SMILES included is a representative of the substance.	1
183658-27-7	-	2-ethylhexyl-2,3,4,5-tetrabromobenzoate	EH-TBB	A			Not preregistered	CCCCC(CC)COC(=O)c1cc(c(c(c1Br)Br)Br)Br		1
19186-97-1	606-254-4	Tri[3-bromo-2,2-bis(bromomethyl)propyl] phosphate.	TTBNPP	A			*	C(C(CBr)(CBr)CBr)OP(=O)(OCC(CBr)(CBr)CBr)OCC(CBr)(CBr)CBr		1
20566-35-2	243-885-0	2-(2-Hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetrabromophthalate	HEEHP-TEBP	A			*	CC(COC(=O)c1c(c(c(c(c1Br)Br)Br)Br)C(=O)OCCOCCO)O		1
23488-38-2	245-688-5	2,3,5,6-Tetrabromo-p-xylene	TBX	A			-	c1(c(c(c(C)c(c1Br)Br)Br)Br)C		1
25327-89-3	246-850-8	1,1'-Isopropylidenebis[4-(allyloxy)-3,5-dibromobenzene]	TBBPA-bAE	A/R		*	-	C(C)(C)(c1cc(c(c(c1)Br)OCC=C)Br)c1cc(c(c(c1)Br)OCC=C)Br		1
25637-99-4	247-148-4	Hexabromocyclododecane	HBCDD	A			*	C1C(CC(CC(CC(CC(CC1Br)Br)Br)Br)Br)Br	SciFinder: incompletely defined substance. ChemSpider ("found by approved synonym"): The SMILES included is a representative, 1,3,5,7,9,11-Hexabromocyclododecane, of the substance.	1
25713-60-4	607-784-9	1,3,5-Triazine, 2,4,6-tris(2,4,6-tribromophenoxy)-	TTBP-TAZ	A			*	c1c(cc(c(c1Br)Oc2nc(nc(n2)Oc3c(cc(cc3Br)Br)Br)Oc4c(cc(cc4Br)Br)Br)Br)Br	Ok according to SciFinder. ChemSpider ("found	1

CAS No	EC No	Substance name	Abbrevia-ted name	R/A[1]	Tox Cast	Tox21	REACH registered	SMILES applied for (Q)SAR prediction	Notes on CAS and SMILES[2]	Source[3]
									by approved synonym"):	
26040-51-7	247-426-5	Bis(2-ethylhexyl) tetrabromophthalate	BEH-TEBP	A	*	*	*	CCCCC(CC)COC(=O)c1c(c(c(c(c1Br)Br)Br)Br)C(=O)OCC(CC)CCCC		1
31780-26-4	250-802-1	Dibromostyrene	DBS	A/R			-	c1(ccccc1)\C=C(\Br)Br		1
3194-55-6	221-695-9	1,2,5,6,9,10-Hexabromocyclododecane	HBCDD	A	*	*	Preregistered – HBCDD registered under CAS No 25637-99-4	C1CC(C(CCC(C(CCC(C1Br)Br)Br)Br)Br)Br		1
32534-81-9	251-084-2	Diphenyl ether, pentabromo derivative	pentaBDE	A		*	-	c1cc(c(cc1Br)Br)Oc2cc(c(cc2Br)Br)Br	SciFinder: incompletely defined substance. The SMILES is a representative of the substance.	1
32536-52-0	251-087-9	Diphenyl ether, octabromo derivative	octaBDE	A		*	-	c1c(c(c(c(c1Br)Br)Br)Br)Oc2cc(c(c(c2Br)Br)Br)Br	SciFinder: incompletely defined substance. The SMILES from ChemSpider is a representative of the substance which is in itself a category!	1
32588-76-4	251-118-6	N,N'-ethylenebis(3,4,5,6-tetrabromophthalimide)	EBTEBPI	A			*	c12c(C(=O)N(C1=O)CCN1C(c3c(c(Br)c(c(c3C1=O)Br)Br)Br)=O)c(c(Br)c(c2Br)Br)Br		1
3278-89-5	221-913-2	2-(allyloxy)-1,3,5-tribromobenzene	TBP-AE	A/R		*	-	O(c1c(cc(cc1Br)Br)Br)CC=C		1
3322-93-8	222-036-8	1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane	DBE-DBCH	A		*	-	C1[C@@H](CC[C@@H]([C@@H]1Br)Br)[C@@H](CBr)Br		1
33798-02-6	251-681-8	4,4'-isopropylidenebis[2,6-dibromophenyl] diacetate	TBBPA-bOAc	A			-	O=C(Oc1c(cc(cc1Br)C(c1cc(c(OC(=O)C)c(Br)c1)Br)(C)C)Br)C		1
34571-16-9	252-097-6	1,2,3,4,7,7-Hexachloro-5- (tetrabromo-	HCTBPH	A			-	Cl[C@@]12C([C@@](Cl)(C[C@@H]1c1c(c(c(c(c1)Br)B		1

CAS No	EC No	Substance name	Abbreviated name	R/A[1]	Tox Cast	Tox21	REACH registered	SMILES applied for (Q)SAR prediction	Notes on CAS and SMILES[2]	Source[3]
		phenyl)bicyclo[2.2.1]hept-2- ene						r)Br)Br)C(=C2Cl)Cl)(Cl)Cl		
35109-60-5	252-372-0	1,3,5-tribromo-2-(2,3-dibromopropoxy)benzene	DPTE	A			-	O(c1c(cc(cc1Br)Br)Br)C[C@@H](Br)CBr		1
3555-11-1	222-610-8	Allyl pentabromophenyl ether	PBPAE	A/R			-	c1(c(c(c(Br)c(c1Br)Br)Br)Br)OCC=C		1
36355-01-8	252-994-2	Hexabromo-1,1'-biphenyl	HexaBB	A			-	c1cc(c(c(c1c2ccc(c(c2Br)Br)Br)Br)Br)Br	SciFinder: incompletely defined substance. The SMILES is a representative of the substance.	1
37853-59-1	253-692-3	1,1'-[ethane-1,2-diylbisoxy]bis[2,4,6-tribromobenzene]	BTBPE	A			-	c1(c(cc(Br)cc1Br)Br)OCCOc1c(cc(Br)cc1Br)Br		1
39569-21-6	254-522-0	Benzene, 1,2,3,4-tetrabromo-5-chloro-6-methyl-	TBCT	A			-	c1(c(c(c(Br)c(c1Br)Br)Br)Cl)C		1
39635-79-5	254-551-9	4,4'-sulphonylbis[2,6-dibromophenol]	TBBPS	A/R			-	S(=O)(=O)(c1cc(c(c(c1)Br)O)Br)c1cc(c(c(c1)Br)O)Br		1
4162-45-2	224-005-4	4,4'-isopropylidenebis(2-(2,6-dibromophenoxy)ethanol)	TBBPA-BHEE	A/R		*		c1(C(c2cc(c(OCCO)c(c2)Br)Br)(C)C)cc(c(OCCO)c(c1)Br)Br		1
42757-55-1	255-929-6	bis[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl] sulphone	TBBPS-BDBPE	A			-	c1c(cc(c(c1Br)OCC(CBr)Br)Br)S(=O)(=O)c2cc(c(c(c2)Br)OCC(CBr)Br)Br		1
51936-55-1	257-526-0	7,8-Dibromo-1,2,3,4,11,11-hexachloro-1,4,4a,5,6,7,8,9,10,10a-decahydro-1,4-methanobenzocyclooctene	DBHCTD	A			-	Br[C@@H]1[C@@H](Br)CC[C@@H]2[C@@]3(C(=C([C@@]([C@@H]2CC1)(Cl)C3(Cl)Cl)Cl)Cl)Cl		1
52434-90-9	257-913-4	1,3,5-Tris(2,3-dibromopropyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione	TDBP-TAZTO	A			-	C(C(CBr)Br)n1c(=O)n(c(=O)n(c1=O)CC(CBr)Br)CC(CBr)Br		1
58965-66-5	261-526-6	1,2,4,5-tetrabromo-3,6-Bis(pentabromophenoxy) benzene	4'-PeBPOB-DE208	A			-	Brc1c(Br)c(Oc2c(Br)c(Br)c(Br)c(Br)c2Br)c(Br)c(Br)c1Oc1c(Br)c(Br)c(Br)c(Br)c1Br		1
615-58-7	210-436-5	2,4-dibromophenol	DBP	A/R			-	c1(c(ccc(c1)Br)O)Br		1

CAS No	EC No	Substance name	Abbreviated name	R/A[1]	Tox Cast	Tox21	REACH registered	SMILES applied for (Q)SAR prediction	Notes on CAS and SMILES[2]	Source[3]
608-71-9	210-167-3	Pentabromophenol	PBP	A/R		*	-	c1(c(c(c(Br)c(c1Br)Br)Br)Br)O		1
79-94-7	201-236-9	2,2',6,6'-Tetrabromo-4,4'-isopropylidenediphenol	TBBPA	A/R	*	*	*	C(c1cc(c(O)c(c1)Br)Br)(c1cc(c(O)c(c1)Br)Br)(C)C		1
84852-53-9	284-366-9	1,1'-(Ethane-1,2-diyl)bis[pentabromobenzene]	DBDPE	A			*	C(Cc1c(c(c(c(c1Br)Br)Br)Br)Br)c2c(c(c(c(c2Br)Br)Br)Br)Br		1
85-22-3	201-593-0	2,3,4,5,6-Pentabromoethylbenzene	PBEB	A		*	-	c1(c(c(c(Br)c(c1Br)Br)Br)Br)CC		1
87-82-1	201-773-9	Hexabromobenzene	HBB	A		*	-	c1(c(c(c(Br)c(c1Br)Br)Br)Br)Br		1
87-83-2	201-774-4	2,3,4,5,6-Pentabromotoluene	PBT	A/R			-	c1(c(c(c(Br)c(c1Br)Br)Br)Br)C		1
1084889-51-9	-	Octabromotrimethyl-phenyl indane	OBTMPI	NA				CC1(CC(c2c1c(c(c(c2Br)Br)Br)Br)(C)c3cc(c(c(c3Br)Br)Br)Br)C	Found in ChemSpider by synonym	2
1522-92-5	-	Tribromoneopentyl alcohol [same substance as CAS No 36483-57-5]	TBNPA	NA		*		C(CO)(CBr)(CBr)CBr	SciFinder: 2 CAS RN's from Table 1 refer to same substance (the other CAS is 36483-57-5)	2
25495-98-1	-	Hexabromocyclodecane	HBCYD	NA				C1(C(C(CCCCCCC1)(Br)Br)(Br)Br)(Br)Br		2
3194-57-8	-	Cyclooctane, 1,2,5,6-tetrabromo	TBCO	NA				C1CC(C(CCC(C1Br)Br)Br)Br		2
37419-42-4	-	Phenol, 4,4′-(1-methylethyli-dene)bis[2,6dibromo-, dipropanoate (9CI)	TBBPA-BP	NA				C(c1cc(c(OC(=O)CC)c(c1)Br)Br)(c1cc(c(OC(=O)CC)c(c1)Br)Br)(C)C	Ok according to SciFinder, SMILES generated	2
38521-51-6	253-985-6	Benzene, 1,2,3,4,5-pentabromo6-(bromomethyl)	PBBB	NA				C(c1c(c(c(c(c1Br)Br)Br)Br)Br)Br		2
497107-13-8	-	Benzene, 1,1′-[oxybis(methylene)]bis [2,3,4,5,6-pentabromo(9CI)	DBDBE	NA				C(c1c(c(c(c(c1Br)Br)Br)Br)Br)OCc2c(c(c(c(c2Br)Br)Br)Br)Br	ChemSpider (found by synonyms):	2
55205-38-4	-	2-Propenoic acid, 1,1′-[(1-methylethylidene) bis(2,6-dibromo-4,1-phenylene)] ester	TBBPA-BA	NA				c1(C(c2cc(c(OC(C=C)=O)c(c2)Br)Br)(C)C)cc(c(OC(C=C)=O)c(c1)Br)Br		2

CAS No	EC No	Substance name	Abbreviated name	R/A[1]	Tox Cast	Tox21	REACH registered	SMILES applied for (Q)SAR prediction	Notes on CAS and SMILES[2]	Source[3]
57829-89-7	-	1-(2,3-Dibromopropyl)-3,5-diallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione	DBP-TAZTO	NA				N1(CC=C)C(=O)N(CC(Br)CBr)C(=O)N(CC=C)C1(=O)	Ok according to SciFinder. SMILES generated.	2
58495-09-3	-	Benzene, 1,2,3,4,5-pentabromo6-(chloromethyl)	PBBC	NA				c1(CCl)c(Br)c(Br)c(Br)c(Br)c1(Br)	Ok according to SciFinder. SMILES generated.	2
607-99-8	-	2,4,6,-tribromoanisol	TBA	NA				c1(c(cc(Br)cc1Br)Br)OC		2
70156-79-5	-	Benzene, 1,1′-sulfonylbis[3,5-dibromo-4-methoxy	TBPPS-BME	NA				S(=O)(=O)(c1cc(c(OC)c(c1)Br)Br)(c1cc(c(OC)c(c1)Br)Br)	Ok according to SciFinder. CAS RN not in ChemID or ChemSpider. SMILES generated	2
75795-16-3	-	1,3-Bis(2,3-dibromopropyl)-5-allyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione	BDBP-TAZTO	NA				N1(CC=C)C(=O)N(CC(Br)CBr)C(=O)N(CC(Br)CBr)C1(=O)	Ok according to SciFinder. SMILES generated.	2
3234-02-4		2,3-Dibromo-2-butene-1,4-diol	DBBD	NA		*		C(C(=C(CO)Br)Br)O	SMILES from ChemSpider	3
26762-91-4		Tribromo-phenyl-allyl-ether, unspecified	AO-TBB	NA				C=CCOc1ccc(c(c1Br)Br)Br	SMILES from Chemspider for Allyl 2,3,4-tribromophenyl ether. The SMILES included is a representative of the substance.	3
52907-07-0		Ethylene-bis(5,6-dibromo-norbornane-2,3-dicarboximide)	EBDBNDC	NA				C1C2C3C(C1C(C2Br)Br)C(=O)N(C3=O)CCN4C(=O)C5C6CC(C5C4=O)C(C6Br)Br	SMILES from ChemSpider	3
55481-60-2		Bis(methyl)tetrabromophtalate	BM-TEBP	NA				COC(=O)c1c(c(c(c(c1Br)Br)Br)Br)C(=O)OC	SMILES from ChemSpider	3
75790-69-1		TBPA, glycol-and propylene-oxide esters ’		NA				c1(c(c(c(c(c1Br)Br)Br)Br)Br)C(=O)O)C(=O)O	Mixed esters with diethylene glycol and propylene glycol, here the SMILES is only for phthalate acid part.	3
96-13-9	202-480-9	Dibromo-propanol	DBPA	NA		*		C(Br)C(Br)CO	SMILES without 3D (note the chiral center)	3
21850-44-2	244-617-5	1,1'-(Isopropylidene) bis[3,5- dibromo-4-(2,3-	TBBPA-BDBPE	R		*	*	c1c(Br)c(c(Br)cc1C(C)(C)c1cc(Br)c(c(c1)Br)OC[C@@H](CBr)Br)OC[C@@H](		1

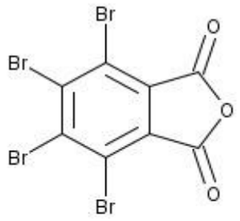
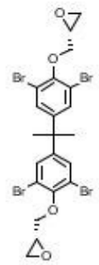
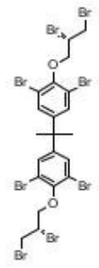
CAS No	EC No	Substance name	Abbreviated name	R/A[1]	Tox Cast	Tox21	REACH registered	SMILES applied for (Q)SAR prediction	Notes on CAS and SMILES[2]	Source[3]
		dibromopropoxy)benzene]						CBr)Br		
3072-84-2	221-346-0	2,2'-[(1-Methylethylidene)bis[(2,6- dibromo-4,1-phenyle- lene)oxymethylene]]bis oxiran e	TBBPA-BGE	R			-	C(C)(C)(c1cc(c(c(c1)Br)OC[C@@H]1OC1)Br)c1cc(c(c(c1)Br)OC[C@@H]1OC1)Br		1
3296-90-0	221-967-7	2,2-bis(bromomethyl)propane-1,3-diol	DBNPG	R	*	*	*	C(CO)(CO)(CBr)CBr		1
36483-57-5	253-057-0	2,2-dimethylpropan-1-ol, tribromo derivative	TBNPA	R			*	BrCC(CBr)(CBr)CO		1
37853-61-5	253-693-9	Benzene, 1,1'-(1-methylethylidene) bis[3,5-dibromo-4-methoxy	TBBPA-BME	R			-	C(c1cc(c(OC)c(c1)Br)Br)(c1cc(c(OC)c(c1)Br)Br)(C)C		1
59447-55-1	261-767-7	(Pentabromophenyl) methyl acrylate	PBB-Acr	R			*	c1(c(c(Br)c(c(c1Br)Br)Br)Br)COC(C=C)=O		1
632-79-1	211-185-4	Tetrabromophthalic anhydride	TEBP-Anh	R		*	*	c12c(c(c(c(c1Br)Br)Br)Br)C(=O)OC2=O		1
66710-97-2	266-455-4	2-Propenoic acid, 1,1'[(1-methylethylidene)bis[(2,6- dibromo-4,1phenylene)oxy-2,1-ethanediyl]] ester	TBBPA-BHEEBA	R			-	O=C(OCCOc1c(cc(cc1Br)C(c1cc(c(OCCOC(=O)C=C)c(Br)c1)Br)(C)C)Br)C=C		1

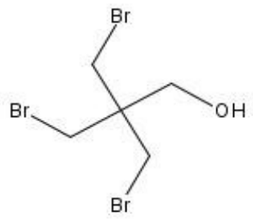
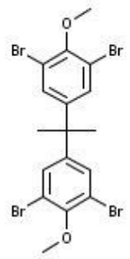
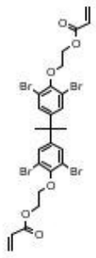
[1] A: Additive and R: Reactive

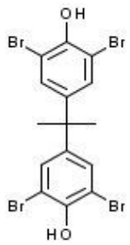
[2] CAS look-up in SciFinder, and SMILES look-up in ChemIDplus and ChemSpider

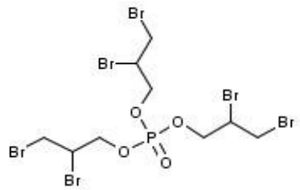
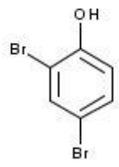
[3] 1 and 2 refer to Table 1 and 2 in the survey report; Danish Ministry of the Environment 2014, and 3 refers to substances from Fujitsu 2015

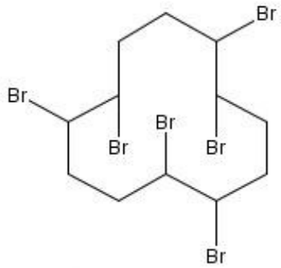
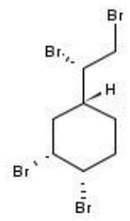
Appendix 2: Start list substances with pictures

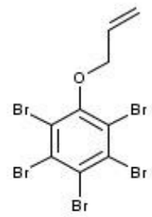
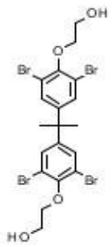
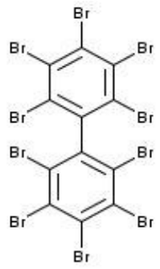
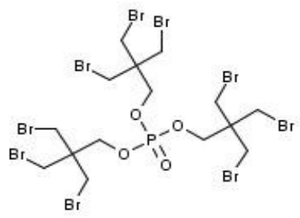
Structure	ALogP	Hydrogen Bond Acceptors	Hydrogen Bond Donors	Lipinski Score	Molecular Weight	Parent Atom Count	Parent Molecular Weight	Polar Surface Area	Rotatable Bonds
 Br1R_632-79-1	4,05	2	0	0	463,7	15	463,7	43,37	0
 Br1R_3072-84-2	6,62	4	0	2	656	29	656	43,52	8
Br1R_3296-90-0	0,75	2	2	0	261,9	9	261,9	40,46	4
 Br1R_21850-44-2	10,14	2	0	2	943,6	31	943,6	18,46	10

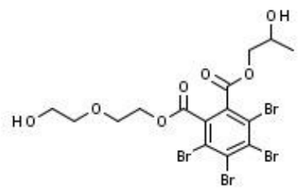
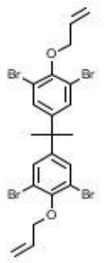
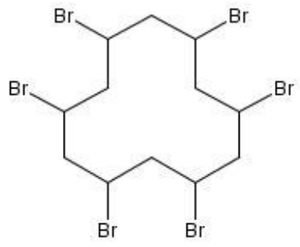
 Br1R_36483-57-5	2,15	1	1	0	324,8	9	324,8	20,23	4
 Br1R_37853-61-5	7,08	2	0	2	571,9	23	571,9	18,46	4
 Br1R_59447-55-1	5,73	1	0	2	556,7	17	556,7	26,3	4
 Br1R_66710-97-2	7,28	4	0	2	740,1	35	740,1	71,06	14

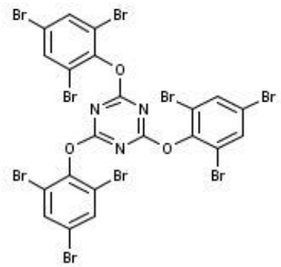
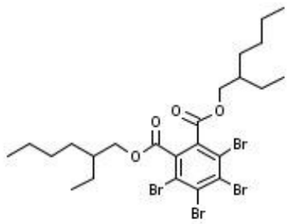
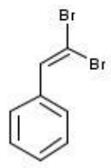
 Br1_79-94-7	6,47	2	2	2	543,9	21	543,9	40,46	2
 Br1_85-22-3	6,06	0	0	2	500,6	13	500,6	0	1
 Br1_87-82-1	6,26	0	0	2	551,5	12	551,5	0	0
 Br1_87-83-2	5,81	0	0	1	486,6	12	486,6	0	0

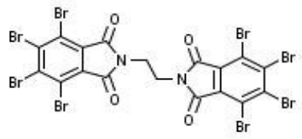
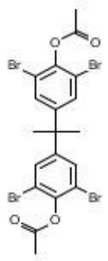
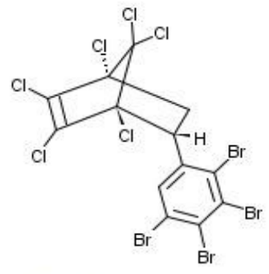
 Br1_118-79-6	3,68	1	1	0	330,8	10	330,8	20,23	0
 Br1_126-72-7	4,55	1	0	1	697,6	20	697,6	44,76	12
 Br1_608-71-9	5,2	1	1	1	488,6	12	488,6	20,23	0
 Br1_615-58-7	2,92	1	1	0	251,9	9	251,9	20,23	0

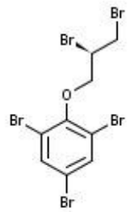
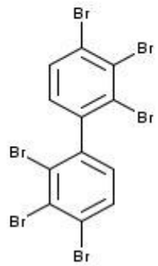
 Br1_1163-19-5	11,1	0	0	2	959,2	23	959,2	9,23	2
 Br1_3194-55-6	6,92	0	0	2	641,7	18	641,7	0	0
 Br1_3278-89-5	4,54	1	0	0	370,9	13	370,9	9,23	3
 Br1_3322-93-8	4,47	0	0	0	427,8	12	427,8	0	2

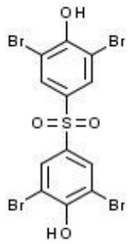
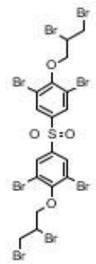
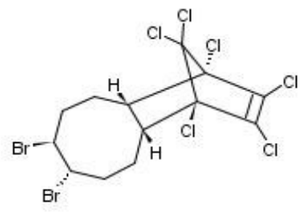
 Br1_3555-11-1	6,06	1	0	2	528,7	15	528,7	9,23	3
 Br1_4162-45-2	5,8	4	2	2	632	27	632	58,92	8
 Br1_13654-09-6	10,98	0	0	2	943,2	22	943,2	0	1
 Br1_19186-97-1	7,59	1	0	2	1018	29	1018	44,76	18

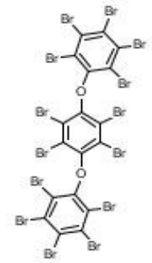
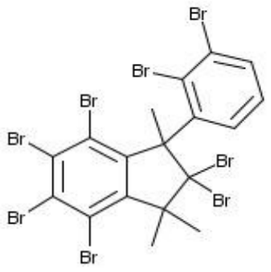
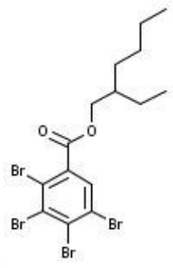
 Br1_20566-35-2	3,44	5	2	1	627,9	26	627,9	102,3	11
 Br1_23488-38-2	5,35	0	0	1	421,8	12	421,8	0	0
 Br1_25327-89-3	8,19	2	0	2	624	27	624	18,46	8
 Br1_25637-99-4	6,92	0	0	2	641,7	18	641,7	0	0

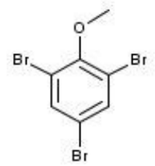
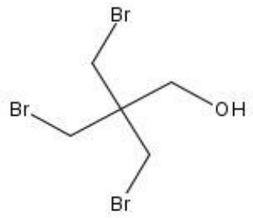
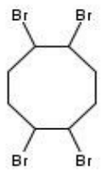
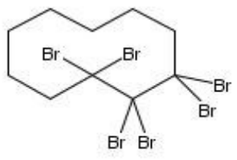
 Br1_25713-60-4	12	3	0	2	1067	36	1067	66,36	6
 Br1_26040-51-7	9,48	2	0	2	706,1	32	706,1	52,6	16
 Br1_31780-26-4	3,77	0	0	0	261,9	10	261,9	0	1
 Br1_32534-81-9	7,29	0	0	2	564,7	18	564,7	9,23	2

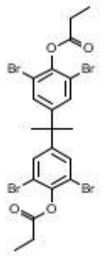
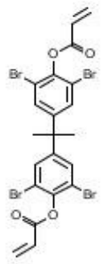
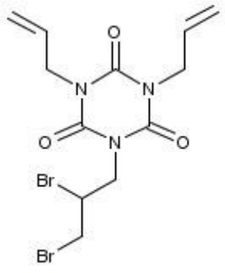
 Br1_32536-52-0	9,58	0	0	2	801,4	21	801,4	9,23	2
 Br1_32588-76-4	7,68	4	0	2	951,5	32	951,5	74,76	3
 Br1_33798-02-6	6,91	2	0	2	627,9	27	627,9	52,6	6
 Br1_34571-16-9	9,06	0	0	2	692,5	23	692,5	0	1

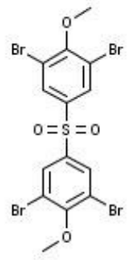
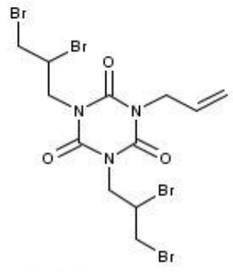
 Br1_35109-60-5	5,51	1	0	2	530,7	15	530,7	9,23	4
 Br1_36355-01-8	7,93	0	0	2	627,6	18	627,6	0	1
 Br1_37853-59-1	7,72	2	0	2	687,6	22	687,6	18,46	5
 Br1_39569-21-6	5,7	0	0	1	442,2	12	442,2	0	0

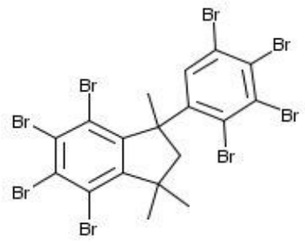
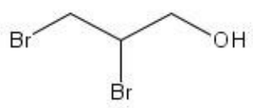
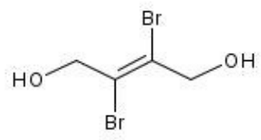
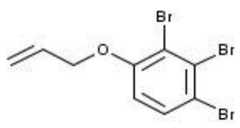
 Br1_39635-79-5	4,98	4	2	1	565,9	21	565,9	74,6	2
 Br1_42757-55-1	8,64	4	0	2	965,6	31	965,6	52,6	10
 Br1_51936-55-1	7,17	0	0	2	540,8	21	540,8	0	0
 Br1_52434-90-9	4,74	3	0	1	728,7	24	728,7	60,93	9

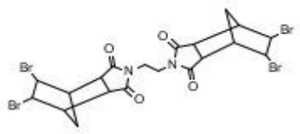
 Br1_58965-66-5	12	0	0	2	1367	34	1367	18,46	4
 Br1_84852-53-9	11,1	0	0	2	971,2	24	971,2	0	3
 Br1_155613-93-7	10,34	0	0	2	867,5	26	867,5	0	1
 Br1_183658-27-7	7,11	1	0	2	549,9	21	549,9	26,3	8

 Br2_607-99-8	3,98	1	0	0	344,8	11	344,8	9,23	1
 Br2_1522-92-5	2,15	1	1	0	324,8	9	324,8	20,23	4
 Br2_3194-57-8	4,61	0	0	0	427,8	12	427,8	0	0
 Br2_25495-98-1	7,19	0	0	2	613,6	16	613,6	0	0

 Br2_37419-42-4	7,69	2	0	2	656	29	656	52,6	8
 Br2_38521-51-6	6,39	0	0	2	565,5	13	565,5	0	1
 Br2_55205-38-4	7,25	2	0	2	652	29	652	52,6	8
 Br2_57829-89-7	2,8	3	0	0	409,1	20	409,1	60,93	7

 Br2_58495-09-3	6,24	0	0	2	521,1	13	521,1	0	1
 Br2_70156-79-5	5,59	4	0	2	593,9	23	593,9	52,6	4
 Br2_75795-16-3	3,77	3	0	1	568,9	22	568,9	60,93	8
 Br2_497107-13-8	11,03	1	0	2	987,2	25	987,2	9,23	4

 Br2_1084889-51-9	10,77	0	0	2	867,5	26	867,5	0	1
 Br3_96-13-9	1,14	1	1	0	217,9	6	217,9	20,23	2
 Br3_3234-02-4	0,97	2	2	0	245,9	8	245,9	40,46	2
 Br3_26762-91-4	4,54	1	0	0	370,9	13	370,9	9,23	3

 Br3_52907-07-0	2,54	4	0	1	672	30	672	74,76	3
 Br3_55481-60-2	4,31	2	0	1	509,8	18	509,8	52,6	4
 Br3_75790-69-1	4,13	4	0	0	481,7	16	481,7	74,6	2

Appendix 3: (Q)SAR models

EPI Suite endpoints (from tabular output only)
Molecular weight
Estimated Log Kow
Estimated Water Sol. (mg/L)
WATERNT frag Water Sol estimate
BIOWIN1 (Linear Model) Probability
BIOWIN2 (Non-Linear Model) Probability
BIOWIN3 numerical output
BIOWIN4 numerical output
BIOWIN5 (Linear MITI Model) Probability
BIOWIN6 (Non-Linear MITI Model) Probability
Ready biodegradability prediction
PBT_P ((BIOWIN2<.5 OR BIOWIN6<.6) AND BIOWIN3<2.2)
Estimated Log BCF
Estimated BCF

PBT fields derived from EPI Suite and LS DTU aquatic toxicity models
PBT_B (BCF>2000)
PBT_vB (BCF>5000)
PBT_PB (PBT_P AND PBT_B)
PBT_vPvB (PBT_P AND PBT_vB)
PBT_PB NOT vPvB
PBT_PBT AQ (PBT_P AND PBT_B AND (DK Daphnia m. 48h EC50<1 mg/L OR DK Fathead m. 96h LC50 <1 mg/L (

Case Ultra (CU) commercial models
HEART_ARRHYTHM (Human cardiac arrhythmia) Model Version: 1.5.2.0.1610.500
HEART_BRADY (Human bradycardia) Model Version: 1.5.2.0.1610.500
HEART_CONDUCT (Human cardiac conduction disorders) Model Version: 1.5.2.0.1610.500
HEART_CORONARY (Human coronary artery disorders) Model Version: 1.5.2.0.1610.500
HEART_ECG (Human electrocardiogram disorders) Model Version: 1.5.2.0.1610.500
HEART_FAIL (Human cardiac failure) Model Version: 1.5.2.0.1610.500
HEART_INFARCT (Human myocardial infarction) Model Version: 1.5.2.0.1610.500
HEART_MYOCARD (Human myocardial disorders) Model Version: 1.5.2.0.1610.500
HEART_PALPIT (Human cardiac palpitations) Model Version: 1.5.2.0.1610.500
HEART_QT (Human cardiac QT-prolongation) Model Version: 1.5.2.0.1610.500
HEART_RATE (Human cardiac rate rhythm disorders) Model Version: 1.5.2.0.1610.500
HEART_TACHY (Human tachycardia) Model Version: 1.5.2.0.1610.500
HEART_TORSADES (Human cardiac Torsades de pointes) Model Version: 1.5.2.0.1610.500

RENAL_NPATHY (Human nephropathy) Model Version: 1.5.2.0.1569.500

A48 (Developmental toxicants, human) Model Version: 1.5.2.0.119.500

RP_AN1 (Female fertility, rodent) Model Version: 1.5.2.0.960.500
RP_AN5 (Female fertility, rat) Model Version: 1.5.2.0.895.500
RP_AN9 (Female fertility, mouse) Model Version: 1.5.2.0.151.500
RP_AO1 (Male fertility, rodent) Model Version: 1.5.2.0.784.500
RP_AO4 (Male fertility, rat) Model Version: 1.5.2.0.715.500
RP_AO7 (Male fertility, mice) Model Version: 1.5.2.0.146.500

RP_AP1 (Sperm toxicity, rodent) Model Version: 1.5.2.0.906.500
RP_AP4 (Sperm toxicity, rat) Model Version: 1.5.2.0.722.500
RP_AP7 (Sperm toxicity, mouse) Model Version: 1.5.2.0.262.500
RP_AQ1 (Newborn behavioral toxicity, rodent) Model Version: 1.5.2.0.666.500
RP_AQ4 (Newborn behavioral toxicity, rat) Model Version: 1.5.2.0.622.500
RP_AQ9 (Newborn behavioral toxicity, mice) Model Version: 1.5.2.0.173.500

SALM (Salmonella mutagenicity (TA97,98,100,1535-1538)) Model Version: 1.5.1.8.10479.500

A2E - Ashby structural alerts for DNA reactivity (NTP data)
A61 Chromosomal aberrations in vitro in CHO cells
A33 - Allergic contact dermatitis in guinea pig and human
A49 - Teratogenic potential in humans

AF1 - Carcinogenicity in male rats
AF2 - Carcinogenicity in female rats
AF3 - Carcinogenicity in male mice
AF4 - Carcinogenicity in female mice
AFU - Carcinogenicity in rodents
AFV - Carcinogenicity in rats
AFW - Carcinogenicity in mice
RCA call

DTU models
Ames (all strains)
Ames_sub_Direct_(S9)
Ames_sub_Potency_>_10x_ctrl.
Ames_sub_Base-pair
Ames_sub_Frame_shift
Mouse_lymphoma, in vitro
Chromosomal_aberrations_CHL, in vitro
UDS_rat_hepatocytes, in vitro
SHE_cell_transformation, in vitro
HGPRT, in vitro
SCE_mouse, in vivo
Mouse_micronucleus, in vivo
Comet_assay, in vivo
Rodent dominant_lethal, in vivo
Drosophila_SLRL
Rodent hepatocarcinogenicity (only)
hERG blocking
MRTD
ER binding, METI all data
ER binding, METI balanced training set
ER agonism, METI agonism data
ER agonism, US EPA CERAPP data
AR antagonism
Thyroid alpha binding
Thyroid beta binding
TPO, Thyroid peroxidase, US EPA data

PXR binding
Airway_allergy
Skin_irritation (severe vs. mild)
CYP_2D6_substrate
CYP_2C9_substrate
Biodegradation
Fathead minnow 96h LC50
Daphnia 48h EC50
72h EC50 (growth)
Pseudokirchneriella subcapitata algae 72h EC50 (growth inhibition)

Leadscope (LS) commercial models
Growth Retard Mouse
Growth Retard Rabbit
Growth Retard Rat
retard rodent (AH1)
Wt Dec Mouse
Wt Dec Rabbit
wt dec rodent (AI1)
weight dec. rat
Fetal Death Mouse
Fetal Death Rabbit
Fetal Death Rat
Fetal Death Rodent
post impl mouse (AG6)
Post Impl Loss Rabbit
Post Impl Loss Rat
post impl rodent (AG1)
Pre Impl Loss Mouse
Pre Impl Loss Rabbit
Pre Impl Loss Rat
Pre Impl Loss Rodent
struct mouse (AL6)
Dysmorph Rabbit
Dysmorph Rat
struct rodent (AL1)
Visc Org Mouse
Visc Org Rat
Visc Org Rodent

Repro Mouse Female
Repro Rat Female
Repro Rodent Female
Repro Mouse Male
Repro Rat Male
Repro Rodent Male
sperm mouse(AP5)
Sperm Eff Rat
Sperm Eff Rodent

Bile Duct Disorder
Gall Bladder Disorder
Jaundice
Liver Damage
Liver Enzyme Abnorm

OECD QSAR Application Toolbox profilers
Simulator name
Metabolite
Database Affiliation
Inventory Affiliation
OECD HPV Chemical Categories
Substance Type
US-EPA New Chemical Categories
Biodeg BioHC half-life (Biowin)
Biodeg primary (Biowin 4)
Biodeg probability (Biowin 1)
Biodeg probability (Biowin 2)
Biodeg probability (Biowin 5)
Biodeg probability (Biowin 6)
Biodeg probability (Biowin 7)
Biodeg ultimate (Biowin 3)
DNA binding by OASIS v.1.3
DNA binding by OECD
DPRA Cysteine peptide depletion
DPRA Lysine peptide depletion
Estrogen Receptor Binding
Hydrolysis half-life (Ka, pH 7)(Hydrowin)
Hydrolysis half-life (Ka, pH 8)(Hydrowin)
Hydrolysis half-life (Kb, pH 7)(Hydrowin)
Hydrolysis half-life (Kb, pH 8)(Hydrowin)
Hydrolysis half-life (pH 6.5-7.4)
Ionization at pH = 1
Ionization at pH = 4
Ionization at pH = 7.4
Ionization at pH = 9
Protein binding by OASIS v1.3
Protein binding by OECD
Protein binding potency
Superfragments
Toxic hazard classification by Cramer (extension)
Toxic hazard classification by Cramer (original)
Ultimate biodeg
Acute aquatic toxicity classification by Verhaar (Modified)
Acute aquatic toxicity MOA by OASIS
Aquatic toxicity classification by ECOSAR
Bioaccumulation - metabolism alerts
Bioaccumulation - metabolism half-lives
Biodegradation fragments (BioWIN MITI)

Carcinogenicity (genotox and nongenotox) alerts by ISS
DART scheme v.1.0
DNA alerts for AMES, MN and CA by OASIS v.1.3
Eye irritation/corrosion Exclusion rules by BfR
Eye irritation/corrosion Inclusion rules by BfR
in vitro mutagenicity (Ames test) alerts by ISS
in vivo mutagenicity (Micronucleus) alerts by ISS
Keratinocyte gene expression
Oncologic Primary Classification
Protein binding alerts for Chromosomal aberration by OASIS v1.1
Protein binding alerts for skin sensitization by OASIS v1.3
Respiratory sensitisation
Retinoic Acid Receptor Binding
rtER Expert System ver.1 - USEPA
Skin irritation/corrosion Exclusion rules by BfR
Skin irritation/corrosion Inclusion rules by BfR
Chemical elements
Groups of elements
Lipinski Rule Oasis
Organic Functional groups
Organic Functional groups (nested)
Organic functional groups (US EPA)
Organic functional groups, Norbert Haider (checkmol)
Tautomers unstable
Repeated dose (HESS)

Appendix 4: (Q)SAR based clusterings

Indhold

Indhold 1
Carcinogenicity (Q)SAR-based clustering 2
Genotoxicity (Q)SAR-based clustering 19
Reproductive toxicity (Q)SAR-based clustering 32
Endocrine (Q)SAR-based clustering 42
Skin sensitization (Q)SAR-based clustering 55

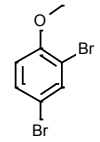
Carcinogenicity (Q)SAR-based clustering

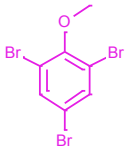
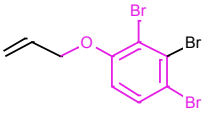
Clustering - Clusters (Run #1)

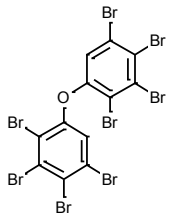
Project Name: Br_67

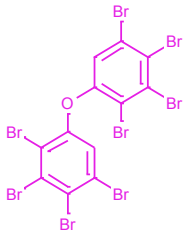
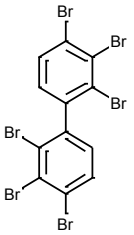
Structure Count: 67

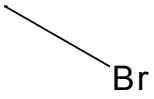
Name for this run: Clusters (Run #1)
Project: Br_67
 # structures: 67
Analysis type: Clustering (Agglomerative Nesting)
Date: Tue Nov 24 11:23:48 CET 2015
Owner: ebawe
Create signatures: true
Group singletons: true
Cluster by: Data (CU_P_Cancer_Female_Mouse CU_P_Cancer_Female_Rat CU_P_Cancer_Male_Mouse CU_P_Cancer_Male_Rat CU_P_Cancer_Mouse CU_P_Cancer_Rat CU_P_Cancer_Rodent TB_120 TB_121 TB_122 TB_123 TB_124 TB_125 TB_126 TB_127 TB_128 TB_50 TB_51 TB_52 TB_53 TB_54 TB_55 TB_56 TB_57 TB_58 TB_59 TB_60 TB_61)
Linkage mechanism: Average Linkage
Cluster height: 0.5

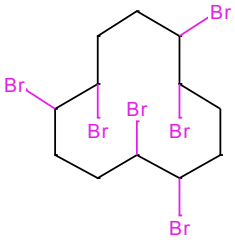
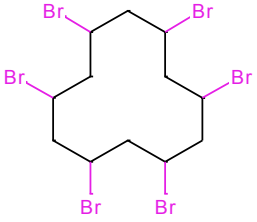
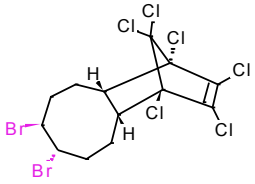
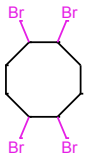
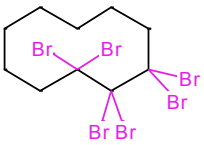
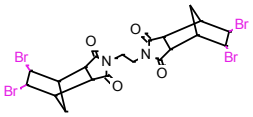
Structure

Cluster 1

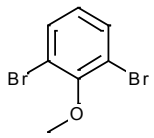
			
Br1_3278-89-5	Br2_607-99-8	Br3_26762-91-4	

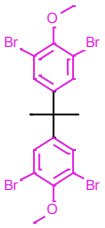
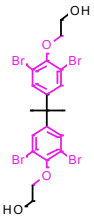
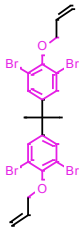
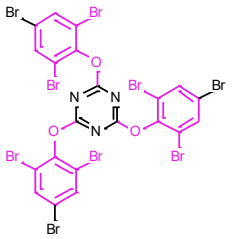
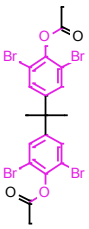
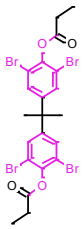
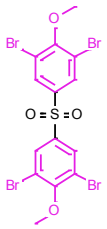
Structure
 Cluster 2

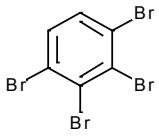
 Br1_32536-52-0	 Br1_36355-01-8		

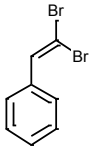
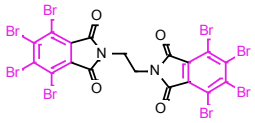
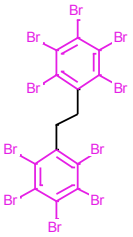
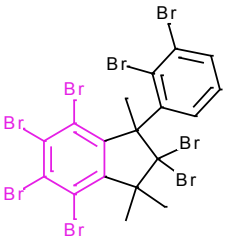
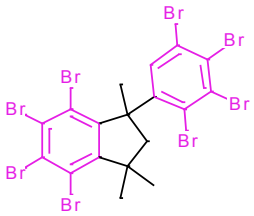
Structure

Cluster 3

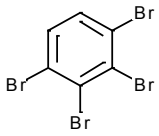
			
Br1_3194-55-6	Br1_25637-99-4	Br1_51936-55-1	Br2_3194-57-8
			
Br2_25495-98-1	Br3_52907-07-0		

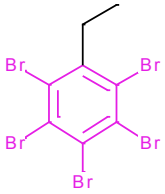
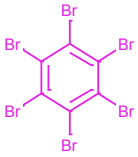
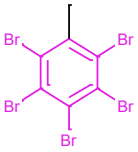
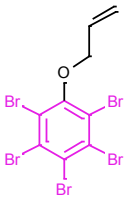
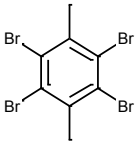
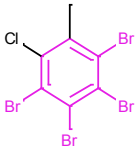
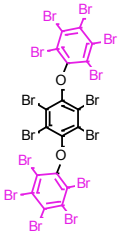
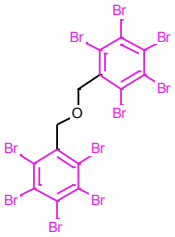
Structure

Cluster 4

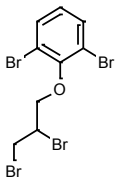
			
Br1R_37853-61-5	Br1_4162-45-2	Br1_25327-89-3	Br1_25713-60-4
			
Br1_33798-02-6	Br1_37853-59-1	Br2_37419-42-4	Br2_70156-79-5

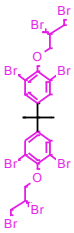
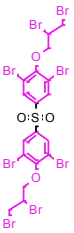
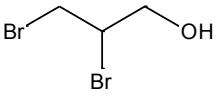
Structure

Cluster 5

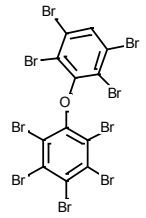
			
Br1_31780-26-4	Br1_32588-76-4	Br1_84852-53-9	Br1_155613-93-7
			
Br2_1084889-51-9			

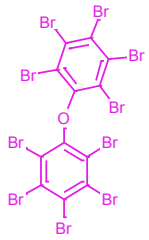
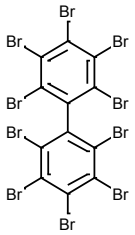
Structure

Cluster 6

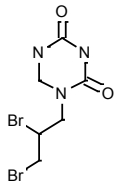
			
Br1_85-22-3	Br1_87-82-1	Br1_87-83-2	Br1_3555-11-1
			
Br1_23488-38-2	Br1_39569-21-6	Br1_58965-66-5	Br2_497107-13-8

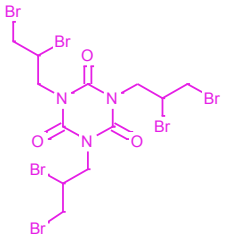
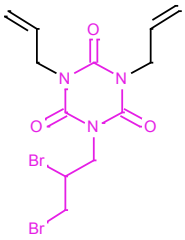
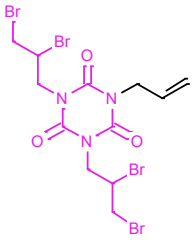
Structure

Cluster 7

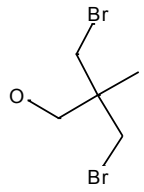
			
Br1R_21850-44-2	Br1_42757-55-1	Br3_96-13-9	

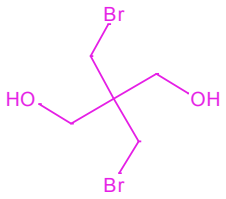
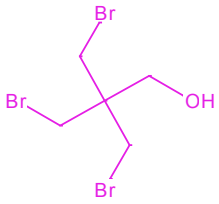
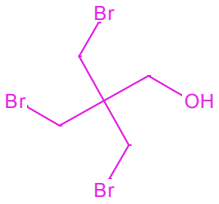
Structure

Cluster 8

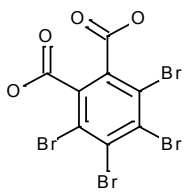
			
Br1_1163-19-5	Br1_13654-09-6		

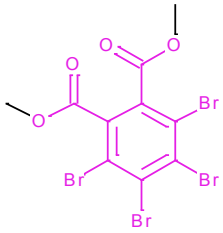
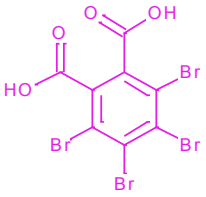
Structure

Cluster 9

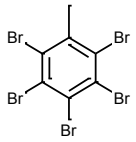
			
Br1_52434-90-9	Br2_57829-89-7	Br2_75795-16-3	

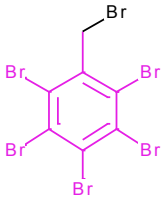
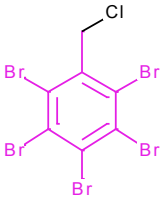
Structure
 Cluster 10

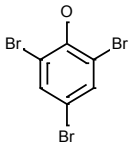
 Br1R_3296-90-0	 Br1R_36483-57-5	 Br2_1522-92-5	

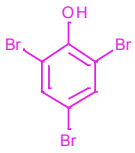
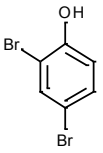
Structure

Cluster 11

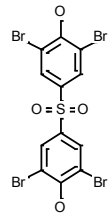
			
Br3_55481-60-2	Br3_75790-69-1		

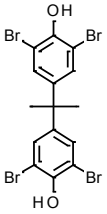
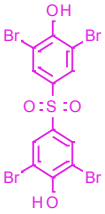
Structure

Cluster 12

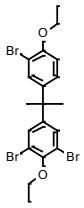
			
Br2_38521-51-6	Br2_58495-09-3		

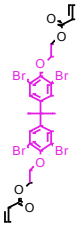
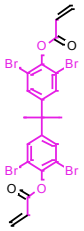
Structure

Cluster 13

			
Br1_118-79-6	Br1_615-58-7		

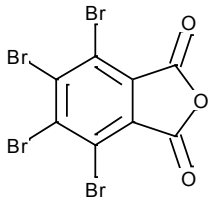
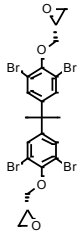
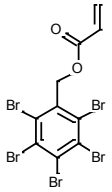
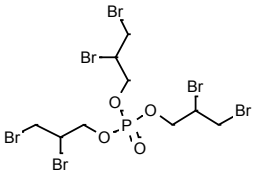
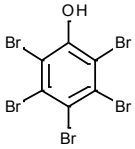
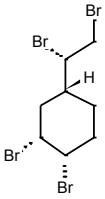
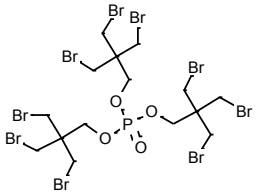
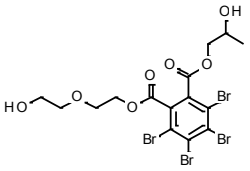
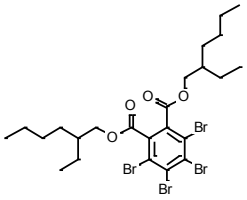
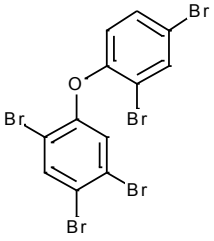
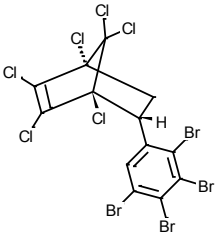
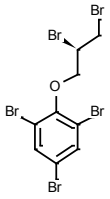
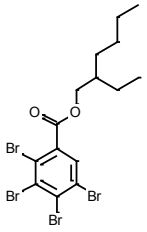
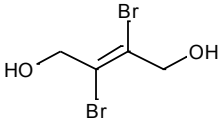
Structure

Cluster 14

			
Br1_79-94-7	Br1_39635-79-5		

Structure

Cluster 15

			
Br1R_66710-97-2	Br2_55205-38-4		

Structure
Singletons

 Br1R_632-79-1	 Br1R_3072-84-2	 Br1R_59447-55-1	 Br1_126-72-7
 Br1_608-71-9	 Br1_3322-93-8	 Br1_19186-97-1	 Br1_20566-35-2
 Br1_26040-51-7	 Br1_32534-81-9	 Br1_34571-16-9	 Br1_35109-60-5
 Br1_183658-27-7	 Br3_3234-02-4		

Genotoxicity (Q)SAR-based clustering

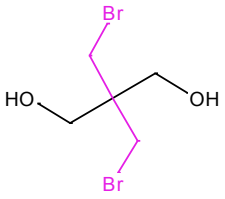
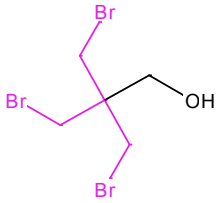
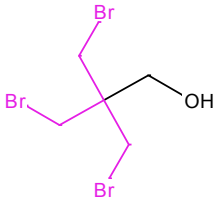
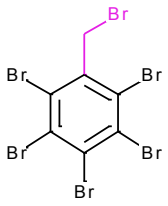
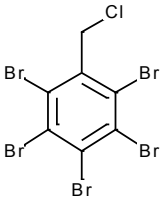
Clustering - Clusters (Run #3)

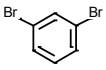
Project Name: Br_67

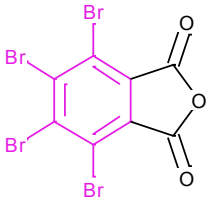
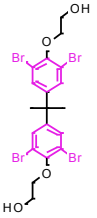
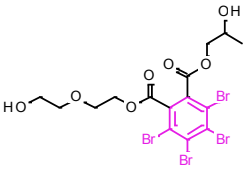
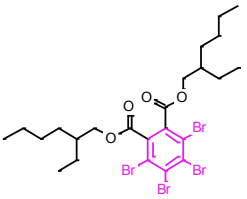
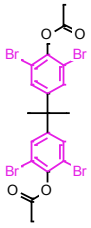
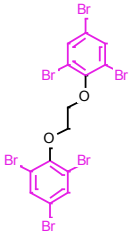
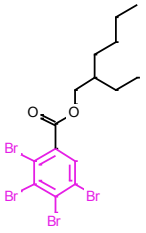
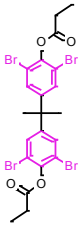
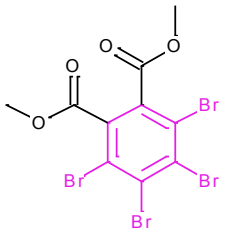
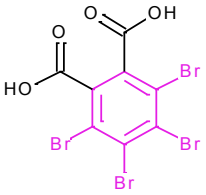
Structure Count: 67

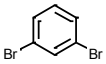
Name for this run: Clusters (Run #3)
Project: Br_67
 # structures: 67
Analysis type: Clustering (Agglomerative Nesting)
Date: Fri Nov 27 18:55:39 CET 2015
Owner: ebawe
Create signatures: true
Group singletons: true
Cluster by: Data (CU_P_Ashby CU_P_CA_CHO CU_P_MN_in_vivo CU_P_SALM LS_R_DK_Ames_test LS_R_DK_Chromosomal_aberrations_CHL LS_R_DK_Comet_assay LS_R_DK_Dominant_lethal LS_R_DK_Drosophila_SLRL LS_R_DK_HGPRT LS_R_DK_Mouse_lymphoma LS_R_DK_Mouse_micronucleus LS_R_DK_SCE_mouse LS_R_DK_SHE_cell_transformation LS_R_DK_UDS_rat_hepatocytes TB_107 TB_108 TB_109 TB_110 TB_111 TB_112 TB_212 TB_213 TB_214 TB_215 TB_216 TB_217 TB_218 TB_219 TB_220 TB_221 TB_222 TB_223 TB_224 TB_225 TB_226 TB_227 TB_228 TB_46 TB_47 TB_48 TB_49 TB_70 TB_71 TB_72 TB_73 TB_74 TB_75 TB_76 TB_77 TB_78 TB_79 TB_80)
Linkage mechanism: Average Linkage
Cluster height: 0.5

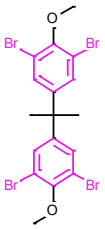
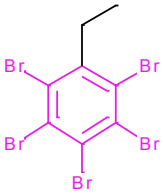
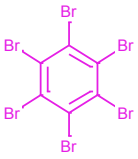
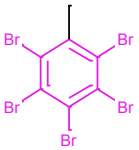
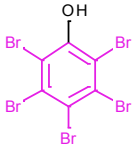
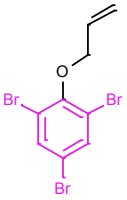
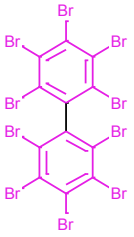
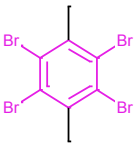
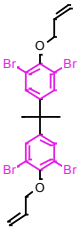
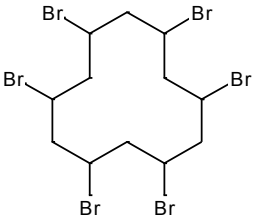
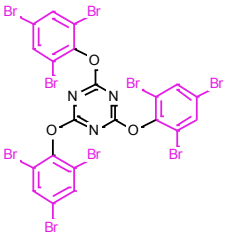
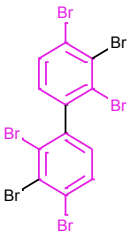
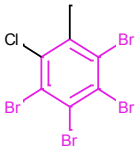
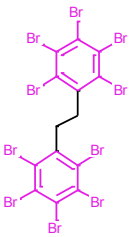
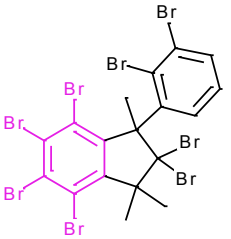
Structure	Frequency
 Cluster 1	5

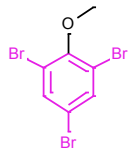
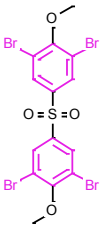
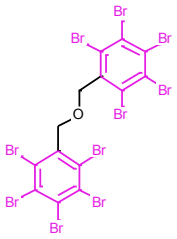
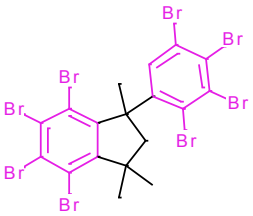
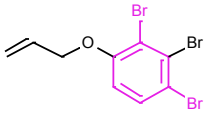
 Br1R_3296-90-0	 Br1R_36483-57-5	 Br2_1522-92-5	 Br2_38521-51-6
 Br2_58495-09-3			

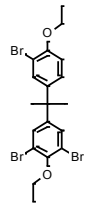
Structure	Frequency
 Cluster 2	10

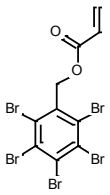
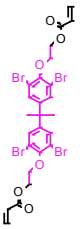
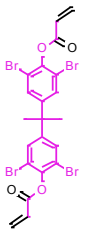
 Br1R_632-79-1	 Br1_4162-45-2	 Br1_20566-35-2	 Br1_26040-51-7
 Br1_33798-02-6	 Br1_37853-59-1	 Br1_183658-27-7	 Br2_37419-42-4
 Br3_55481-60-2	 Br3_75790-69-1		

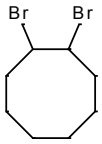
Structure	Frequency
 Cluster 3	21

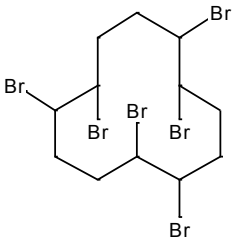
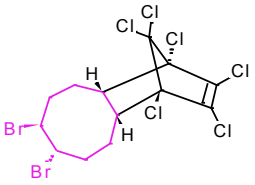
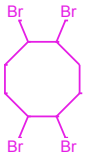
 Br1R_37853-61-5	 Br1_85-22-3	 Br1_87-82-1	 Br1_87-83-2
 Br1_608-71-9	 Br1_3278-89-5	 Br1_3555-11-1	 Br1_13654-09-6
 Br1_23488-38-2	 Br1_25327-89-3	 Br1_25637-99-4	 Br1_25713-60-4
 Br1_36355-01-8	 Br1_39569-21-6	 Br1_84852-53-9	 Br1_155613-93-7

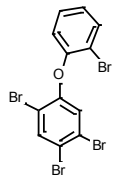
 Br2_607-99-8	 Br2_70156-79-5	 Br2_497107-13-8	 Br2_1084889-51-9
 Br3_26762-91-4			

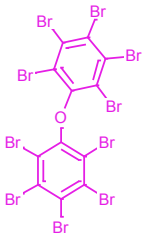
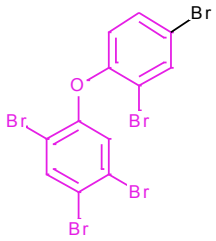
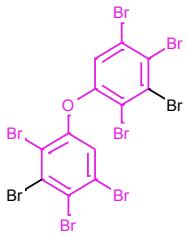
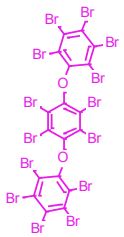
Structure	Frequency
 Cluster 4	3

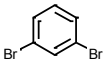
 Br1R_59447-55-1	 Br1R_66710-97-2	 Br2_55205-38-4	

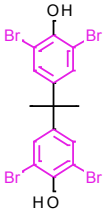
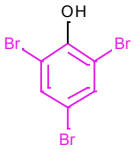
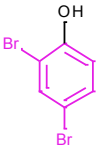
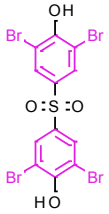
Structure	Frequency
 Cluster 5	3

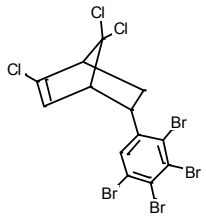
 Br1_3194-55-6	 Br1_51936-55-1	 Br2_3194-57-8	

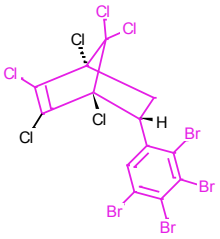
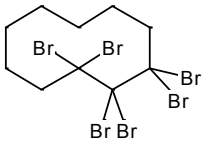
Structure	Frequency
 Cluster 6	4

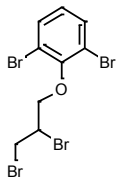
 Br1_1163-19-5	 Br1_32534-81-9	 Br1_32536-52-0	 Br1_58965-66-5

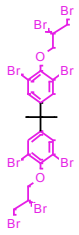
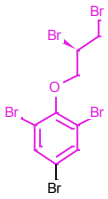
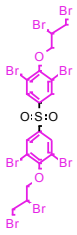
Structure	Frequency
 Cluster 7	4

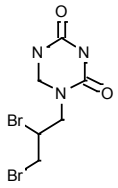
 Br1_79-94-7	 Br1_118-79-6	 Br1_615-58-7	 Br1_39635-79-5

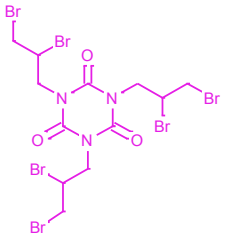
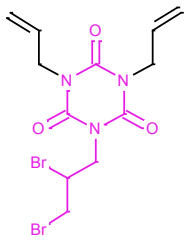
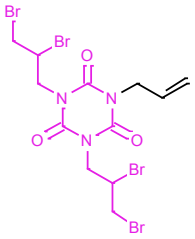
Structure	Frequency
 Cluster 8	2

 Br1_34571-16-9	 Br2_25495-98-1		

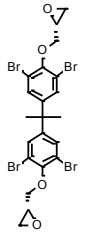
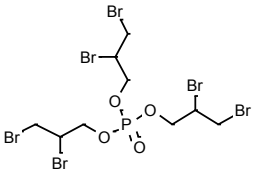
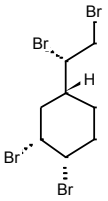
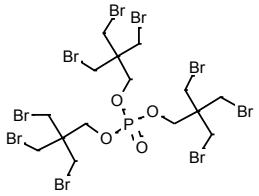
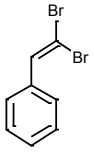
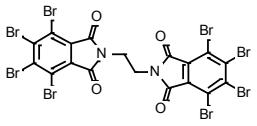
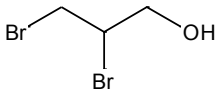
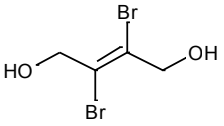
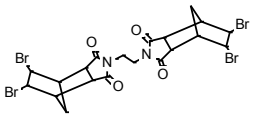
Structure	Frequency
 Cluster 9	3

 Br1R_21850-44-2	 Br1_35109-60-5	 Br1_42757-55-1	

Structure	Frequency
 Cluster 10	3

 Br1_52434-90-9	 Br2_57829-89-7	 Br2_75795-16-3	

Structure	Frequency
Singletons	9

 Br1R_3072-84-2	 Br1_126-72-7	 Br1_3322-93-8	 Br1_19186-97-1
 Br1_31780-26-4	 Br1_32588-76-4	 Br3_96-13-9	 Br3_3234-02-4
 Br3_52907-07-0			

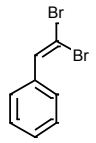
Reproductive toxicity (Q)SAR-based clustering

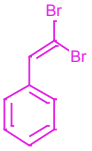
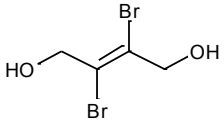
Clustering - Clusters (Run #3)

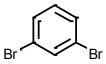
Project Name: Br_67

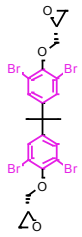
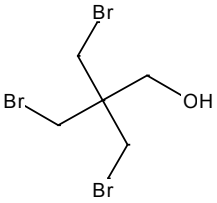
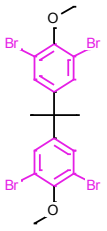
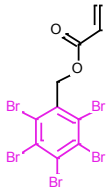
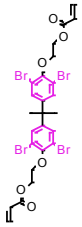
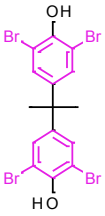
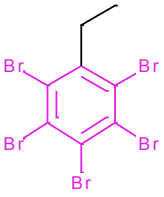
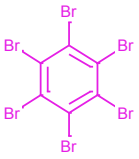
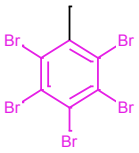
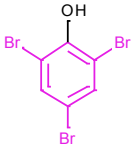
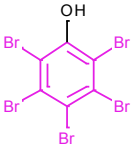
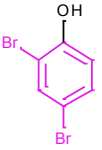
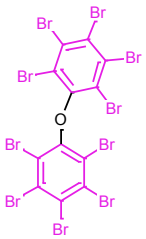
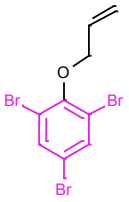
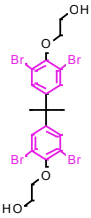
Structure Count: 67

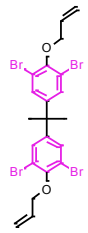
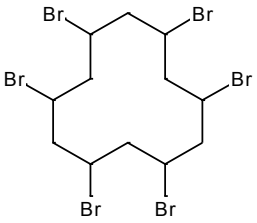
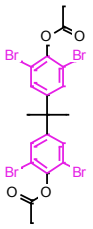
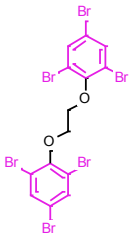
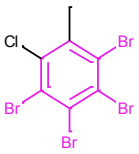
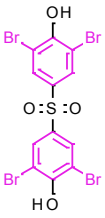
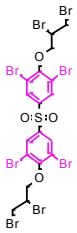
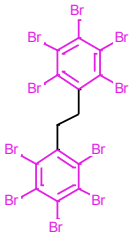
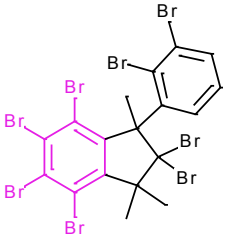
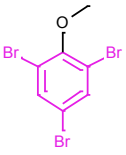
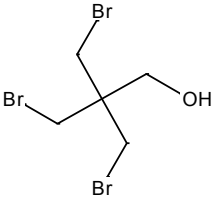
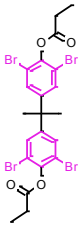
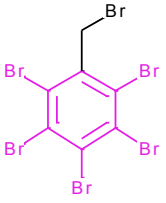
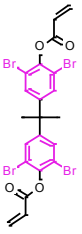
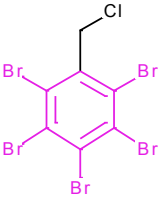
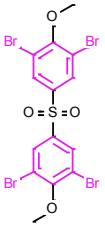
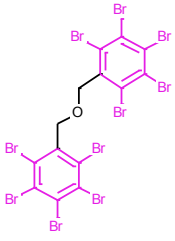
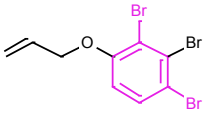
Name for this run: Clusters (Run #3)
Project: Br_67
 # structures: 67
Analysis type: Clustering (Agglomerative Nesting)
Date: Fri Nov 27 18:25:52 CET 2015
Owner: ebawe
Create signatures: true
Group singletons: true
Cluster by: Data (CU_P_RP_Female_fertility_mouse CU_P_RP_Female_fertility_rat CU_P_RP_Female_fertility_rodent CU_P_RP_Male_fertility_mouse CU_P_RP_Male_fertility_rat CU_P_RP_Male_fertility_rodent CU_P_RP_Newborn_behavioral_mouse CU_P_RP_Newborn_behavioral_rat CU_P_RP_Newborn_behavioral_rodent CU_P_RP_Sperm_toxicity_mouse CU_P_RP_Sperm_toxicity_rat CU_P_RP_Sperm_toxicity_rodent CU_P_Teratogenicity LS_R_Dysmorph_Rabbit LS_R_Dysmorph_Rat LS_R_Fetal_Death_Mouse LS_R_Fetal_Death_Rabbit LS_R_Fetal_Death_Rat LS_R_Fetal_Death_Rodent LS_R_Growth_Retard_Mouse LS_R_Growth_Retard_Rabbit LS_R_Growth_Retard_Rat LS_R_Post_Impl_Loss_Rabbit LS_R_Post_Impl_Loss_Rat LS_R_post_impl_mouse_(AG6) LS_R_post_impl_rodent_(AG1) LS_R_Pre_Impl_Loss_Mouse LS_R_Pre_Impl_Loss_Rabbit LS_R_Pre_Impl_Loss_Rat LS_R_Pre_Impl_Loss_Rodent LS_R_Repro_Mouse_Female LS_R_Repro_Mouse_Male LS_R_Repro_Rat_Female LS_R_Repro_Rat_Male LS_R_Repro_Rodent_Female LS_R_Repro_Rodent_Male LS_R_retard_rodent_(AH1) LS_R_Sperm_Eff_Rat LS_R_Sperm_Eff_Rodent LS_R_sperm_mouse(AP5) LS_R_struct_mouse_(AL6) LS_R_struct_rodent_(AL1) LS_R_Visc_Org_Mouse LS_R_Visc_Org_Rat LS_R_Visc_Org_Rodent LS_R_weight_dec._rat LS_R_Wt_Dec_Mouse LS_R_Wt_Dec_Rabbit LS_R_wt_dec_rodent_(AI1) TB_113 TB_114 TB_115 TB_116 TB_117 TB_118 TB_119)
Linkage mechanism: Average Linkage
Cluster height: 0.5

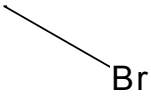
Structure	Frequency
 Cluster 1	2

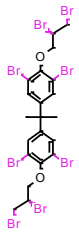
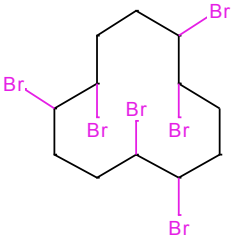
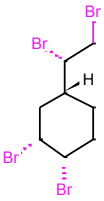
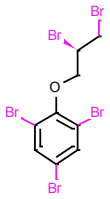
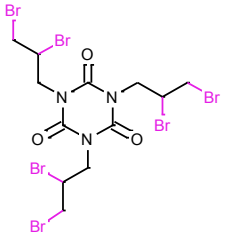
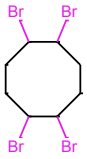
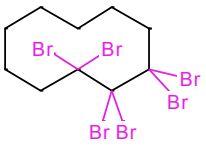
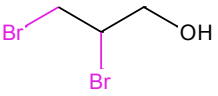
 Br1_31780-26-4	 Br3_3234-02-4		

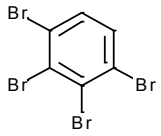
Structure	Frequency
 Cluster 2	34

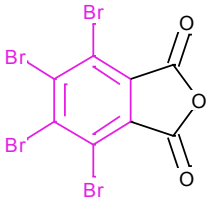
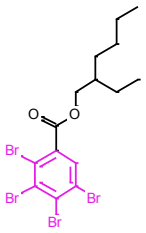
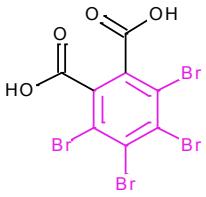
 Br1R_3072-84-2	 Br1R_36483-57-5	 Br1R_37853-61-5	 Br1R_59447-55-1
 Br1R_66710-97-2	 Br1_79-94-7	 Br1_85-22-3	 Br1_87-82-1
 Br1_87-83-2	 Br1_118-79-6	 Br1_608-71-9	 Br1_615-58-7
 Br1_1163-19-5	 Br1_3278-89-5	 Br1_3555-11-1	 Br1_4162-45-2

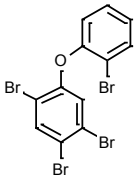
 Br1_25327-89-3	 Br1_25637-99-4	 Br1_33798-02-6	 Br1_37853-59-1
 Br1_39569-21-6	 Br1_39635-79-5	 Br1_42757-55-1	 Br1_84852-53-9
 Br1_155613-93-7	 Br2_607-99-8	 Br2_1522-92-5	 Br2_37419-42-4
 Br2_38521-51-6	 Br2_55205-38-4	 Br2_58495-09-3	 Br2_70156-79-5
 Br2_497107-13-8	 Br3_26762-91-4		

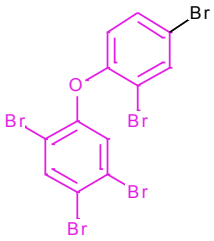
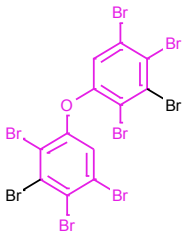
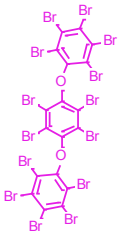
Structure	Frequency
 Cluster 3	8

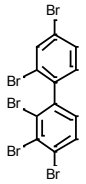
 Br1R_21850-44-2	 Br1_3194-55-6	 Br1_3322-93-8	 Br1_35109-60-5
 Br1_52434-90-9	 Br2_3194-57-8	 Br2_25495-98-1	 Br3_96-13-9

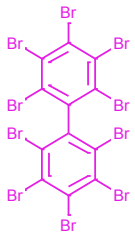
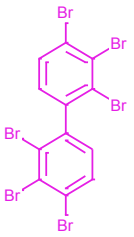
Structure	Frequency
 Cluster 4	3

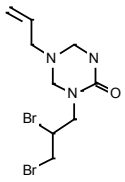
 Br1R_632-79-1	 Br1_183658-27-7	 Br3_75790-69-1	

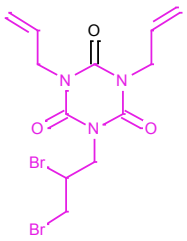
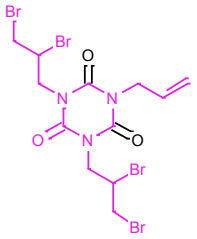
Structure	Frequency
 Cluster 5	3

 Br1_32534-81-9	 Br1_32536-52-0	 Br1_58965-66-5	

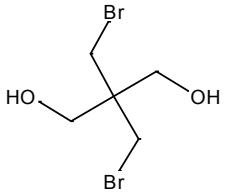
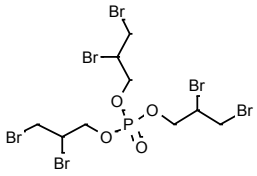
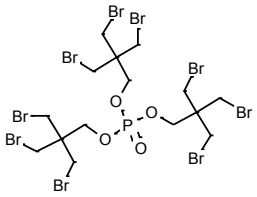
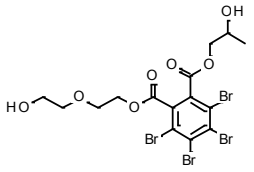
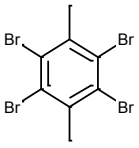
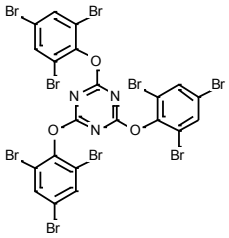
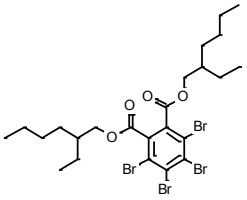
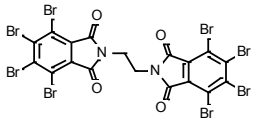
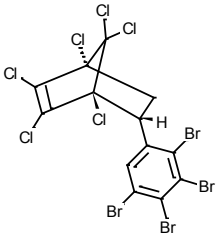
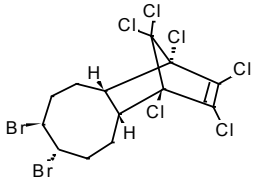
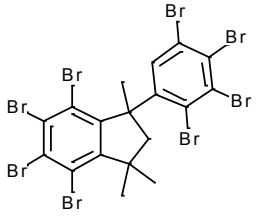
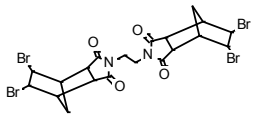
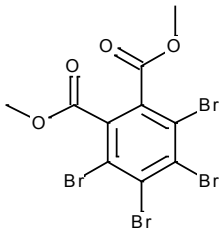
Structure	Frequency
 Cluster 6	2

 Br1_13654-09-6	 Br1_36355-01-8		

Structure	Frequency
 Cluster 7	2

 Br2_57829-89-7	 Br2_75795-16-3		

Structure	Frequency
Singletons	13

 Br1R_3296-90-0	 Br1_126-72-7	 Br1_19186-97-1	 Br1_20566-35-2
 Br1_23488-38-2	 Br1_25713-60-4	 Br1_26040-51-7	 Br1_32588-76-4
 Br1_34571-16-9	 Br1_51936-55-1	 Br2_1084889-51-9	 Br3_52907-07-0
 Br3_55481-60-2			

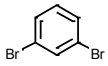
Endocrine (Q)SAR-based clustering

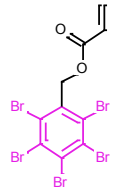
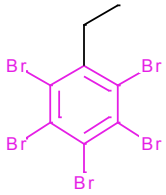
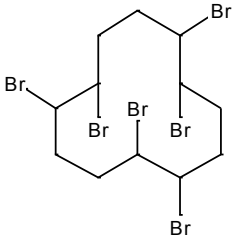
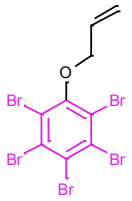
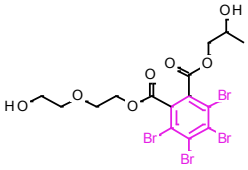
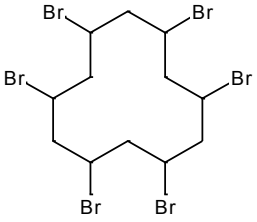
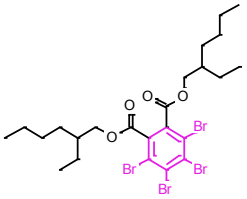
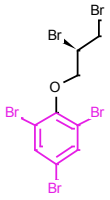
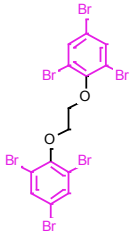
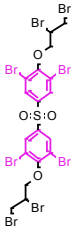
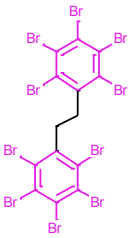
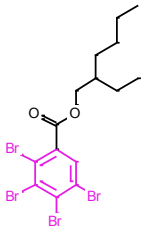
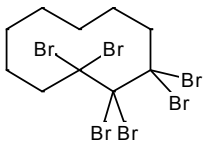
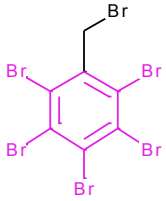
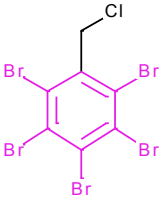
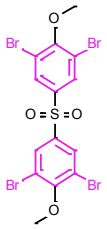
Clustering - Clusters (Run #3)

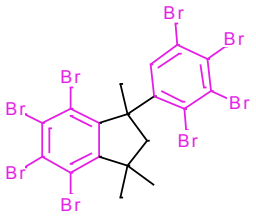
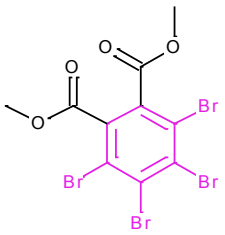
Project Name: Br_67

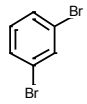
Structure Count: 67

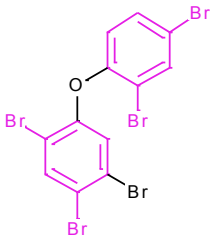
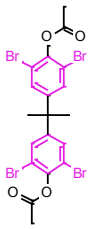
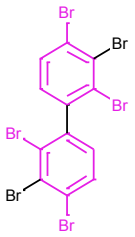
Name for this run: Clusters (Run #3)
Project: Br_67
 # structures: 67
Analysis type: Clustering (Agglomerative Nesting)
Date: Sat Nov 28 22:02:42 CET 2015
Owner: ebawe
Create signatures: true
Group singletons: true
Cluster by: Data (LS_R_DK_Anti-androgenicity LS_R_DK_CERAPP_agonist LS_R_DK_Estrogen_binding_all LS_R_DK_Estrogen_binding_bal. LS_R_DK_Estrogen_reporter_gene LS_R_DK_PXR LS_R_DK_TPO TB_196 TB_197 TB_198 TB_199 TB_200)
Linkage mechanism: Average Linkage
Cluster height: 0.5

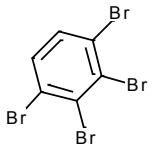
Structure	Frequency
 Cluster 1	18

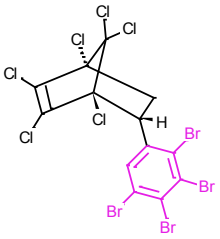
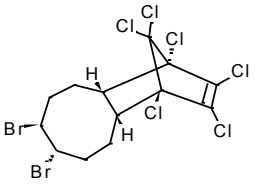
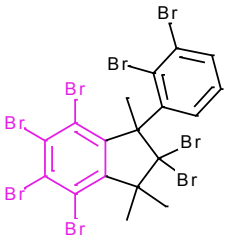
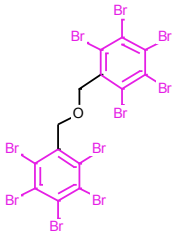
 Br1R_59447-55-1	 Br1_85-22-3	 Br1_3194-55-6	 Br1_3555-11-1
 Br1_20566-35-2	 Br1_25637-99-4	 Br1_26040-51-7	 Br1_35109-60-5
 Br1_37853-59-1	 Br1_42757-55-1	 Br1_84852-53-9	 Br1_183658-27-7
 Br2_25495-98-1	 Br2_38521-51-6	 Br2_58495-09-3	 Br2_70156-79-5

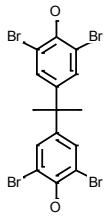
 Br2_1084889-51-9	 Br3_55481-60-2		

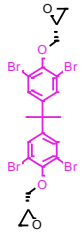
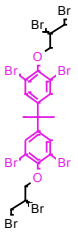
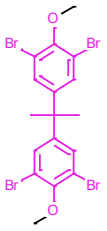
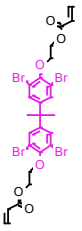
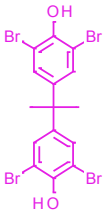
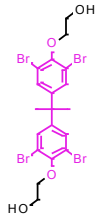
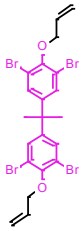
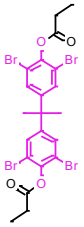
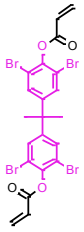
Structure	Frequency
 Cluster 2	3

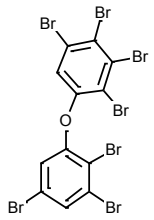
 Br1_32534-81-9	 Br1_33798-02-6	 Br1_36355-01-8	

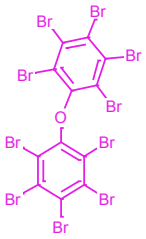
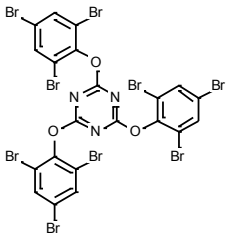
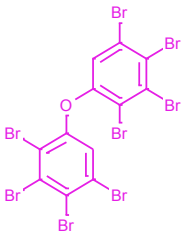
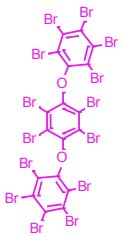
Structure	Frequency
 Cluster 3	4

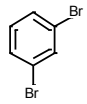
 Br1_34571-16-9	 Br1_51936-55-1	 Br1_155613-93-7	 Br2_497107-13-8

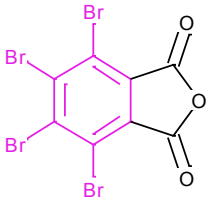
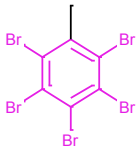
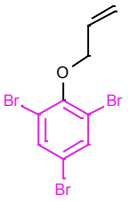
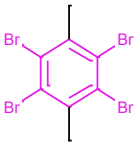
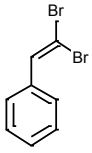
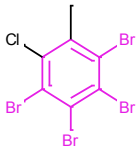
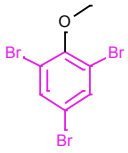
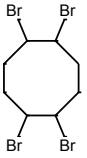
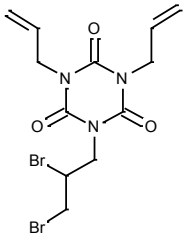
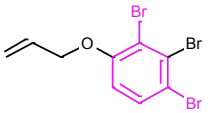
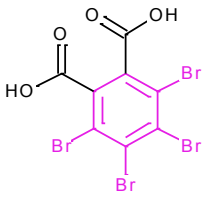
Structure	Frequency
 Cluster 4	9

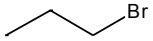
 Br1R_3072-84-2	 Br1R_21850-44-2	 Br1R_37853-61-5	 Br1R_66710-97-2
 Br1_79-94-7	 Br1_4162-45-2	 Br1_25327-89-3	 Br2_37419-42-4
 Br2_55205-38-4			

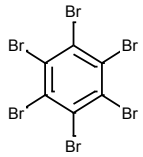
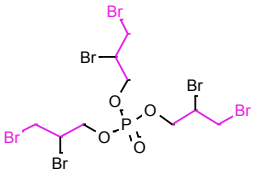
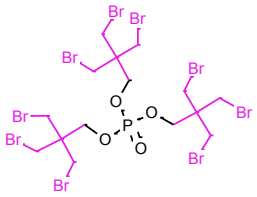
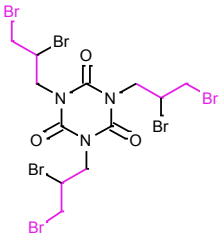
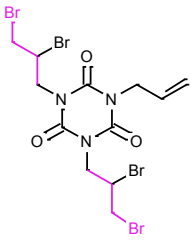
Structure	Frequency
 Cluster 5	4

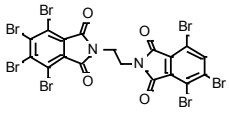
 Br1_1163-19-5	 Br1_25713-60-4	 Br1_32536-52-0	 Br1_58965-66-5

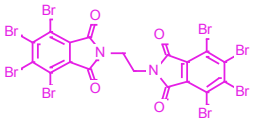
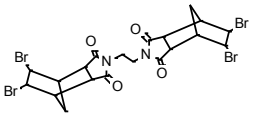
Structure	Frequency
 Cluster 6	11

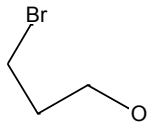
 Br1R_632-79-1	 Br1_87-83-2	 Br1_3278-89-5	 Br1_23488-38-2
 Br1_31780-26-4	 Br1_39569-21-6	 Br2_607-99-8	 Br2_3194-57-8
 Br2_57829-89-7	 Br3_26762-91-4	 Br3_75790-69-1	

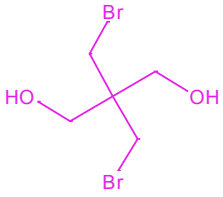
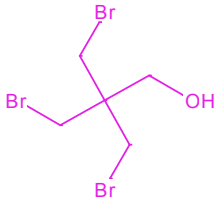
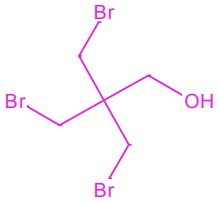
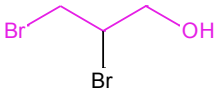
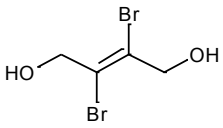
Structure	Frequency
 Cluster 7	5

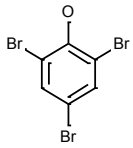
 Br1_87-82-1	 Br1_126-72-7	 Br1_19186-97-1	 Br1_52434-90-9
 Br2_75795-16-3			

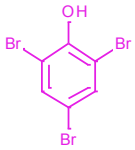
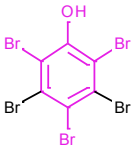
Structure	Frequency
 Cluster 8	2

 Br1_32588-76-4	 Br3_52907-07-0		

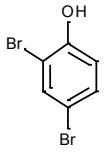
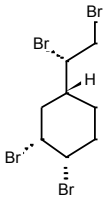
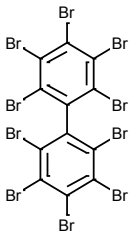
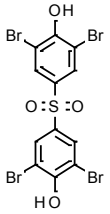
Structure	Frequency
 Cluster 9	5

 Br1R_3296-90-0	 Br1R_36483-57-5	 Br2_1522-92-5	 Br3_96-13-9
 Br3_3234-02-4			

Structure	Frequency
 Cluster 10	2

 Br1_118-79-6	 Br1_608-71-9		

Structure	Frequency
Singletons	4

			
Br1_615-58-7	Br1_3322-93-8	Br1_13654-09-6	Br1_39635-79-5

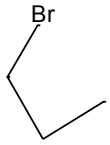
Skin sensitization (Q)SAR-based clustering

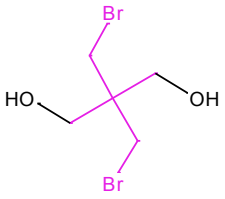
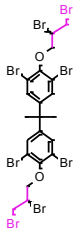
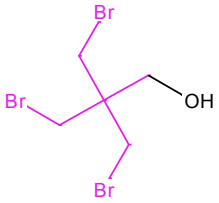
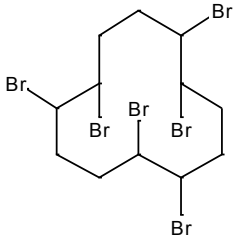
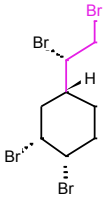
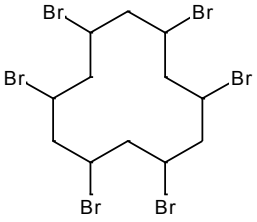
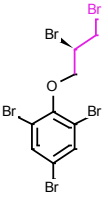
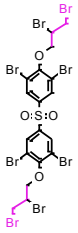
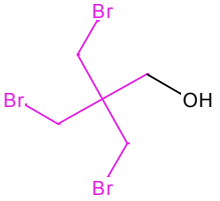
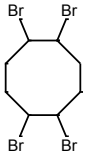
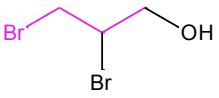
Clustering - Clusters (Run #3)

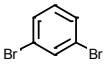
Project Name: Br_67

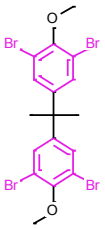
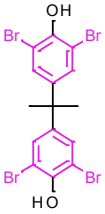
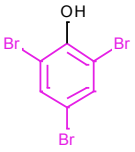
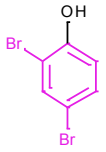
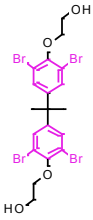
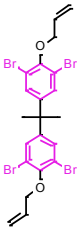
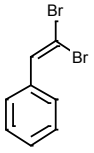
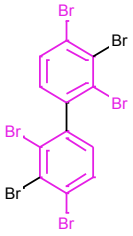
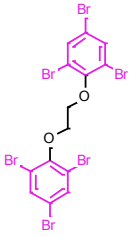
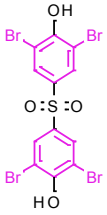
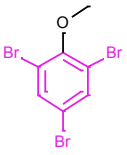
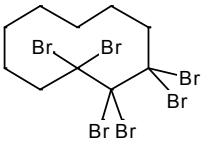
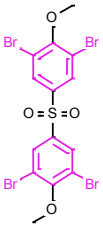
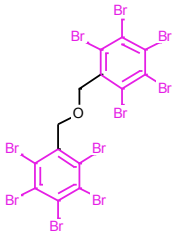
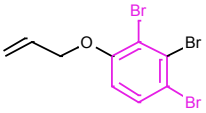
Structure Count: 67

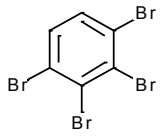
Name for this run: Clusters (Run #3)
Project: Br_67
 # structures: 67
Analysis type: Clustering (Agglomerative Nesting)
Date: Fri Nov 27 18:17:02 CET 2015
Owner: ebawe
Create signatures: true
Group singletons: true
Cluster by: Data (CU_P_Skin_allergy TB_163 TB_164 TB_165 TB_166 TB_167 TB_168 TB_169 TB_170 TB_171 TB_172 TB_173 TB_174 TB_175 TB_176 TB_177 TB_178 TB_179 TB_180 TB_181 TB_182 TB_183 TB_184 TB_185 TB_186 TB_187 TB_188 TB_189 TB_190 TB_191 TB_192 TB_193 TB_194 TB_195 TB_201 TB_202 TB_203 TB_204 TB_205 TB_206 TB_207 TB_208 TB_209 TB_210 TB_211 TB_33 TB_34 TB_35 TB_36 TB_37 TB_38 TB_39 TB_40 TB_41 TB_42 TB_43 TB_44 TB_45 TB_62 TB_63 TB_64 TB_65 TB_66 TB_67 TB_68 TB_69)
Linkage mechanism: Average Linkage
Cluster height: 0.5

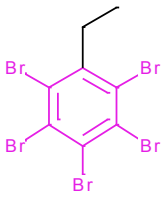
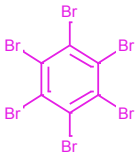
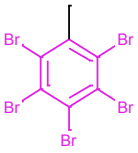
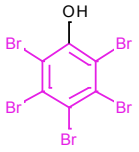
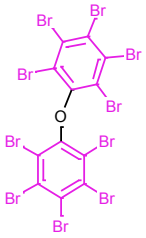
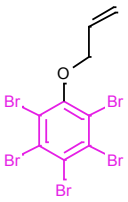
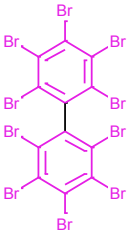
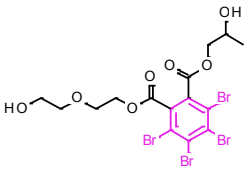
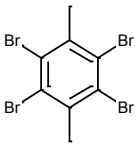
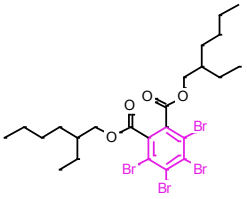
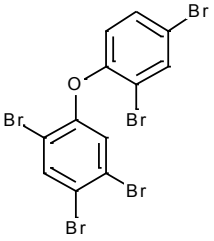
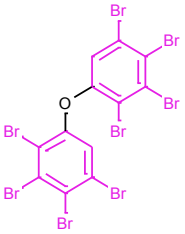
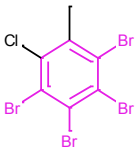
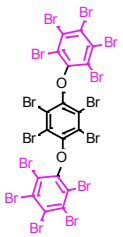
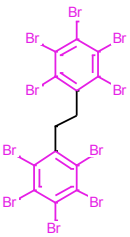
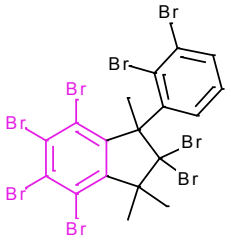
Structure	Frequency
 Cluster 1	11

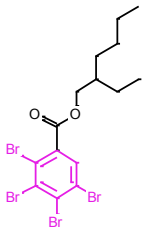
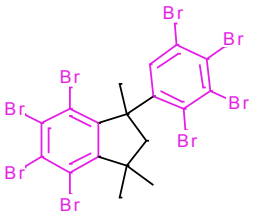
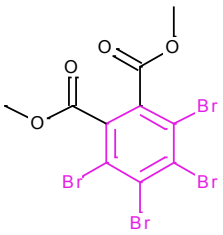
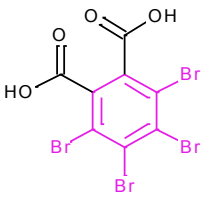
 Br1R_3296-90-0	 Br1R_21850-44-2	 Br1R_36483-57-5	 Br1_3194-55-6
 Br1_3322-93-8	 Br1_25637-99-4	 Br1_35109-60-5	 Br1_42757-55-1
 Br2_1522-92-5	 Br2_3194-57-8	 Br3_96-13-9	

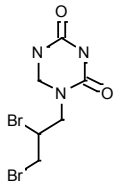
Structure	Frequency
 Cluster 2	16

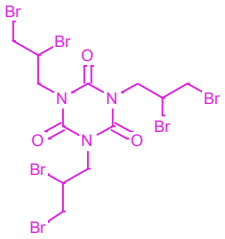
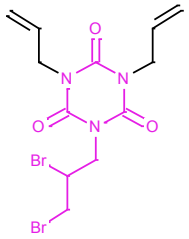
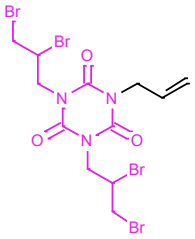
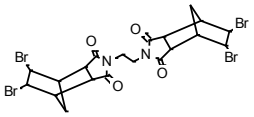
 Br1R_37853-61-5	 Br1_79-94-7	 Br1_118-79-6	 Br1_615-58-7
 Br1_3278-89-5	 Br1_4162-45-2	 Br1_25327-89-3	 Br1_31780-26-4
 Br1_36355-01-8	 Br1_37853-59-1	 Br1_39635-79-5	 Br2_607-99-8
 Br2_25495-98-1	 Br2_70156-79-5	 Br2_497107-13-8	 Br3_26762-91-4

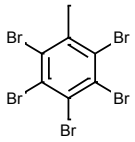
Structure	Frequency
 Cluster 3	20

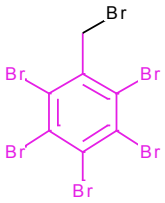
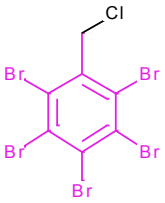
 Br1_85-22-3	 Br1_87-82-1	 Br1_87-83-2	 Br1_608-71-9
 Br1_1163-19-5	 Br1_3555-11-1	 Br1_13654-09-6	 Br1_20566-35-2
 Br1_23488-38-2	 Br1_26040-51-7	 Br1_32534-81-9	 Br1_32536-52-0
 Br1_39569-21-6	 Br1_58965-66-5	 Br1_84852-53-9	 Br1_155613-93-7

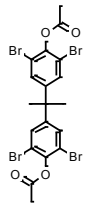
			
Br1_183658-27-7	Br2_1084889-51-9	Br3_55481-60-2	Br3_75790-69-1

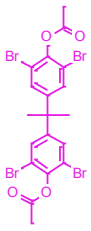
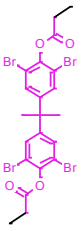
Structure	Frequency
 Cluster 4	4

 Br1_52434-90-9	 Br2_57829-89-7	 Br2_75795-16-3	 Br3_52907-07-0

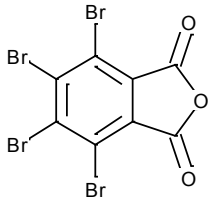
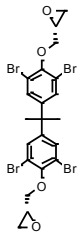
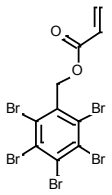
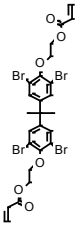
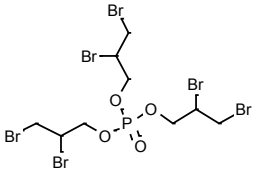
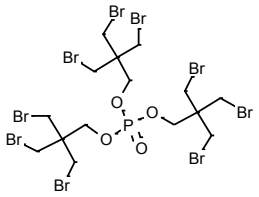
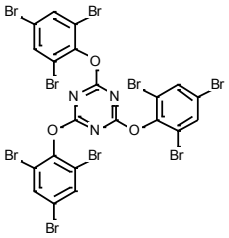
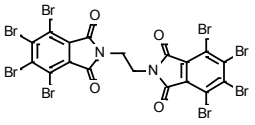
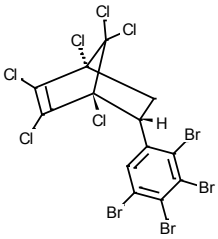
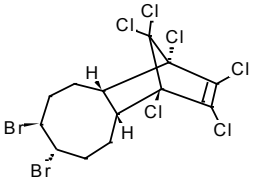
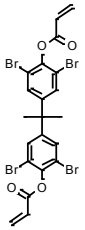
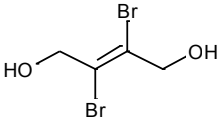
Structure	Frequency
 Cluster 5	2

 Br2_38521-51-6	 Br2_58495-09-3		

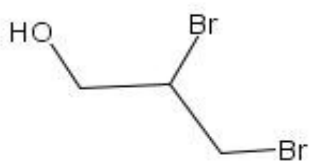
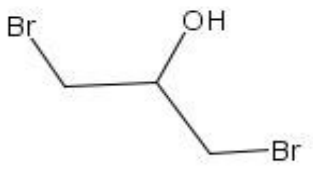
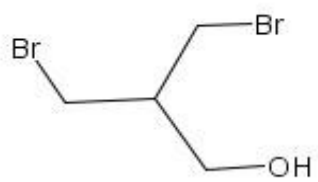
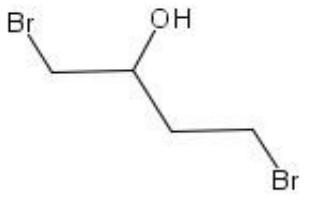
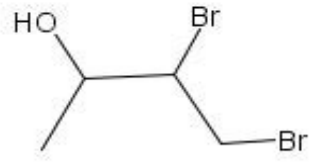
Structure	Frequency
 Cluster 6	2

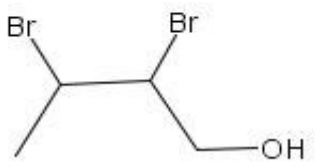
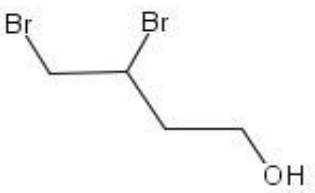
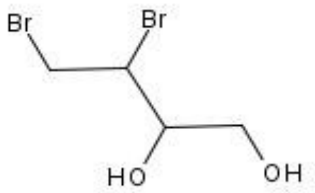
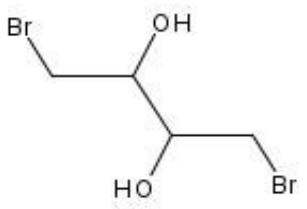
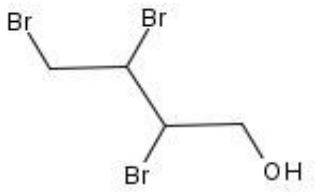
 Br1_33798-02-6	 Br2_37419-42-4		

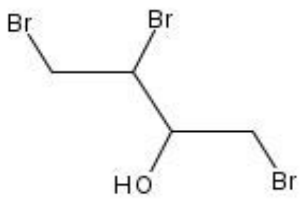
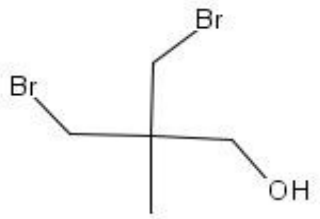
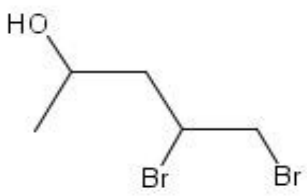
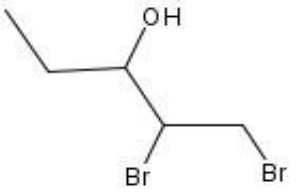
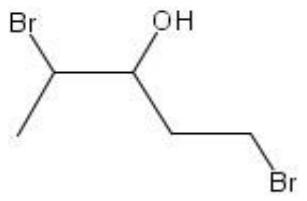
Structure	Frequency
Singletons	12

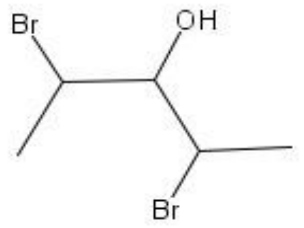
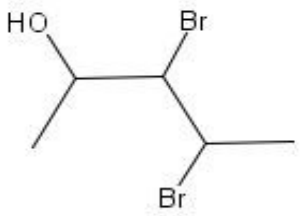
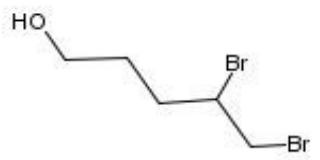
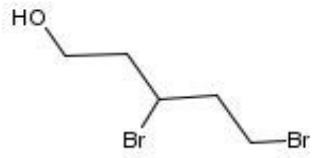
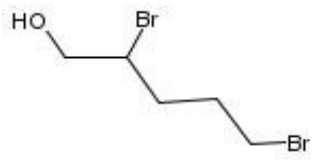
 Br1R_632-79-1	 Br1R_3072-84-2	 Br1R_59447-55-1	 Br1R_66710-97-2
 Br1_126-72-7	 Br1_19186-97-1	 Br1_25713-60-4	 Br1_32588-76-4
 Br1_34571-16-9	 Br1_51936-55-1	 Br2_55205-38-4	 Br3_3234-02-4

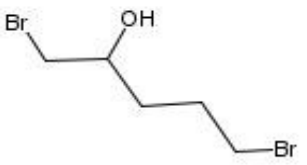
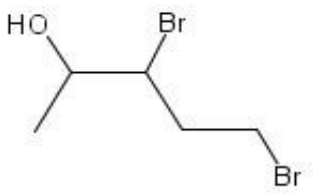
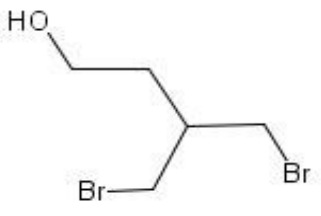
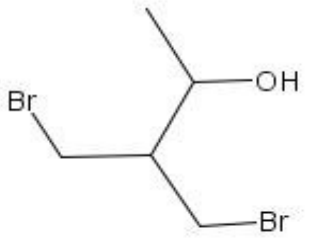
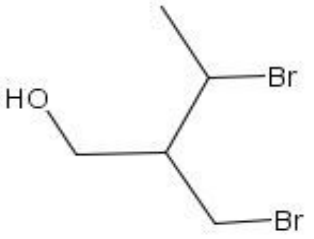
Appendix 5: Category members

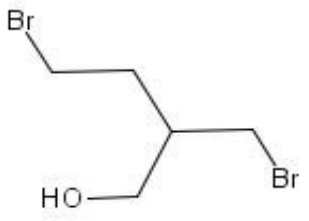
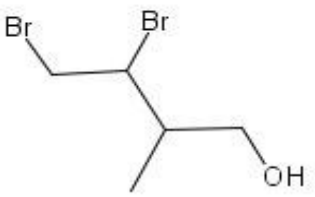
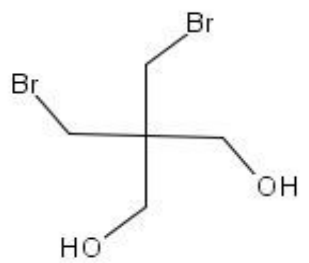
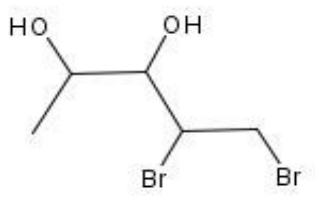
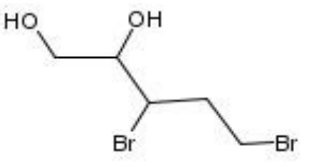
Structure	ALogP	H-bond Acceptors	H-bond Donors	Lipinski Score	Mol. Weight	Polar Surface Area	Rotatable Bonds
 BFR_32102_96-13-9	1,14	1	1	0	217,9	20,23	2
 BFR_32103_96-21-9	1,14	1	1	0	217,9	20,23	2
 BFR_42104_106023-63-6	1,38	1	1	0	231,9	20,23	3
 BFR_42105_19398-47-1	1,53	1	1	0	231,9	20,23	3
 BFR_42106_79033-40-2	1,53	1	1	0	231,9	20,23	2

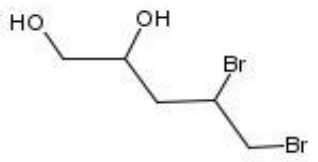
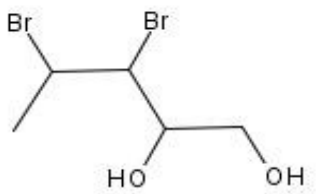
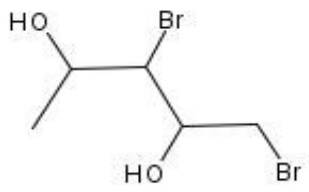
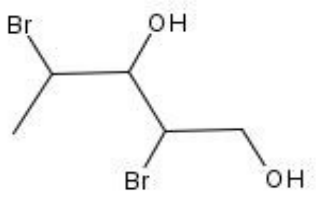
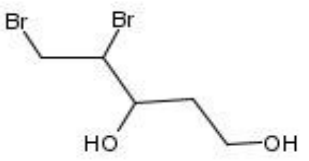
 BFR_42107_4021-75-4	1,53	1	1	0	231,9	20,23	2
 BFR_42108_87018-30-2	1,53	1	1	0	231,9	20,23	3
 BFR_42209_35330-59-7	0,5	2	2	0	247,9	40,46	3
 BFR_42210_14396-65-7	0,5	2	2	0	247,9	40,46	3
 BFR_43111_855236-37-2	1,9	1	1	0	310,8	20,23	3

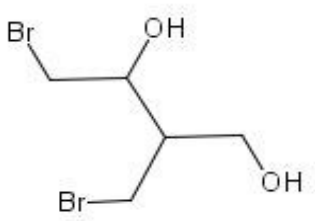
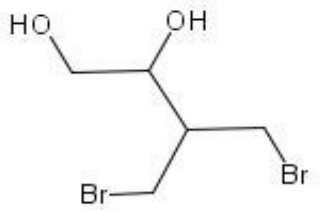
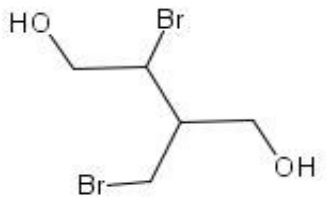
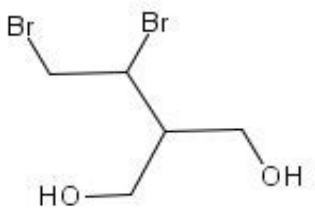
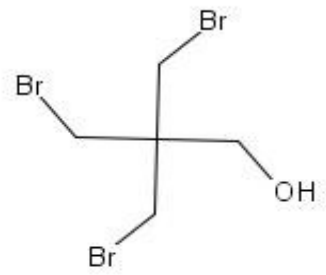
 BFR_43112_87018-38-0	1,9	1	1	0	310,8	20,23	3
 BFR_52113_105100-80-9	1,77	1	1	0	245,9	20,23	3
 BFR_52114_213821-22-8	1,92	1	1	0	245,9	20,23	3
 BFR_52115_408319-76-6	1,92	1	1	0	245,9	20,23	3
 BFR_52116_159475-15-7	1,92	1	1	0	245,9	20,23	3

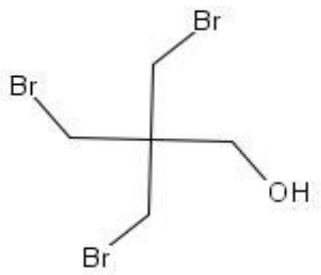
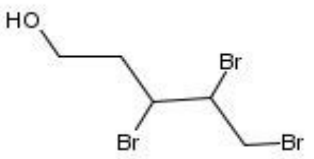
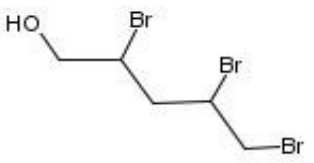
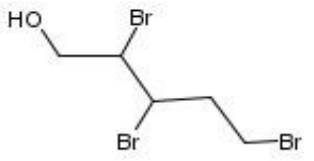
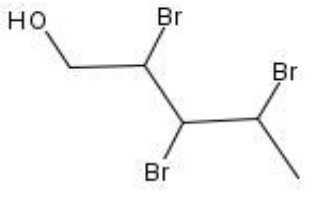
 BFR_52117_343268-04-2	1,91	1	1	0	245,9	20,23	2
 BFR_52118_76377-07-6	1,91	1	1	0	245,9	20,23	2
 BFR_52119_59287-66-0	1,92	1	1	0	245,9	20,23	4
 BFR_52120_1	1,92	1	1	0	245,9	20,23	4
 BFR_52121_856991-78-1	1,92	1	1	0	245,9	20,23	4

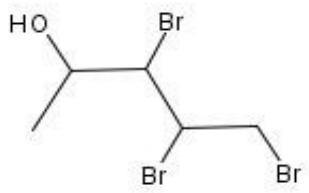
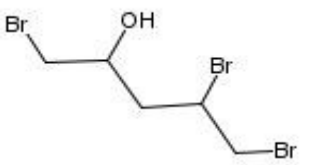
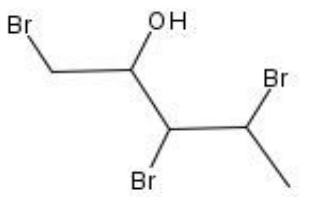
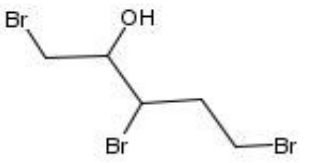
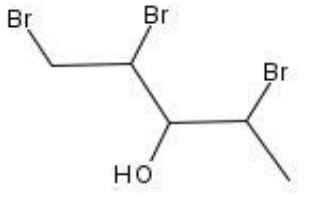
 BFR_52122_100606-66-4	1,92	1	1	0	245,9	20,23	4
 BFR_52123_213821-20-6	1,92	1	1	0	245,9	20,23	3
 BFR_52124_2	1,77	1	1	0	245,9	20,23	4
 BFR_52125_3	1,77	1	1	0	245,9	20,23	3
 BFR_52126_4	1,77	1	1	0	245,9	20,23	3

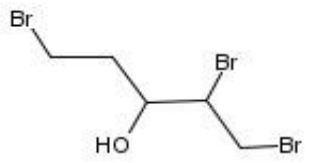
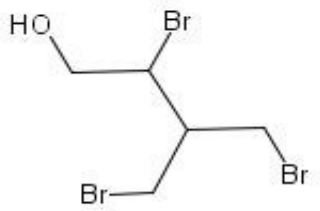
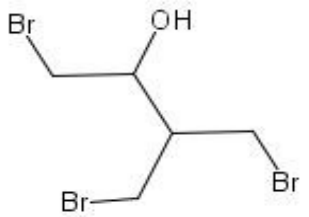
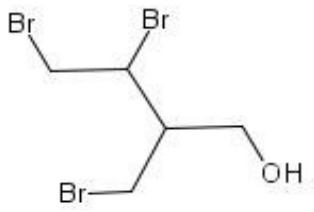
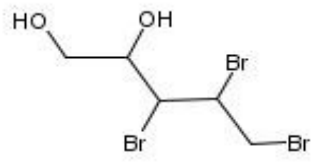
 BFR_52127_98069-26-2	1,77	1	1	0	245,9	20,23	4
 BFR_52128_5	1,77	1	1	0	245,9	20,23	3
 BFR_52229_3296-90-0	0,75	2	2	0	261,9	40,46	4
 BFR_52230_20	0,89	2	2	0	261,9	40,46	3
 BFR_52231_21	0,89	2	2	0	261,9	40,46	4

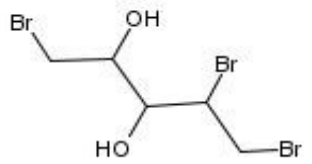
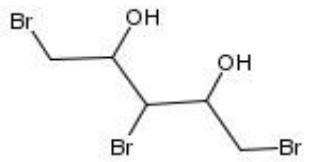
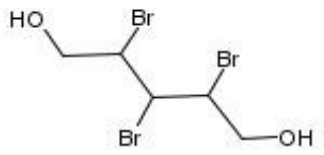
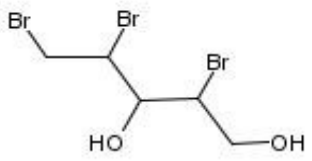
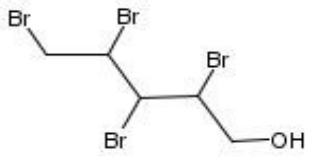
 BFR_52232_22	0,89	2	2	0	261,9	40,46	4
 BFR_52233_23	0,89	2	2	0	261,9	40,46	3
 BFR_52234_24	0,89	2	2	0	261,9	40,46	3
 BFR_52235_25	0,89	2	2	0	261,9	40,46	3
 BFR_52236_26	0,89	2	2	0	261,9	40,46	4

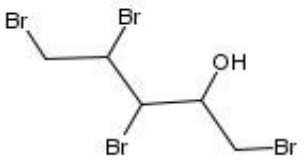
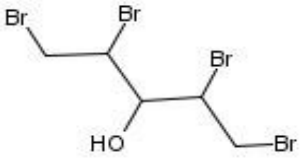
 BFR_52237_44804-46-8	0,75	2	2	0	261,9	40,46	4
 BFR_52238_27	0,75	2	2	0	261,9	40,46	4
 BFR_52239_28	0,75	2	2	0	261,9	40,46	4
 BFR_52240_29	0,75	2	2	0	261,9	40,46	4
 BFR_53141_1522-92-5	2,15	1	1	0	324,8	20,23	4

 BFR_53142_36483-57-5	2,15	1	1	0	324,8	20,23	4
 BFR_53143_6	2,29	1	1	0	324,8	20,23	4
 BFR_53144_7	2,29	1	1	0	324,8	20,23	4
 BFR_53145_8	2,29	1	1	0	324,8	20,23	4
 BFR_53146_9	2,29	1	1	0	324,8	20,23	3

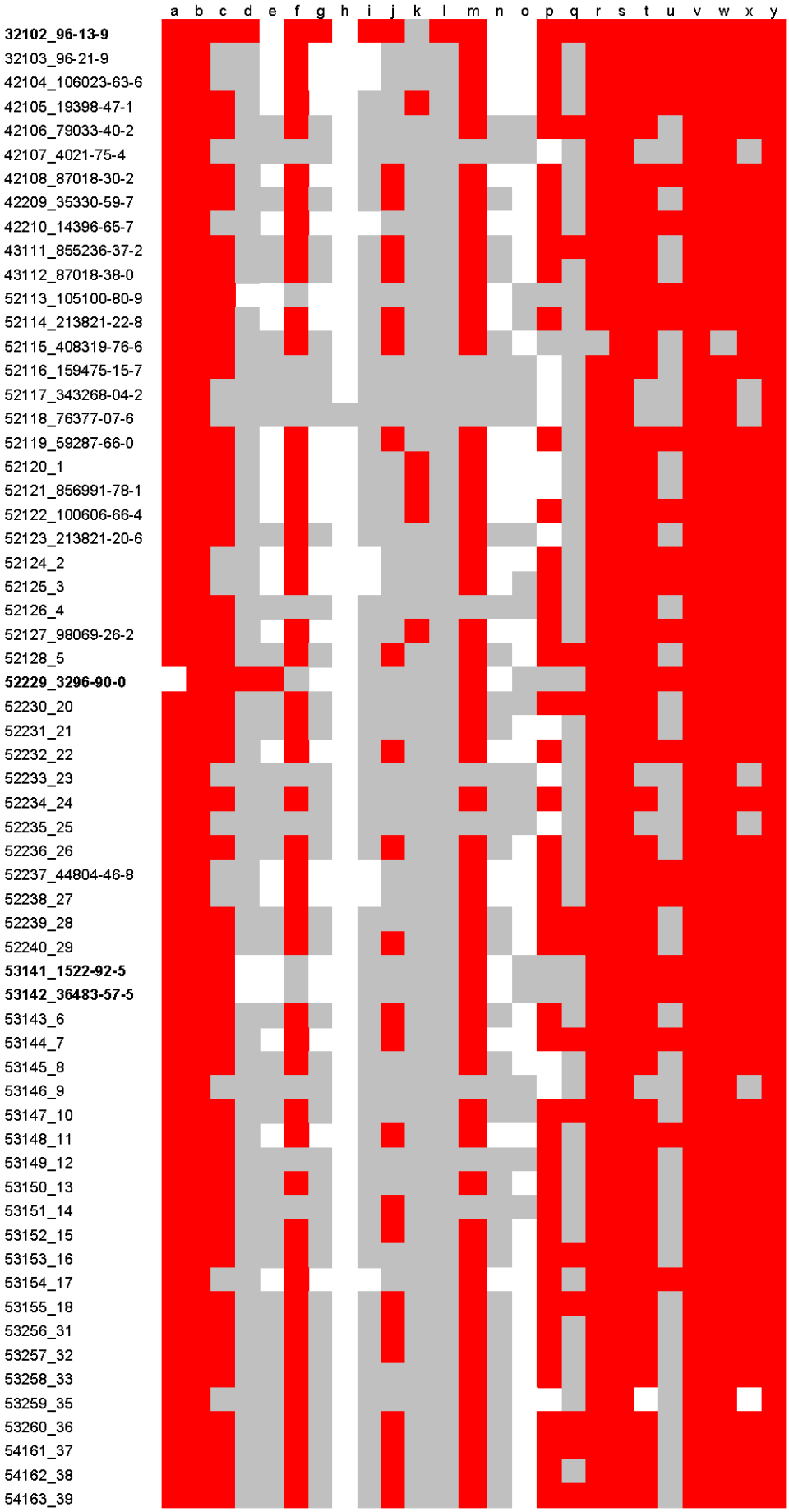
 BFR_53147_10	2,29	1	1	0	324,8	20,23	3
 BFR_53148_11	2,29	1	1	0	324,8	20,23	4
 BFR_53149_12	2,29	1	1	0	324,8	20,23	3
 BFR_53150_13	2,29	1	1	0	324,8	20,23	4
 BFR_53151_14	2,29	1	1	0	324,8	20,23	3

 BFR_53152_15	2,29	1	1	0	324,8	20,23	4
 BFR_53153_16	2,15	1	1	0	324,8	20,23	4
 BFR_53154_17	2,15	1	1	0	324,8	20,23	4
 BFR_53155_18	2,15	1	1	0	324,8	20,23	4
 BFR_53256_31	1,26	2	2	0	340,8	40,46	4

 BFR_53257_32	1,26	2	2	0	340,8	40,46	4
 BFR_53258_33	1,26	2	2	0	340,8	40,46	4
 BFR_53259_35	1,26	2	2	0	340,8	40,46	4
 BFR_53260_36	1,26	2	2	0	340,8	40,46	4
 BFR_54161_37	2,66	1	1	0	403,7	20,23	4

 BFR_54162_38	2,66	1	1	0	403,7	20,23	4
 BFR_54163_39	2,66	1	1	0	403,7	20,23	4

Appendix 6: Heat maps of (Q)SAR results


a b c d e f g h i j k l m n o p q r s t u v w x y
32102_96-13-9
32103_96-21-9
42104_106023-63-6
42105_19398-47-1
42106_79033-40-2
42107_4021-75-4
42108_87018-30-2
42209_35330-59-7
42210_14396-65-7
43111_855236-37-2
43112_87018-38-0
52113_105100-80-9
52114_213821-22-8
52115_408319-76-6
52116_159475-15-7
52117_343268-04-2
52118_76377-07-6
52119_59287-66-0
52120_1
52121_856991-78-1
52122_100606-66-4
52123_213821-20-6
52124_2
52125_3
52126_4
52127_98069-26-2
52128_5
52229_3296-90-0
52230_20
52231_21
52232_22
52233_23
52234_24
52235_25
52236_26
52237_44804-46-8
52238_27
52239_28
52240_29
53141_1522-92-5
53142_36483-57-5
53143_6
53144_7
53145_8
53146_9
53147_10
53148_11
53149_12
53150_13
53151_14
53152_15
53153_16
53154_17
53155_18
53256_31
53257_32
53258_33
53259_35
53260_36
54161_37
54162_38
54163_39

APPENDIX 6/FIGURE 1 HEAT MAP OF (Q)SAR PREDICTIONS OF CARCINOGINICITY AND GENOTOXICITY

DTU and commercial CASE Ultra (CU) (Q)SAR models:	
a	CU Ashby structural alerts
b	CU SALM, Salmonella mutagenicity (TA97,98,100,1535-1538) (*in vitro*)
c	DTU Ames Salmonella (TA98, 100, 1535 and either TA1537 or TA97) (*in vitro*)
d	DTU Ames sub-model Direct (S9 not required) (*in vitro*)
e	DTU Ames sub-model Potency > 10x ctrl. (*in vitro*)
f	DTU Ames sub-model Base-pair (*in vitro*)
g	DTU Ames sub-model Frame shift (*in vitro*)
h	CU Chromosomal aberrations CHO (*in vitro*)
i	DTU Chromosomal aberrations CHL (*in vitro*)
j	DTU UDS rat hepatocytes (*in vitro*)
k	DTU HGPRT (*in vitro*)
l	DTU SHE cell transformation (*in vitro*)
m	DTU SCE mouse (*in vivo*)
n	DTU Mouse micronucleus (bone marrow) (*in vivo*)
o	DTU Dominant lethal (*in vivo*)
p	DTU Drosophila SLRL (*in vivo*)
q	DTU Comet assay (*in vivo*)
r	CU FDA RCA cancer male rat (*in vivo*)
s	CU FDA RCA cancer female rat (*in vivo*)
t	CU FDA RCA cancer male mouse (*in vivo*)
u	CU FDA RCA cancer female mouse (*in vivo*)
v	CU FDA RCA cancer rodent (*in vivo*)
w	CU FDA RCA cancer rat (*in vivo*)
x	CU FDA RCA cancer mice (*in vivo*)
y	CU FDA RCA overall cancer call

APPENDIX 6/TABLE 1 EXPLANATION OF COLUMNS IN THE HEAT MAP OF (Q)SAR PREDICTIONS OF CARCINOGINICITY AND GENOTOXICITY. CU: CASE ULTRA, DTU: TECHNICAL UNIVERSITY OF DENMARK, FDA: U.S. FOOD AND DRUG ADMINISTRATION, RCA: RESEARCH COLLABORATION AGREEMENT

The heat map above shows (Q)SAR genotoxicity and carcinogenicity hits for the 61 category members with associated X and Y legend information in the table. Substances marked in bold originate from the initial group. Red means 'hit', white means 'not hit' and grey means 'out-of-the-applicability-domain'.

APPENDIX 6/FIGURE 2 HEAT MAP OF OECD (Q)SAR APPLICATION TOOLBOX PROFILER ALERTS

DNA binding by OECD	A1	Aliphatic halides
	A2	1,2-Dihaloalkanes
	A3	Mono aldehydes
	A4	Epoxides
	A5	Mustards
DNA binding by OASIS v.1.3	B1	Haloalkanes Containing Heteroatom

	B2	Haloalkane Derivatives with Labile Halogen
	B3	Vicinal Dihaloalkanes
	B4	Epoxides and Aziridines
	B5	Haloalcohols
Protein binding alerts for Chromosomal aberration by OASIS v1.1	C1	Halogenated Vicinal Hydrocarbons
	C2	Alpha-Activated Haloalkanes
In vitro mutagenicity (Ames test) alerts by ISS	D1	Aliphatic halogens
	D2	Simple aldehyde
	D3	Epoxides and aziridines
DNA alerts for AMES, MN and CA by OASIS v.1.3	E1	Haloalkane Derivatives with Labile Halogen
	E2	Vicinal Dihaloalkanes
	E3	Haloalcohols
	E4	Epoxides and Aziridines
In vivo mutagenicity (Micronucleus) alerts by ISS	F1	Aliphatic halogen
	F2	Epoxides and aziridines
	F3	H-acceptor-path3-H-acceptor
	F4	Simple aldehyde
Oncologic Primary Classification	G1	Aldehyde Type Compounds
	G2	Alpha, beta-Haloether Reactive Functional Groups
	G3	Reactive Ketone Reactive Functional Groups
	G4	Epoxide Reactive Functional Groups
Carcinogenicity (genotox and nongenotox) alerts by ISS	H1	(Poly) Halogenated Cycloalkanes (Nongenotox)
	H2	Aliphatic halogens (Genotox)
	H3	Epoxides and aziridines (Genotox)
	H4	Simple aldehyde (Genotox)
	H5	Substituted n-alkylcarboxylic acids (Nongenotox)

APPENDIX 6/TABLE 2 EXPLANATION OF COLUMNS IN THE HEAT MAP OF OECD (Q)SAR APPLICATION TOOLBOX PROFILER ALERTS

The heat map above shows the identified OECD (Q)SAR Application Toolbox profiler structural alerts hits for the 61 category members with associated X legend information in the table. Substances marked in bold originate from the initial group. The rows of the matrix represent structural alerts identified either in the parent compound or in one or more metabolites, and each associated with one or more possible mechanisms in the OECD (Q)SAR Application Toolbox individual alerts explanations provided in Appendix 6.

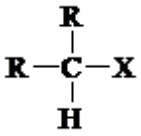
Appendix 7: OECD (Q)SAR Application Toolbox profiler alerts explanations

Table of contents

A	DNA Binding by OECD	2
B	DNA Binding by OASIS	10
C	Protein binding alerts for Chromosomal aberration by OASIS v1.1	36
D	In vitro mutagenicity (Ames test) alerts by ISS	43
E	DNA alerts for AMES, MN and CA by OASIS v.1.3	50
F	In vivo mutagenicity (Micronucleus) alerts by ISS	71
G	Oncologic Primary Classification	79
H	Carcinogenicity (genotox and nongenotox) alerts by ISS	83

A. Profiler: DNA Binding by OECD

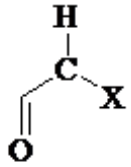
Structural alert: Aliphatic halide



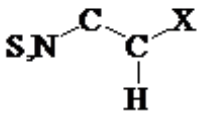
R = hydrogen, any carbon except the following:

X = Cl, Br, I

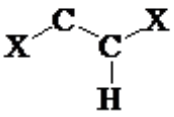
Excluded structures (alternative alert as shown):



α-halocarbonyls



mustards



1,2-dihaloalkanes

X = halogen

Mechanism

An S_N2 mechanism has been proposed as the primary method of DNA alkylation (Sobol et al 2007).

Nu = biological nucleophile

Structural alert mitigating factors

- The carbon being attacked by the biological nucleophile cannot be tertiary

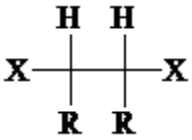
- Fluorine is excluded due to the strength of the C-F bond

- 1,2-dihaloalkanes are excluded as they react via an episulfonium ion

References

Sobol Z et al (2007) Mutation Research 633, 80-94

Structural alert: 1,2-Dihaloalkanes

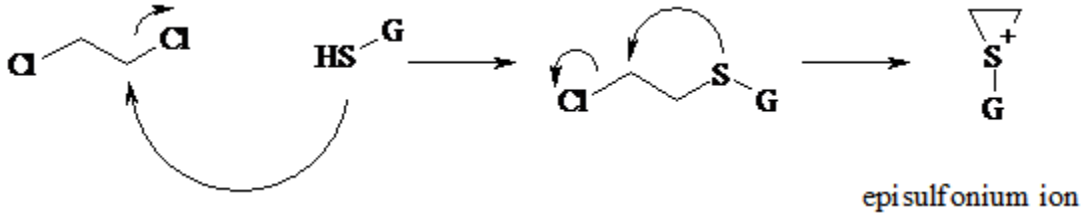


X = Cl, Br, I

R = hydrogen, sp3 carbon

Mechanism

It has been suggested that 1,2-dihaloalkanes undergo an initial attack by glutathione followed by internal cyclisation resulting in the formation of a reactive episulfonium ion. This ion can then undergo an S_N2 type ring opening reaction (Granville et al 2005).



episulfonium ion

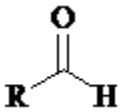
Structural alert mitigating factors

- The carbon being attacked by the biological nucleophile cannot be tertiary
- Fluorine is excluded due to the strength of the C-F bond

References

Granville CA et al (2005) Mutation Research, 572, 98-112

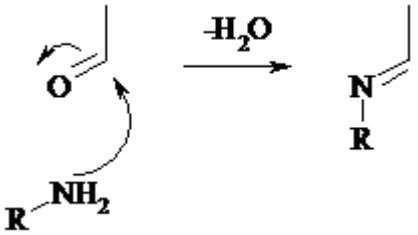
Structural alert: Mono-aldehydes



R = sp3 carbon, hydrogen

Mechanism

Mono aldehydes undergo Schiff base formation (Garcia et al 2009, Hecht et al 2001).



R = DNA chain

Sructural alert mitigating factors

- No mitigating factors have been reported for the chemicals in this mechanistic alert.

References

Garcia CL et al (2009) Mutation Research, 662, 3-9

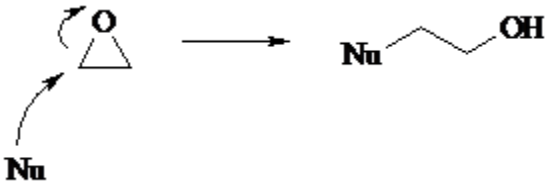
Mechanistic Alert: Direct Acting Epoxides and Related

Several structural alerts have been identified as being able to form DNA adducts via a ring opening S_N2 reaction. These structural alerts are as follows:

Structural alert: Epoxides Epoxides and related

Mechanism

Alkylation occurs via an S_N2 ring opening mechanism (Sawatari et al 2001).

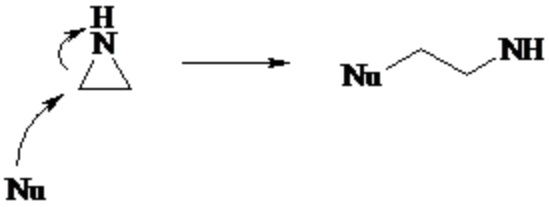


Nu = biological nucleophile

Structural alert: Aziridines

Mechanism

Alkylation occurs via an S_N2 ring opening mechanism (Sawatari et al 2001).

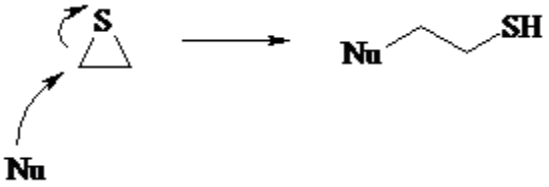


Nu = biological nucleophile

Structural alert: Sulfuranes

Mechanism

Alkylation occurs via an S_N2 ring opening mechanism (Sawatari et al 2001).



Nu = biological nucleophile

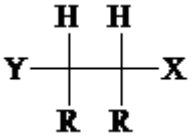
Mechanistic alert mitigating factors

- All structural alerts in this mechanistic alert: The carbon being attacked by the biological nucleophile cannot be tertiary

References

Sawatari KY et al (2001) Industrial Health, 39, p341-345

Structural alert: Mustards



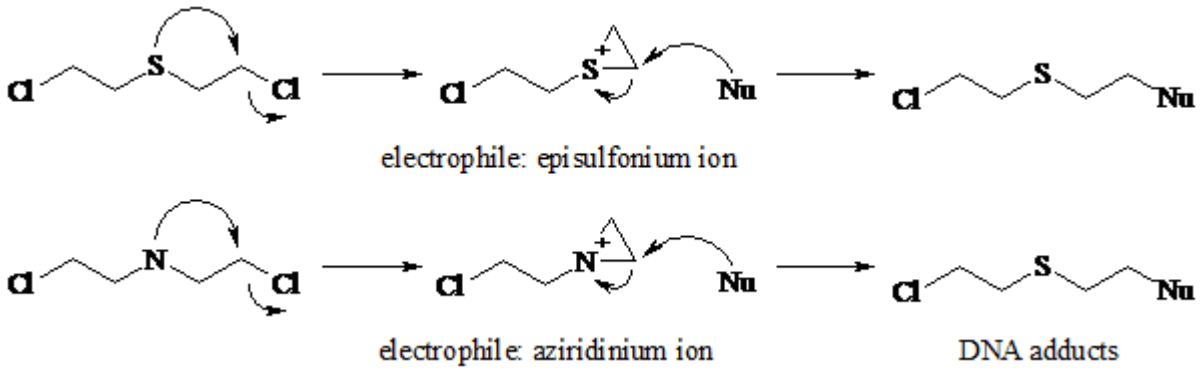
Y = nitrogen, sulphur (any oxidation state of sulphur is allowed as long as a lone pair remains free for the cyclisation reaction)

X = Cl, Br, I

R = sp3 carbon, hydrogen

Mechanism

Mustards have been suggested to undergo an intra-molecular cyclisation to form an electrophilic reactive episulfonium ion. The episulfonium ion is then susceptible to S_N2 attack by biological nucleophiles (Noll et al 2006, Smith et al 1995).



Nu = biological nucleophile.

Structural alert mitigating factors

- The carbon being attacked by the biological nucleophile cannot be tertiary
- Fluorine is excluded due to the strength of the C-F bond

References

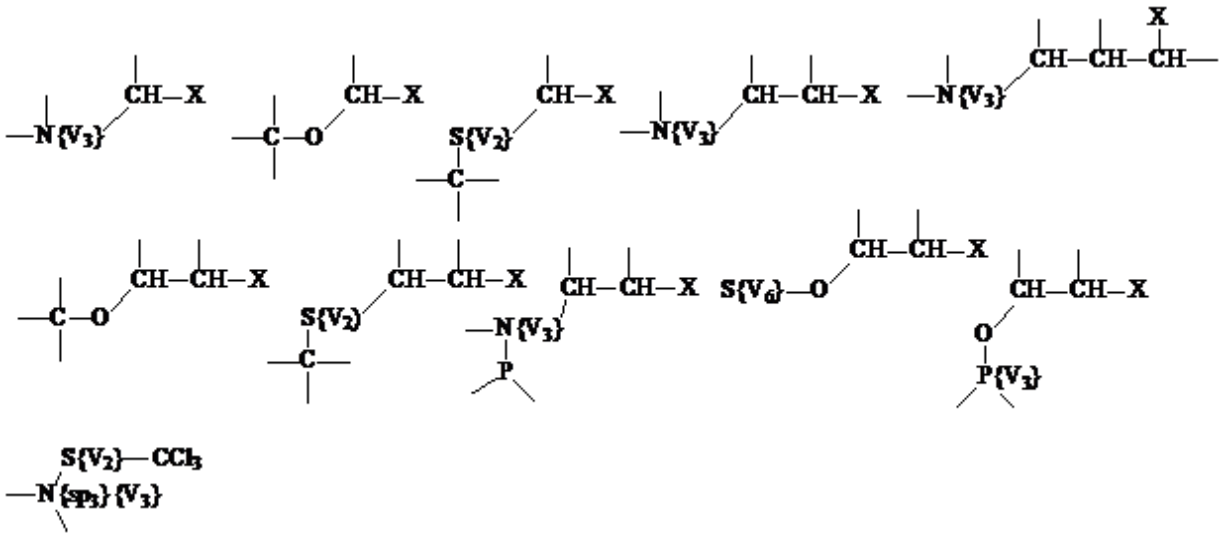
Noll DM et al (2006) Chemical Reviews, 106, 277-301

Smith KJ et al (1995) Journal of the American Academy of Dermatology, 32, 765-776.

B. Profiler: DNA Binding by OASIS

Haloalkanes Containing Heteroatom

Principal and characteristic active structural fragments:



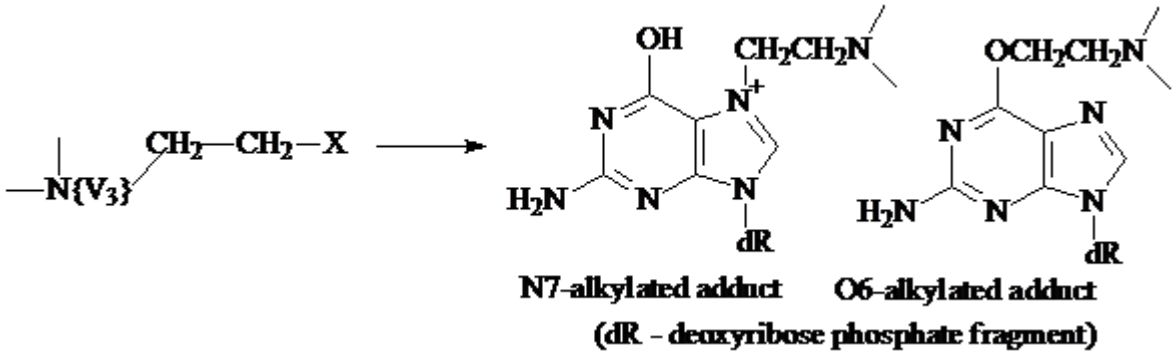
(X = Cl, Br, I)

Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, nucleophilic substitution at sp3 carbon atom

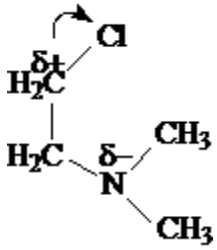
Mechanistic Domain: Radical
Mechanistic Alert: Generation of ROS by glutathione depletion (indirect)

A. Compounds with halogen at beta-position with respect to a heteroatom

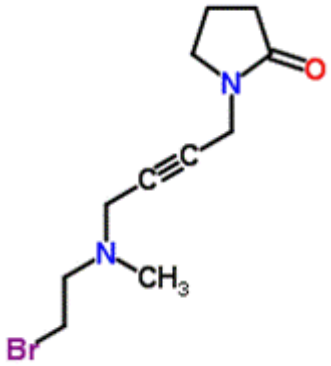
Mustards and beta-haloethers belong to this sub-class of compounds, and are alkylating agents. Generally, there is no need for metabolic activation, and the presence of labile halogen at α- or β-position with respect to a heteroatom in the open chain determines biological activity. The primary mechanism of action is an electrophilic attack on the nucleophilic sites of DNA. Most nitrogen and oxygen positions on DNA bases can be alkylated under appropriate conditions but guanine O6 or N7 (preferably), cytosine O2 and adenine N1 and N3 are the most vulnerable targets [1 - 3]. Some examples for the formation of such adducts with guanosine are given below:



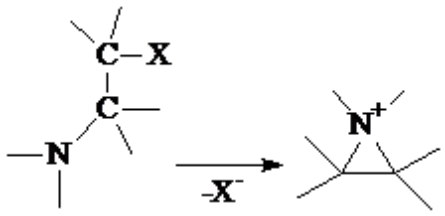
2-Chloro-N,N-dimethylethylamine is structurally similar to nitrogen mustards and possesses both the mutagenic and carcinogenic potency [4]. The stabilization of the transition state, responsible for the DNA alkylating activities can be outlined as follows:



On the other hand, 2-(β)-haloalkylamines such as the compound N-[4-(2-bromoethylmethylamino)-2-butynyl]-2-pyrrolidone:



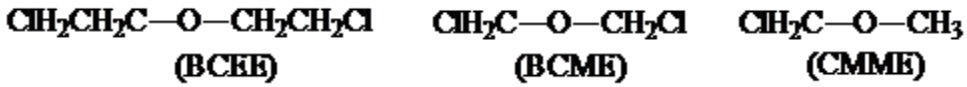
also show biological activity, due to a rapid cyclization to the pharmacologically active aziridinium ion, according to the following scheme:



Thus the genotoxicity of this compound when administered to mouse has been explained (see also ***Nitrogen Mustards***) [5].

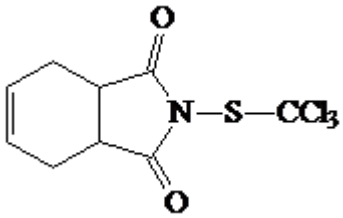
B. Compounds with Halogen in Alpha-Position with Respect to a Heteroatom

The general mechanism of action of such chemicals is believed to be similar to that of mustards and other direct-acting alkylating agents (see above). For example, bis(2-chloroethyl) ether (BCEE), bis(chloromethyl) ether (BCME) and chloromethyl methyl ether (CMME) are chemicals from a large class known as chloroalkyl ethers:

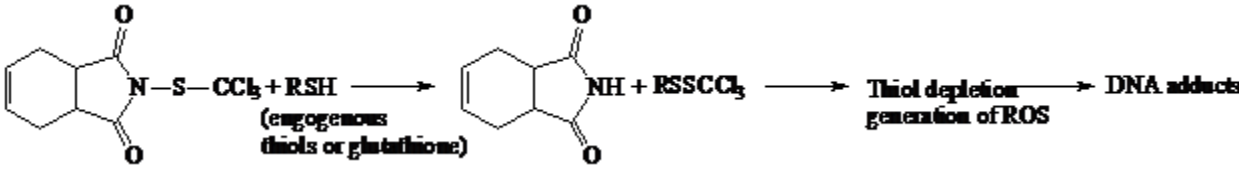


Carcinogenicity studies in experimental animals (mice and rats) exposed to, e.g., BCME have shown significantly elevated incidence of pulmonary adenomas and respiratory tumours. In mice, inhalation exposure also indicated evidence of lung tumours. The chemicals are mutagens in the *Ames* test [6]. The structure of bis(chloromethyl) ether predicts that it would be direct alkylating agent, which is consistent with its ability to react *in vitro* with DNA bases [7].

The fungicide captan:



takes specific position among this class of chemicals. It is likely to exert its mutagenicity as parent chemical [8] by different mechanism, i.e., probably by generation of reactive oxygen species (ROS) by thiols (glutathione) depletion [9, 10]:



REFERENCES

1. Kovacic, P., A. Cooksy, *Iminium Metabolite Mechanism for Nicotine Toxicity and Addiction: Oxidative Stress and Electron Transfer,* Medical Hypoth.**64** (2005), 104 - 111.

2. *Evidence on the Carcinogenicity of Technical Grade Bis(2-Chloro-1-Methylethyl) Ether*, Final November 1999 (Reproductive and Cancer Hazard Assessment Section Office of Environmental Health Hazard Assessment, California EPA; http://www.oehha.ca.gov/prop65/pdf/BCMEEf.pdf

3. Dacre, J. C., R. Beers, M. Goldman (Geo-Centers Inc. Newton Centre, MA), *Toxicology and Pharmacology of the Chemical Warfare Agent Sulfur Mustard – A Review* (1995); http://www.stormingmedia.us/72/7294/A729492.html

4. Theiss, J. C., M. B. Shimkin, L. A. Poirer, *Induction of Pulmonary Adenomas in Strain A Mice by Substituted Organohalides*, Canc. Res. **39** (1979), 391-395.

5. B. Ringdahl, D. J. Jenden**,** *Muscarinic Actions of an N-Methyl-N-2-Bromoethylamino Analog of Oxotremorine (BR 401) in the Mouse***,** Pharmacol. Exper. Ther. **240 (**2) (1987), 370-375.

6. *Selected Chloroalkyl Ethers*, World Health Organization, International Programme on Chemical Safety, Environmental Health Criteria 201, (1998); http://www.inchem.org/documents/ehc/ehc/ehc201.htm.

7. Van Duuren, B. L., *Carcinogenic Epoxides, Lactones, and Halo-Ethers and Their Mode of Action*, Ann. New York Acad. Sci **163** Biological Effects of Alkylating Agents No. 2 (1969), 633 – 650; http://onlinelibrary.wiley.com.sci-hub.org/doi/10.1111/j.1749-6632.1969.tb24883.x/pdf.

8. Ruiz, M. J., D. Marzin, *Genotoxicity of Six Pesticides by Salmonella Mutagenicity Test and SOS Chromotest*, Mutat. Res. **390** (1997), 245 – 255.

9. DeBaun, J. R., J. B. Miaullis, J. Knarr, *The Fate of N-Trichloro[^{14}C]methylthio-4-Cyclohexene-1,2-Dicarboxamide ([^{14}C]Captan) in the Rat*, Xenobiotica **4**(2) (1974), 101-119.

10. D. Morte, R., G. R. Villiani, E. Di Martino, C. Squillacioti, L. De Marco, P. Vuotto, M. A. Belisario, N. Staiano, *Glutathione Depletion Induced in Rat Liver by Seven Pesticides*, Boll. Soc. Ital. Biol. Sper. **70**(8-9) (1994), 185 – 192 (Abtsract); http://www.ncbi.nlm.nih.gov/pubmed/7893475.

Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, nucleophilic substitution at sp3-carbon atom

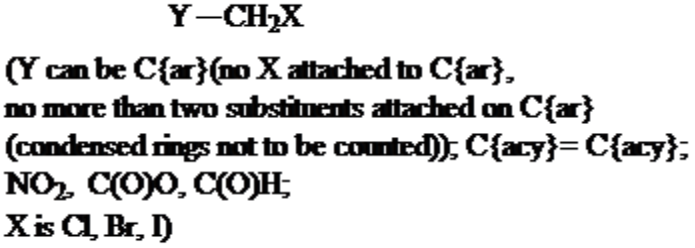
Mechanistic Domain: A_N2
Mechanistic Alert: Shiff base formation for aldehydes

Mechanistic Domain: S_N2
Mechanistic Alert: Acylation involving a leaving group

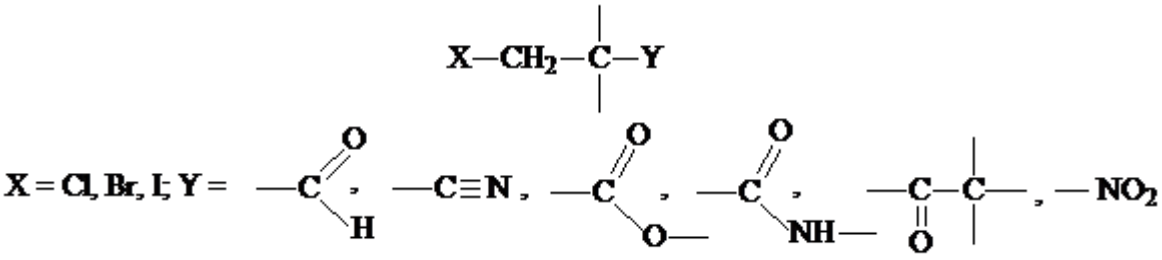
Structural alert: Haloalkane Derivatives with Labile Halogen

Principal and characteristic active structural fragments:

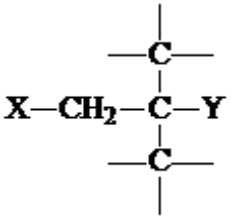
A. Primary haloalkane derivatives with labile halogen at alpha-position towards other groups:



B. Primary haloalkane derivatives with labile halogen at beta-position towards other groups:



"Mask":



Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, nucleophilic substitution at sp3-carbon atom

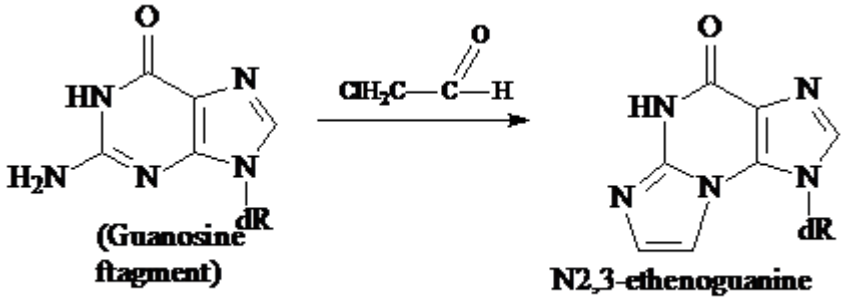
Mechanistic Domain: A_N2
Mechanistic Alert: Shiff base formation for aldehydes

Mechanistic Domain: S_N2
Mechanistic Alert: Acylation involving a leaving group

A. Haloalkane derivatives with labile halogen at alpha-position towards other groups

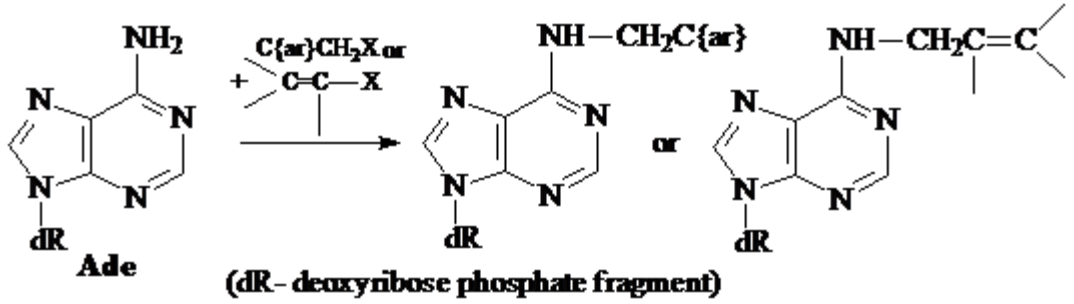
Many of these compounds represent disinfection by-products, some of which are rather toxic. They include halonitriles, some halocarbonyl- and halocarboxyl-compounds, halonitroalkanes, etc. Short-chain monohalogenated alkanes and their derivatives, containing such electron-withdrawing substituents are potential direct-acting alkylating agents, particularly if the halogen is at the terminal end of the carbon chain or at allylic (benzylic) position (primary halogenoalkane derivatives). For example, acetonitrile is not carcinogenic in rodents and is only weakly or marginally mutagenic. For halogenated acetonitriles, positive mutagenicity is expected, due to the strong –I effect of the neighboring nitrile group, however, bacterial toxicity hinders the expression of positive mutagenic potential.

Generally, the introduction of halogen to alpha- or terminal carbon atoms with the above-mentioned structural neighbors is expected to increase the genotoxic potential by making it an alkylating and/or cross-linking agent. On the basis of the alkylating activity, the brominated compounds are expected to be more reactive than chlorinated ones, and iodoacetic acid is the most toxic and genotoxic disinfection by-product in mammalian cells reported [1, 3]. For example, bromoacetic acid is far more mutagenic than the chloroacetic acid [2]. The toxicity of brominated and chlorinated acetic acids, however, decreases as the number of halo-substituents increases [4]. Haloaldehydes with mono-substitution at the alpha- or terminal carbon atom are also expected to be potential alkylating agents [1]. For example, the reaction of the strong mutagen, chloroacetaldehyde, a reactive metabolite of the carcinogenic vinyl chloride (and bifunctional compound) with DNA produces 1,N6-ethenoadenine, 3,N4-ethenocytosine and, particularly, N2,3-ethenoguanine [5]. Therefore, if the substituent Y represents the reactive aldehyde (e.g., formyl) group), the following scheme of formation of one of the DNA adducts has been proposed [6]:



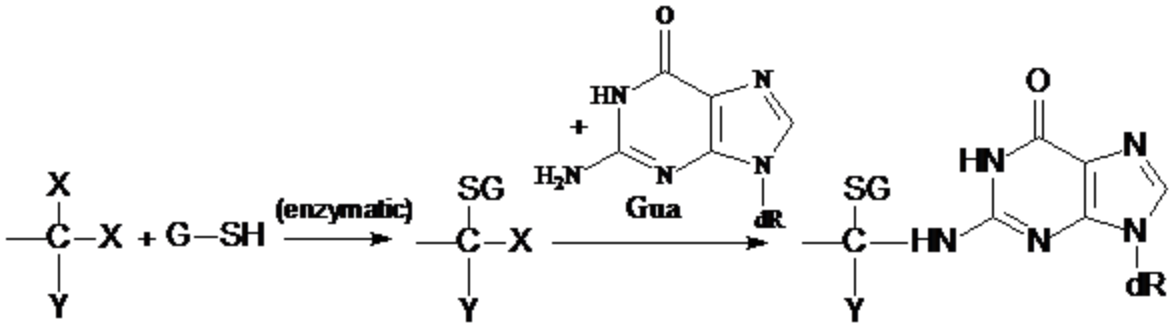
In this case, the reactivities of both the formyl and chlorine functional groups have been involved in the formation of the DNA adduct.

If Y is allylic or benzylic moiety, direct attack on DNA has also been suggested [7, 8]:



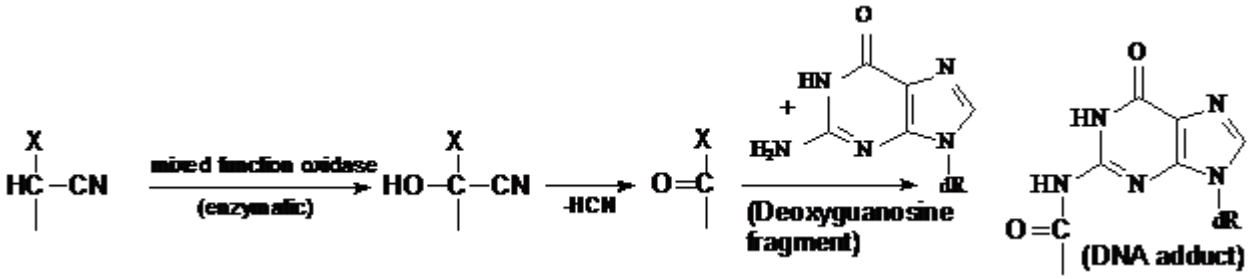
Unfortunately, little information has been available, concerning directly formed DNA adducts for the cases, where Y represent $–NO_2$, –COOH/COOR, –CN and other electron-withdrawing substituents. Chloroacetonitrile has been proposed to possess direct-acting DNA-alkylating capability, which does not manifest itself to bacterial mutagenicity, due to cytotoxicity. However, metabolic activation is also possible [9]. As far as benzyl- and allyl halides are concerned, which also belong to this class of compounds, benzyl chloride has been found to show mutagenicity in the *Salmonella*/microsome mutagenesis assay [17]. On the other hand, allyl halides such as allyl chloride and allyl bromide showed positive results in the *Ames* mutagenicity assay and have established DNA alkylating properties [18 - 20]. Similarly to the mechanistic schemes outlined above, direct attack on the purine/pyrimidine bases [18, 19] ("alkylation") has been suggested. For these compounds, minimum steric hindrance effects are important in order to show direct mutagenicity.

Bromonitromethane and chloronitromethane have been found to be mutagenic in the *Salmonella typhimurium* bioassay in the presence of S9 system. The halonitromethanes (e.g. chloropicrin) are significantly more potent mutagens and toxicants than the dihalomethanes in the *Salmonella typhimurium TA100* preincubation assay, and conjugation with glutathione is also involved [10, 11]. The following mechanism for the glutathione-dependent (enzymatic) bioactivation and attack on DNA could be suggested, by analogy with the published data [11]:

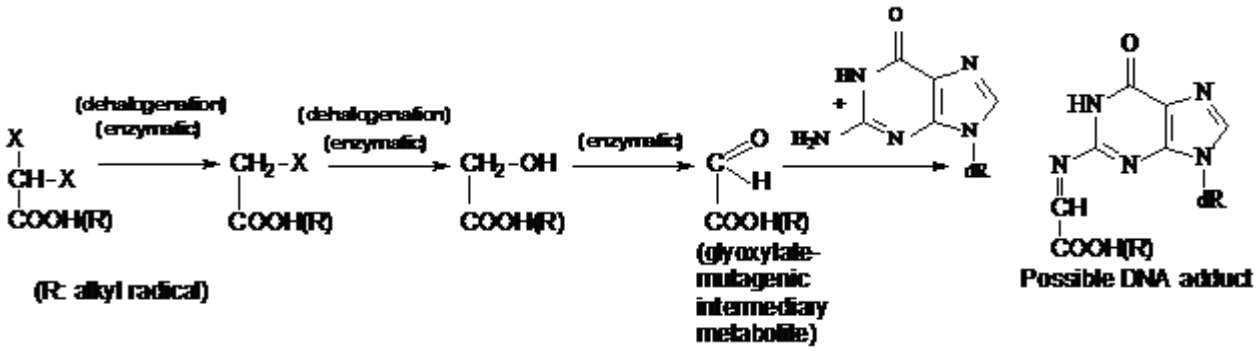


Halogenated acetonitriles, on the other hand, can be metabolized *via* oxidative dehalogenation, and this first step of the biotransformation is catalyzed by a mixed function oxidase such as cytochrome P450. It has been proposed that halocyanoalcohols formed by the

oxidative dehalogenation are converted to haloformaldehydes (formyl halides), which are mutagenic metabolites [1, 9]:

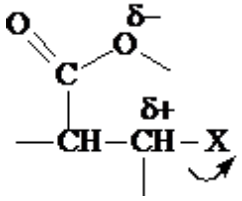


Monobromoacetic acid was evaluated as mutagenic in *Salmonella typhimurium* [12]. Possible metabolic transformation of haloacetic acids such as dichloroacetic acid takes place *via* dehalogenation and one of the intermediary metabolites is glyoxylate, which is mutagenic. The primary metabolic pathway for dichloroacetic acid involves oxidative dechlorination to form glyoxylate. This reaction, once thought to be microsomal, Cytochrome P-450 mediated, has now been shown to be NADPH- and GSH-dependent, and occurs predominantly in the cytosol [13, 14]. Based upon the above considerations, the following scheme for possible bioactivation can be expertly assumed for haloacetates:

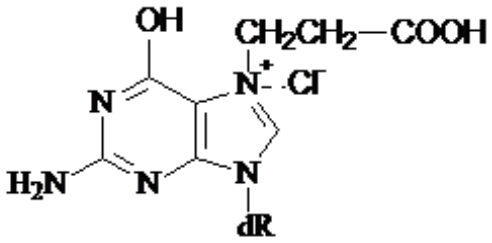


B. <u>Haloalkane derivatives with labile halogen at beta-position towards other groups</u>

These chemicals are assumed to be direct-acting mutagens, eliciting mutagenicity by alkylation of DNA fragments. For example, 3-chloropropionic acid binds DNA in mouse skin. Mutagenicity has also been suggested for methyl 3-bromopropionate, 3-chlorobutanoic acid, 3-chloropropanoic acid, etc. These compounds contain a nucleophilic center such a carboxyl ester group in beta-position with respect to the halogen. In each case, a conformation may be envisioned, in which the nucleophilic substituent stabilizes the transition state of the biological alkylating agent and the possible alkylation of DNA sub-fragments is facilitated [15]:

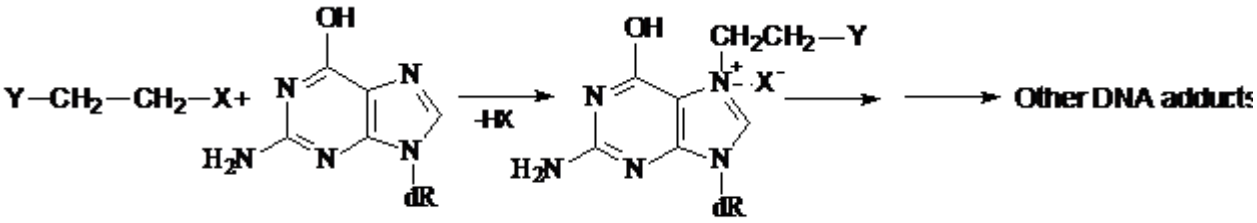


7-Carboxyethylguanine was suggested a possible DNA adduct for 3-chloropropanoic acid [16]:



(N7-Carboxyethylguanine fragment)

The following scheme for formation of DNA adducts can be expertly suggested:



(Deoxyguanosine fragment) (N7-Alkylated DNA adduct)

REFERENCES

1. Woo, Y. T., D. Lai, J. L. McLain, M. Ko Manibusan, V. Dallarco, *Use of Mechanism-Based Structure-Activity Relationships in Carcinogenic Potential Ranking for Drinking Water Disinfection By-Products*, Environ. Health Persp. **110** (2002), 75 – 87.
2. Kargalioglu, Y., B. J. McMillan, R. A. Minear, M. J. Plewa, *Analysis of the Cytotoxicity and Mutagenicity of Drinking Water Disinfection By-Products in Salmonella typhimurium*, Teratog. Carcinog. Mutag. **22**(2) (2002), 113-128; http://onlinelibrary.wiley.com/doi/10.1002/tcm.10010/abstract;jsessionid=BFC80F2B0989BAF938AE8DA5D8B15DDE.d01t01.

3. Plewa, M. J., E. D. Wagner, S. D. Richardson, A. D. Thruston, Y. T. Woo, A. B. McKague, *Chemical and Biological Characterization of Newly Discovered Iodoacid Drinking Water Disinfection By-Products*, Environ. Sci Technol. **38**(18), 2004, pp. 4713-4722; http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=86035.

4. Giller, S., F. Le Curtieux, F. Erb, D. Marzin, *Comparative Genotoxicity of Halogenated Acetic Acids Found in Drinking Water*, Mutagenesis **12**(5) (1997), 321 - 328.

5. Oesch, Fr., G. Doerjer, *Detection of N2, 3-Ethenoguanine in DNA After Treatment With Chloroacetaldehyde In Vitro*, Carcinogenesis **3** (6) (1982), 663 – 665; http://carcin.oxfordjournals.org/content/3/6/663.abstract.

6. Cheng, K. C., B. D. Preston, D. S. Cahill, M. K. Dosanjuh, B. Singer, L. A. Loeb, *The Vinyl Chloride DNA Derivative N2,3-Ethenoguanine Produces G → A Transitions in Escherichia Coli*, Proc. Natl. Acad. Sci USA **88** (1991), 9974 - 9978.

7. Fall, M., H. Haddouk, J. P. Morin, R. Forster, *Mutagenicity of Benzyl Chloride in the Salmomella/Microsome Mutagenesis Assay Depends on Exposure Conditions*, Mutat. Res. 633(1) (2007), 13 – 20; http://www.ncbi.nlm.nih.gov/pubmed/17631040

8. Eder, E., D. Henschler, T. Neudecker, *Mutagenic Properties of Allylic and Alpha,beta-Unsaturated Compounds: Consideration of Alkylating Mechanisms* Xenobiotica **12**(12), 1982, 831-848; http://pubget.com/paper/6763406.

9. Lin, E. L. C., F. B. Daniel, S. L. Herren-Freund, M. A. Pereira, *Haloacetonitriles: Metabolism, Genotoxicity, and Tumor-Initiating Activity*, Environ. Health Persp. **69** (1986), 67 – 71.

10. Kundu, B., S. D. Richardson, P. D. Swartz, P. P. Matthews, A. M. Richard, D. M. DeMarini, *Mutagenicity in Salmonella of Halonitromethanes: A Recently Recognized Class of Disinfection By-Products in Drinking Water*, Mutat. Res. **562**(1-2) (2004), 39-65.

11. Schneider, M., G. B. Quistad, J. E. Casida, *Glutathione Activation of Chloropicrin in the Salmonella Mutagenicity Test*, Mutat. Res. **439**(2) (1999), 233 - 238.

12. Brominated Acetic Acids in Drinking Water (Background Document for Development of WHO Guidelines for Drinking Water Quality, WHO/SDE/WSH/03.04/79 (2004); http://www.who.int/water_sanitation_health/dwq/chemicals/brominatedaceticacids.pdf

13. *Toxicological Review of Dichloroacetic Acid (CAS No. 79-43-6)*, In Support of Summary Information on the Integrated Risk Information Systme (IRIS), US EPA, Washington DC, August 2003; http://www.epa.gov/iris/toxreviews/0654tr.pdf.

14. *Monochloroacetic Acid in Drinking Water* (Background Document for Development of WHO Guidelines for Drinking Water Quality), WHO/SDE/WSH/03.04/85, WHO, 2004; http://www.who.int/water_sanitation_health/dwq/chemicals/monochloroaceticacid.pdf.

15. Theiss, J. C., M. B. Shimkin, L. A. Poirer, *Induction of Pulmonary Adenomas in Strain A Mice by Substituted Organohalides*, Canc. Res. **39**, 1979, 391 - 395.

16. Colburn, N. H., R. K. Boutwell, *The Binding of Beta-Propiolactone and Some Related Alkylating Agent to DNA, RNA, and Protein of Mouse Skin; Relation Between Tumor-Initiating Power of Alkylating Agents and Their Binding to DNA*, Canc. Res. **28** (1968), 653 – 660.

17. Fall, M., H. Haddouk, J. P. Morin, R. Fortun, *Mutagenicity of Benzyl Chloride in the Salmonella/Microsome Mutagenesis Assay Depends on Exposure Conditions*, Mutat. Res. 633(1) (2007), 13 – 21; http://www.ncbi.nlm.nih.gov/pubmed/17631040.

18. *Allyl Bromide CAS No. 106-95-6, CSWG Evaluation* (12/16/94); http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/AllylBromide.pdf

19. Eder, E., D. Henschler, T. Neudecker, *Mutagenic Properties of Allylic and Alpha,beta-Unsaturated Compounds: Consideration of Alkylating Mechanisms* Xenobiotica 12(12), 1982, 831-848; http://pubget.com/paper/6763406.

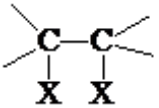
20. McCoy, E. C., L. Burrows, H. S. Rosenkranza, *Genetic Activity of Allyl Chloride*, Mutat. Res./Fund. Molec. Mechan. Mutag. 57(1) (1978), 11 – 15; http://www.sciencedirect.com/science/article/pii/0027510778902294.

Mechanistic Domain: S_N2
Mechanistic Alert: Internal S_N2 reaction with aziridinium and/or cyclic sulfonium ion formation (enzymatic)

Mechanistic Domain: S_N2
Mechanistic Alert: DNA alkylation

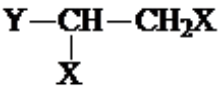
Structural alert: Vicinal Dihaloalkanes

Principal structural fragments:



(X = Cl, Br, I

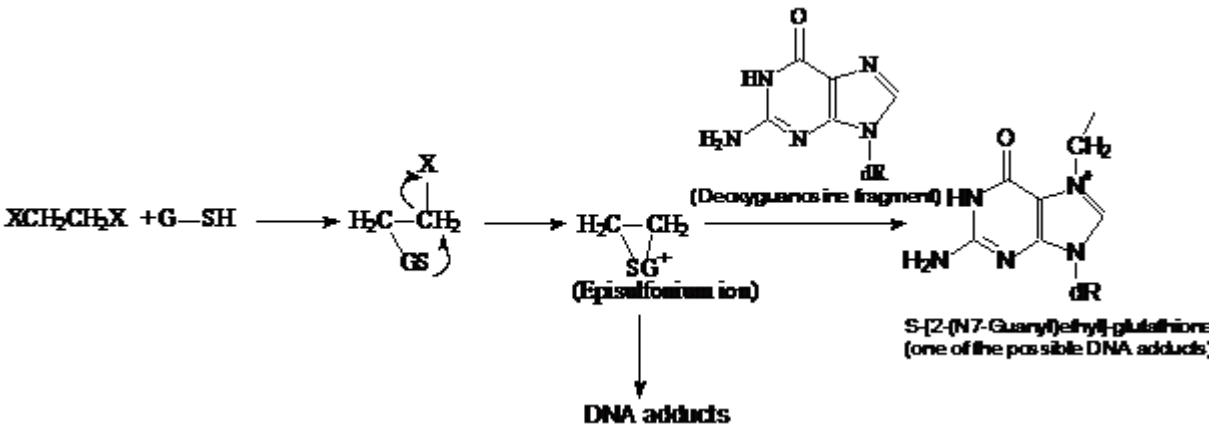
Characteristic active fragments:



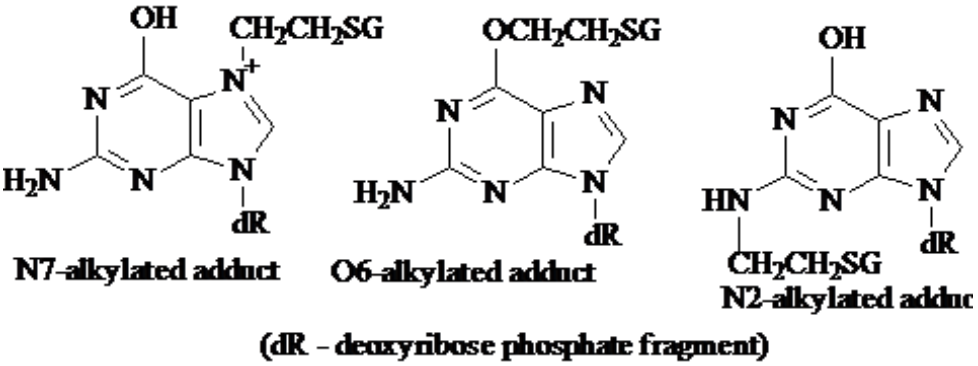
(Y is –H, $-(CH_2)_nH$ (n = 1, 2), $-O(CH_2)_nH$ (n = 0 -2), $-CH_2-O-$, C{acy}{sp2};
No other halogens bound to Y)

In vitro metabolic activation (bioactivation) is believed to be the main mutagenicity-eliciting process *via* glutathione-mediated generation of electrophilic species.

For example, 1,2-dichloroethane is reasonably anticipated to be a human carcinogen, based on sufficient evidence of carcinogenicity in experimental animals. *In vivo* and *in vitro* studies in rodents have revealed that the primary metabolic pathway for 1,2-dichloroethane probably involves conjugation with glutathione, and the compound shows bacterial mutagenicity. This is S_N2 (bimolecular nucleophilic attack) of glutathione GSH on the electron-deficient carbon of 1,2-dichloroethane (also for 1,2-dibromoethane, 1,2-dichloropropane, etc.) and S-(2-chloroethyl)-glutathione adduct is formed. One of the further possible metabolic pathways is the loss of chloride ion with the formation of *episulfonium ion*, which is highly reactive. This ion is believed to be the reactive *electrophilic* intermediate that results in covalent reaction with biopolymers such as DNA, and is believed to determine the mutagenic potential of this class of organic halides [1 – 4, 6]:

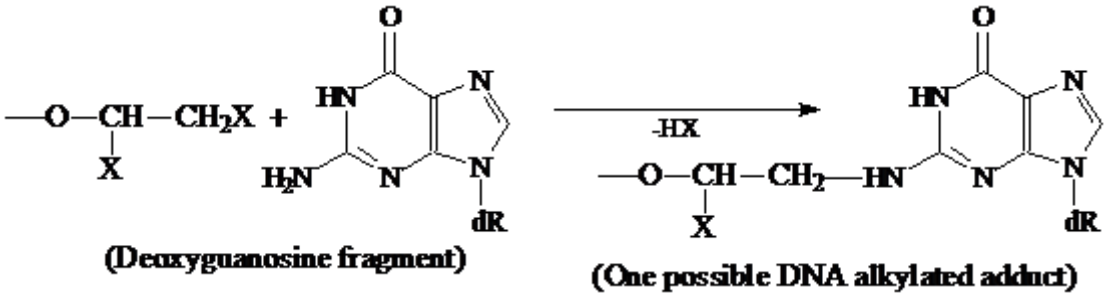


The major product of this reaction is *S*-[2-(*N*^{7}-guanyl)ethyl]glutathione, but *N*^{2}- and *O*^{6}-guanyl adducts are also formed, and all three adducts are potentially mutagenic [3]:



Similar mechanism of *in vitro* metabolic activation by forming episulfonium cation as reactive intermediate has also been suggested for structurally similar short-chain compounds such as 1,2-dibromo-3-chloropropane [5].

Beside 1,2-dichloroethane, 1,2-dibromoethane belonging to this class of compounds was also found to possess bacterial mutagenicity [7]. Short-chain vicinal dihalolakanes with halogen attached to terminal carbon atom are assumed to act by direct alkylation mechanism, too. Other short-chain vicinal haloalkane derivatives with electron-withdrawing heteroatoms adjacent to the –CHX fragment such as 1-methoxy-1,2-dichloroethane, 2,3-dibromo-propanol, etc., are believed to cause also direct mutagenicity by alkylation mechanism:



REFERENCES

1. Anders, M. W., *Glutathione-Dependent Bioactivation of Haloalkanes and Haloalkenes*, Drug Metabol. Rev. **36** (3 – 4) (2004), 583 – 594.

2. *Public Health Goal for 1,2-Dichloropropane in Drinking Water*, Office of Environmental Health Hazard Assessment, California EPA, February 1999;

   http://www.oehha.ca.gov/water/phg/pdf/12dcp_f.pdf.

3. Guengerich, F. P., L. A. Peterson, J. L. Cmarik, N. Koga, Ph. B. Inskeep, *Activation of Dihaloalkanes by Glutathione Conjugation and Formation of DNA Adducts*, Environ. Health Persp. **76** (1987), 15 – 18.

4. Liu, L., A. E. Pegg, K. M. Williams, F. P. Guengerich, *Paradoxal Enhancement of the Toxicity of 1,2-Dibromoethane by O6-Alkylguanine-DNA Alkyltransferase*, J. Biol. Chem. **277** (40) (2002), 37920 - 37928.

5. Miller, G. E., M. J. Brabec, A. P. Kulkarni, *Mutagen Activation of 1,2-Dibromo-3-Chloropropane by Cytsololic Glutathione S-Transferases and Microsomal Enzymes*, J. Toxicol. Environ. Health: Current Issues **19**(4) (1986), 503 – 518.

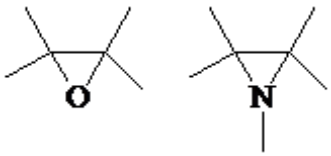
6. Rannug, U., A. Sundvall, C. Ramel, *The Mutagenic Effect of 1,2-Dichloroethane on Salmonella Typhimurium I. Activation Through Conjugation with Glutathion In Vitro*, Chem.-Biol. Interact. **20** (1978), 1 – 16.

7. Strubel, K., T. Grummt, *Aliphatic and Aromatic Halocarbons as Potential Mutagens in Drinking Water. III. Halogenated Ethanes and Ethenes*, Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128.

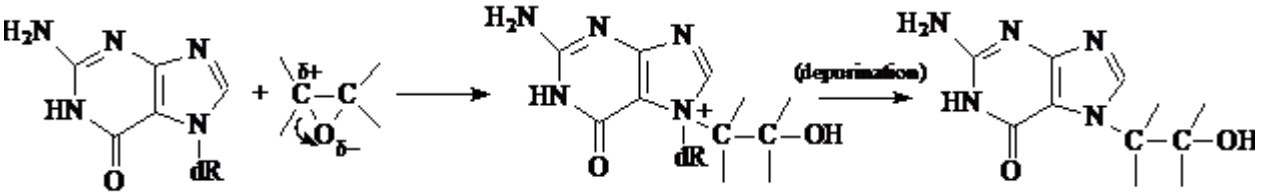
Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, direct acting epoxides and related

Structural alert: Epoxides and Aziridines

Principal structural fragments:

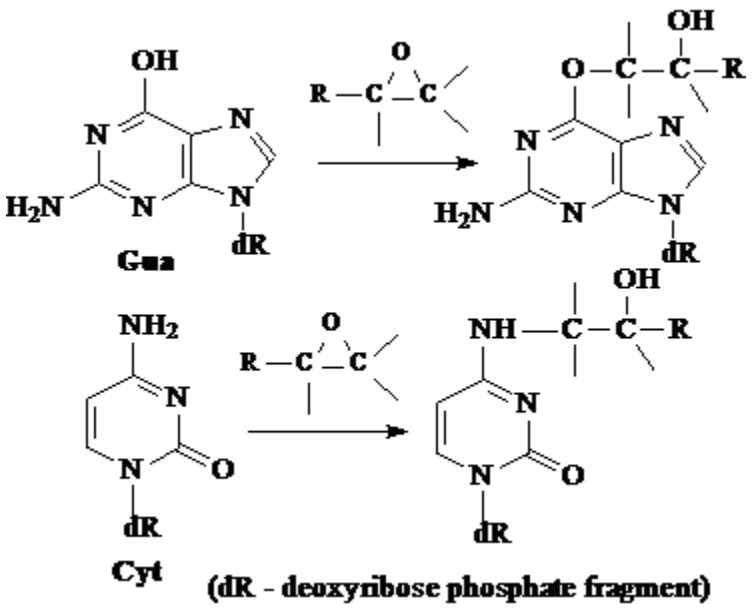


Mono-substituted epoxides have two elecrophilic carbon atoms (α- and β-carbons). The site of nucleophilic attack on alkyl epoxides under physiological conditions occurs principally at the less substituted, sterically more accessible β-carbon atom. However, epoxides with vicinal aromatic or vinyl group such as styrene epoxide and butadiene monoepoxide can react through both C-atoms, due to substituent effects leading to increase of positive charge on the α-carbon. Under physiological conditions, the main alkylation sites for simple alkyl epoxides are N7 guanine, N1-and N3-adenine, and N3-cytosine, since these heterocyclic ring nitrogens are the most nucleophilic sites for attack. Therefore, for the epoxide structural fragments, *direct alkylation* mechanisms have been proposed. Epoxides such as ethylene oxide, propylene oxide and glycydol are known carcinogens that are widely used in industrial chemistry. Mutagenic and carcinogenic epoxides can also be formed metabolically from alkenes such as ethylene, butadiene, propylene and styrene and from vinyl monomers like acrylonitrile and acrylamide. Simple epoxides react with nucleic acid bases to form 2-hydroxy-2-alkyl adducts. These are fairly unstable, due to the presence of a charged quaternary nitrogen at the site of alkylation and frequently undergo "depurination" to remove the charge. This leads to the formation of highly mutagenic sites. The final form of adduct is uncharged and stable. It is mutagenic and contributes to the toxicological hazards of exposure to simple epoxides [1]:

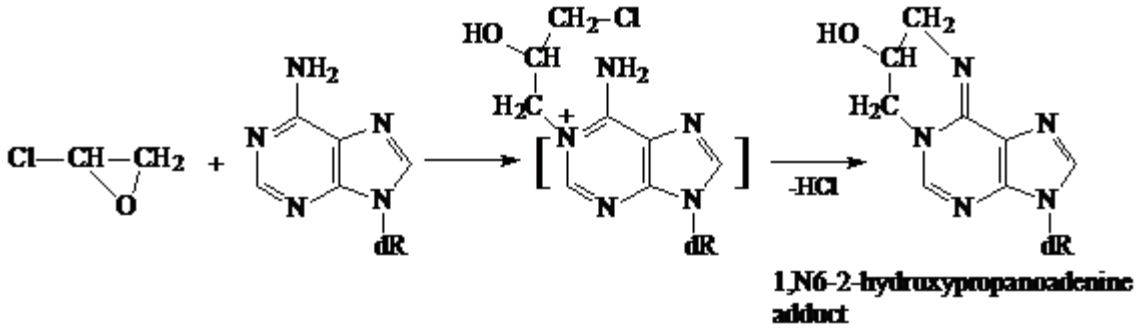


(DNA fragment)
(dR - deoxyribose phosphate fragment)

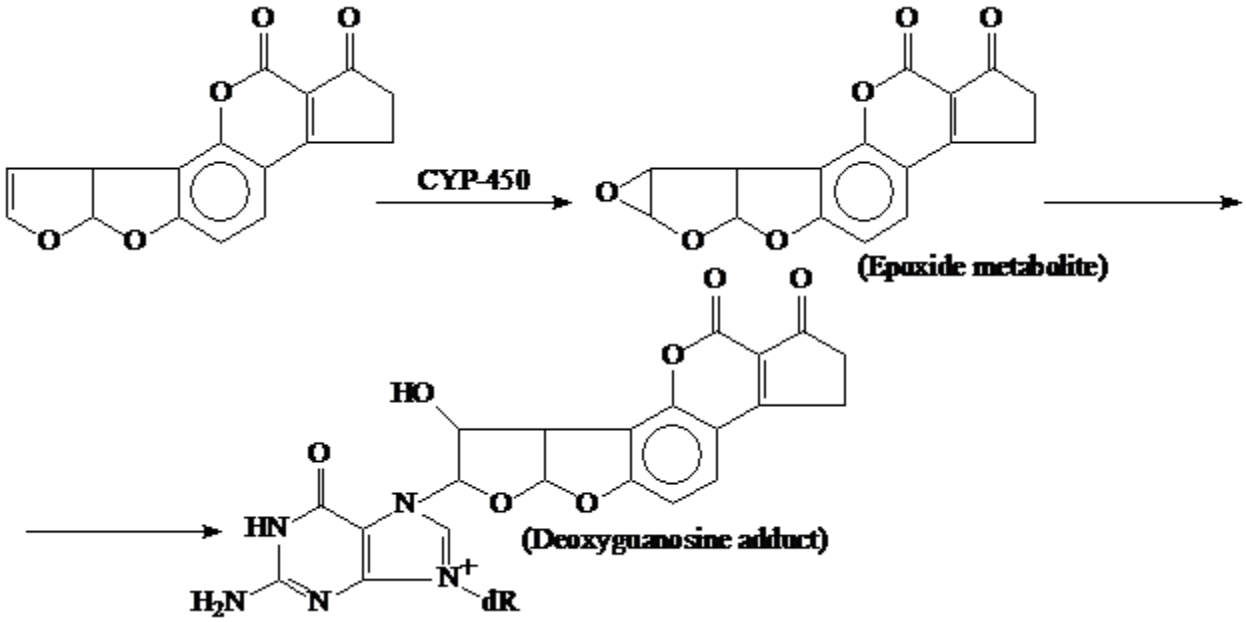
Epoxides such as styrene oxide and butadiene monoepoxide can also modify exocyclic groups as shown below [1]:



However, epichlorohydrin, unlike many other epoxides, acts as bifunctional alkylating agent. For alkylation of adenine, for example, the epoxide undergoes first ring opening; then cyclization and loss of HCl *via* the attack on N6, and the carbon, carrying chlorine contributes to the formation of 1,N6-2-hydroxypropanoadenine adduct [2]:



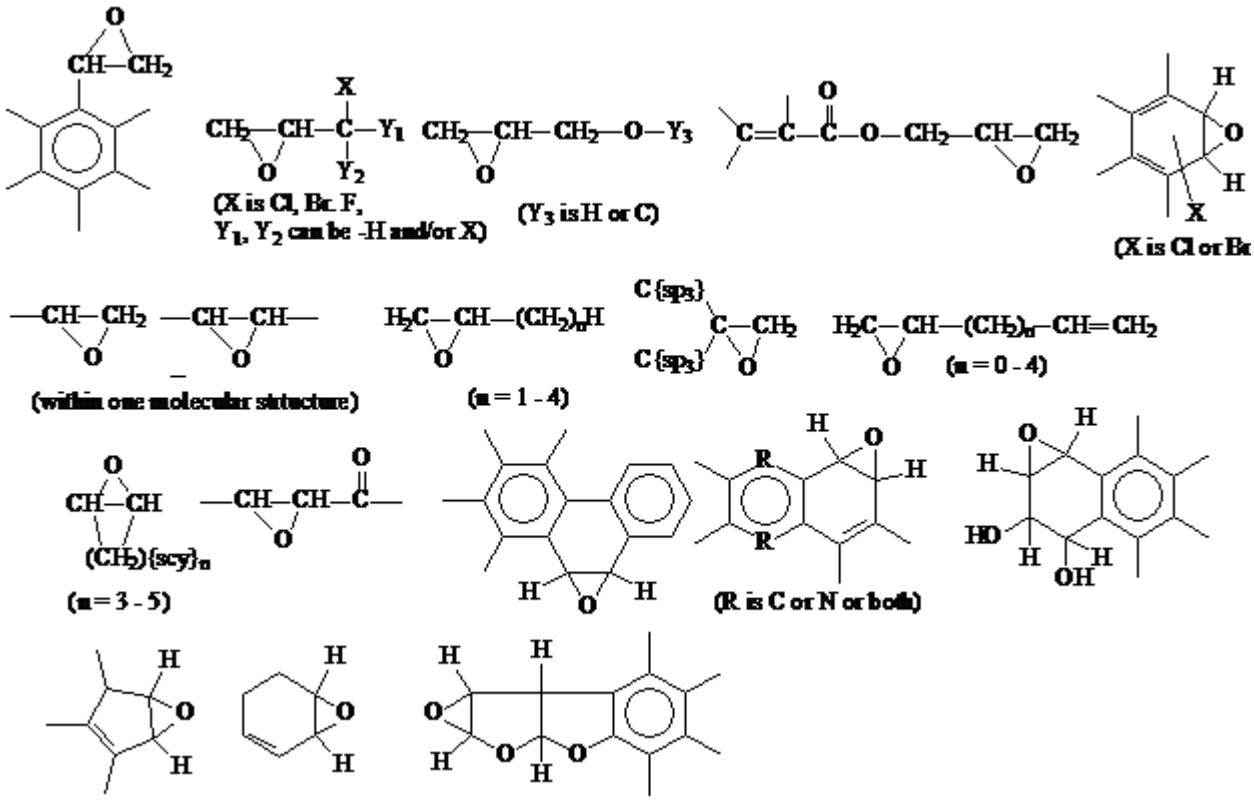
In substructures, about which more than ten test results have been reported, the epoxide fragment is most highly correlated with mutagenicity, and the percentage of positive compounds was found to be 65 – 70. Therefore, compounds that contain or are metabolically activated to products with the highly-strained epoxide fragment in the structure can be considered as suspect carcinogens [3]. For example, the well-known carcinogen (and bacterial mutagen) Aflatoxin B1 is metabolically activated to aflatoxin B1 exo-8,9-epoxide by CYP 450 monooxygenase, which then reacts with N7 of guanine to form the primary DNA adduct [4]:



The mutagenicity of a series of 13 epoxide compounds has been studied. Mono-substituted epoxides such as allyl glycidyl ether, n-butyl glycidyl ether, vinyl cyclohexene diepoxide, glycidol, glycidaldehyde, diglycidyl ether, diepoxybutane and diglycidyl ether of substituted glycerine were mutagenic in TA100 strain. The mutagenic compounds had linear structure, without any groups causing steric hindrance. On the contrary, dieldrin was inactive, due to its relatively high molecular weight, specific molecular structural envelope, and, possibly, large number of chlorine atoms causing excessive hydrophobicity. Higher-molecular weight derivatives of diglycidyl bisphenol-A were also negative in the *Ames* bacterial mutagenicity test, due to the high molecular mass and branched structure [5]. Similar results, associated with the molecular structure of epoxides were obtained, according to another publication, dealing with 45 epoxides of large structural diversity, of which mono-substituted epoxides such as epichlorohydrin, epibromohydrin, 1,2-epoxibutane, propylene oxide, glycidol, glycidaldehyde, 1,2,3,4-diepoxybutane, styrene oxide, etc. produced positive *Ames* test results [6].

According to another publication, halogenated derivatives of simple monosubstituted epoxides such as epichlorohydrin and epibromohydrin had much stronger DNA alkylating capability and bacterial mutagenicity than propylene oxide, butylene oxide, etc. The electron-withdrawing effects of the haloalkyl groups in the halogenated derivatives apparently contributes to higher epoxide reactivity [7].

The *in vitro* genotoxicity of 51 epoxides has been studied by employing the *Ames* test with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. Approximately, 75 – 80 % of all tested epoxides (mostly mono-substituted ones) were found to show positive bacterial mutagenicity [8], and their principal sub-structures are given below:

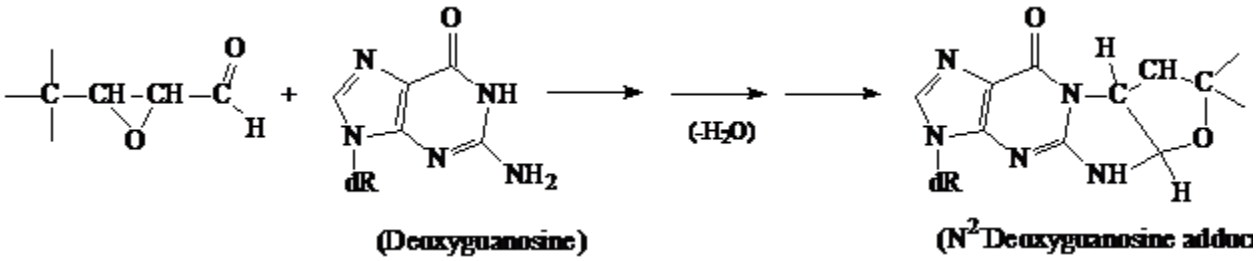


1,2-Disubstituted cycloaliphatic epoxides with one or two oxirane ring fragments such as cyclopentane- and cyclohexane oxide, diepoxycyclooctane, vinylcyclohexane dioxide, cyclopentene- and cyclohexene-3,4-epoxides, etc., were also found to show, at least, weak bacterial mutagenicity effects in the base-pair indicator strains TA100 and TA1535 [9]. Some 1,1- and 1,2-disubstituted epoxides such as 2,3-epoxybutane and oxaspiro-epoxides have also shown *Ames* mutagenicity, and the structural requirements associated with minimal steric hindrance in the vicinity of epoxide group fully apply [10, 11].

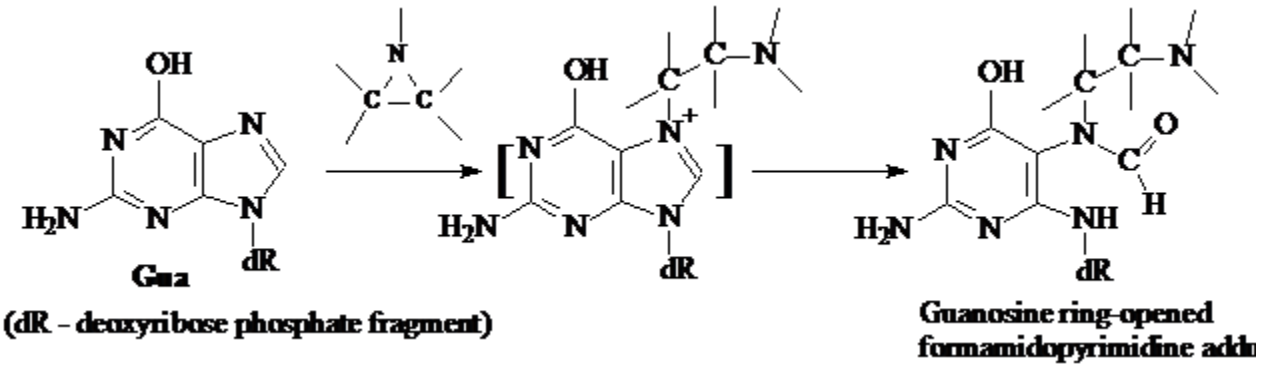
According to another publication, none of the tested chemicals such as epoxysteroids, vitamin K epoxides, pesticides (dieldrin, eldrin, heptachlor epoxides), and, also, some natural products, metabolites and antibiotics such as oleandomycin, anticapsin and asperlin, carbamazepine-9,10-oxide, diethylstilbestrol-α,β-oxide, scopolamine, etc. showed any mutagenic activity in the *Ames* test. This indicates that epoxides with complex molecular structures and more than one substituent at the epoxide moiety, which are relatively more inert and stable, and, especially, more sterically hindered, are inactive in the *Ames* mutagenicity assay. In contrast, benzo[a]pyrene 4,5-oxide, benzo[e]pyrene 4,5-oxide and 7,12-dimethylbenz[a]-anthracene 5,6-oxide, derived from polycyclic aromatic hydrocarbons (PAH) are potent mutagens [12].

Halogenated epoxides as metabolites of some haloalkenes such as vinylidene chloride and vinyl chloride, are also bacterial mutagens [13]. Non-allylic chloropropenes and their homologues, being chloro-substituted in vinyl position are mutagenic in the presence of S9 metabolic activation system. This has been explaineded by the polarizing –I and M-effects of halogen and the alkyl substituents bound to the C=C bond. The metabolic activation of such chemicals was associated with the epoxide formation [14]. In these cases, the lack of significant steric hindrance effects is essential for the mutagenic activity.

According to another publication, 2,3-epoxyaldehydes are also genotoxic chemicals. These compounds may exist as parents but, more probably, can be formed as metabolites of *alpha-beta*-unsaturated aldehydes. The structure of their adducts with guanosine can be outlined as follows [15]:



Aziridine (ethyleneimine) reacts with DNA *in vitro* mainly at the N7 position of guanine and N3 of adenine; then imidazole ring opening of the modified guanine fragment results in the formation of formamidopyrimidine adducts [16]. Possible scheme for the formation of such adducts [17] is outlined below:

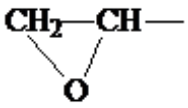


Arene imines, also containing aziridine structural fragment were shown to be very strong mutagens in bacterial and mammalian cells [18].

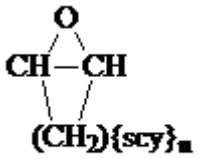
Conclusions:

I. **Structurally generalized active alerting groups for epoxides:**

- Monosubstituted epoxides:

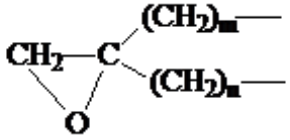


- Simple cycloaliphatic epoxides:

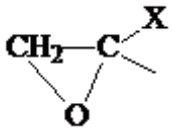


(n = 2 - 6)

- 1,1-Disubstituted epoxides and spiro-epoxides:

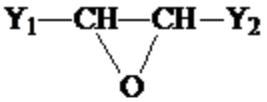


(m = 1 - 3; n = 1 - 3)
(If (CH_2) is acyclic, the terminal group is $-CH_3$)



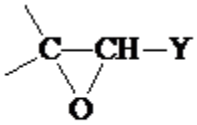
(X is Cl or Br

- 1,2-Disubstitured epoxides (including cycloaliphatic epoxides):



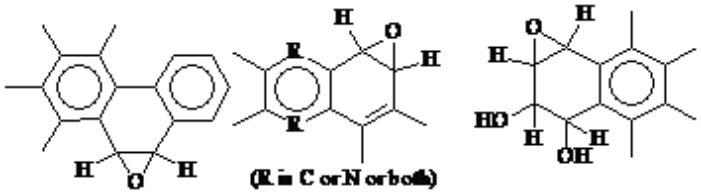
Y_1 and Y_2 can be the following structural moeties:
(a) $(-CH_2)_nH$ (n = 1 - 2)
(b) CH_2{scy} and -CH{scy}=CH{scy}-
(c) -CH{sp3}{scy} and O{scy} or -NH{scy}
(d) Y_1 is Cl or Br; Y_2 is C

- Other terminal polarized epoxides:

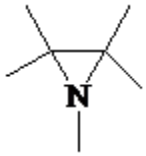


(Y can be Cl, Br or -CHO)

- Polycyclic Aromatic Hydrocarbons (PAH)-derived epoxides:



II. **Structurally-generalized active alerting groups for aziridines:**



REFERENCES

1. Koskinen, M., K. Plna, *Specific DNA Adducts Induced by Some Mono-Substituted Epoxides In Vitro and In Vivo*, Chem.-Biol. Interact. **129** (2000), 209 – 229.

2. Singh, U. S., K. D. Samuelian, J. J. Solomon, *Reaction of Epichlorohydrin with 2'-Deoxynucleosides: Characterization of Adducts*, Chem. Biol. Interact. **99** (1996), 109 – 128.

3. Sawatari, K., Y. Nakanishi, T. Matsushima, *Relationship Between Chemical Structures and Mutagenicity: A Preliminary Survey for a Database of Mutagenicity Test Results of New York Place Chemicals*, Industrial Health **39** (2001), 341 – 345.

4. Raney, V. M., Th. M. Harris, M. P. Stone*, DNA Conformation Mediates Aflatoxin B_1 – DNA Binding and the Formation of Guanine N_7 Adducts by Aflatoxin B1 8,9-exo-Epoxide*, Chem. Res. Toxicol. **6** (1993), 64 – 68.

5. Wade, M. J., J. W. Moyer, Ch. H. Hine, *Mutagenic Action of a Series of Epoxides*, Mutat. Res. **66** (1979), 367 – 371.

6. Voogd, C. E., J. J. van der Stel, J. J. J. A. A. Jacobs*, The Mutagenic Action of Aliphatic Epoxides*, Mutat. Res. **89** (1981), 269 – 282.

7. Hemminki, K., K. Falck, H. Vainio, *Comparison of Alkylation Rates and Mutagenicity of Directly Acting Industrial and Laboratory Chemicals: Epoxides, Glycidyl Ethers, Methylating and Ethylating Agents, Halogenated Hydrocarbons, Hydrazine Derivatives, Aldehydes, Thiuram and Dithiocarbamate Derivatives*, Arch. Toxicol. **46** (1980), 277 – 285.

8. Von der Hude, W., A. Seelbach, A. Basler, Epoxides: *Comparison of the Induction of SOS Repair in Escherichia coli PQ37 and the Bacterial Mutagenicity in the Ames Test*, Mutat. Res. **231** (1990), 205 – 218.

9. Frantz, S. W., J. E. Sinsheimer, *Bacterial Mutagenicity and Toxicity of Cycloaliphatic Epoxides*, Mutat. Res. **90** (1981), 67 – 78.

10. Meester, C. De, *Increase of 2,3-Epoxybutane Mutagenicity by Glutathione-S-Transferases*, Toxicol. Lett. **224** (1984), 255 – 262.

11. Sinsheimer, J. E., P. K. Chakraborty, E. A. Messerly, V. Gaddamidi, *Mutagenicity of Oxaspiro Compounds with Salmonella*, Mutat. Res. **224** (1989), 171 – 175.

12. Glatt, H., R. Jung, Fr. Oesch, *Bacterial Mutagenicity Investigation of Epoxides: Drugs, Drug Metabolites, Steroids and Pesticides*, Mutat. Res. **11** (1983), 99 – 118.

13. *Vinylidene Chloride*, Chemical Carcinogenesis Research Informartion System (CCRIS), US National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS.

14. Neudecker, T., W. Dekant, M. Jorns, E. Eder, D. Henschler, *Mutagenicity of Chloroolefins in the Salmonella/Mammalian Microsome Test – II. Structural Requirements for the Metabolic Activation of Non-Allylic Chloropropenes and Methylated Derivatives via Epoxide Formation*, Biochem. Pharmacol. **35**(2) (1986), 195 – 200.

15. Petrova, K. V., R. S. Jalluri, I. D. Kozekov, C. J. Rizzo, *Mechanism of 1,N^2-Etheno-2'-Deoxyguanosine Formation from Epoxyaldehydes*, Chem. Res. Toxicol. **20** (2007), 1685 – 1692.

16. Cussac, C., F. Laval, *Reduction of the Toxicity and Mutagenicity of Aziridine in Mammalian Cells Harboring the Eschericia coli frg Gene*, Nucleic Acids Res. **24**(9) (1996), 1742 -1746.

17. Tudek, B., *Imidazole Ring-Opened DNA Purines and Their Biological Significance*, J. Biochem. Molec. Biol. **36**(1) (2003), 12 – 19.

18. Glatt, H., G. Ludewig, K. L. Platt, et al., *Arene Imines, a New Class of Exceptionally Potent Mutagens in Bacterial and Mammalian Cells*, Canc. Res. **45** (1985), 2600 – 2607.

Haloalcohols

Principal structural fragments and alerting groups eliciting *in vitro* bacterial mutagenicity:

(X = Cl, Br, J)

Mechanistic Domain: E2 (elimination reaction)

Mechanistic Alert: E2 reaction with epoxide formation

Mechanistic Domain: Radical

Mechanistic Alert: ROS formation after GSH depletion

A.1. Direct-acting mutagens: alkylation of DNA by epoxide formed by bacterial dehalogenase – no external S9 metabolic activation system required

The metabolism of 1,3-dichloropropan-2-ol is likely to produce a reactive epoxide intermediate that could damage DNA, and this compound was found to be mutagenic to *Salmonella typhimurium* strains TA1535 and/or TA 100. 2,3 Dichloropropan-1-ol, on the other hand, was also mutagenic *in vitro* in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation [1]. The formation of epoxide intermediate (mutagenicity alert group) can be influenced by *haloalcohol dehalogenases* which are bacterial enzymes that catalyze the cofactor-independent dehalogenation of vicinal haloalcohols. Typical example in this respect is again the genotoxic environmental pollutant 1,3-dichloro-2-propanol, which produces epoxide, chloride ion and proton [2]. Then the epoxide is likely to exert its DNA alkylation capability [3]:

A.2. Mutagens requiring metabolic activation (S9, etc.).

Some authors have assumed genotoxicity mechanism, associated with glutathione depletion as glutathione S-transferase was used as the enzyme source, especially with bromohydrins such as 1,3-dibromopropanol [4]. It is likely that the protection afforded by glutathione against the toxicity of this chemical is mediated through the activity of cytosolic glutathione S-transferase. While 1,3-dichloro-2-propanol is relatively poor substrate for glutathione S-transferase, the dibromo-analogue causes extensive glutathione depletion [4]. According to another study, dichloropropanols such as 1,3-dichloropropan-2-ol, 2,3-dichloropropan-1-ol, 1,3-dibromopropan-2-ol, 1,4-dibromopropan-2-ol, 1-bromopropan-2-ol, other haloalcohols and their metabolites such as epichlorohydrin have been proved to deplete glutathione when incubated with liver fractions obtained from rats. However, difluoropropanols did not deplete glutathione [5].

It is therefore expertly assumed that glutathione depletion would further give rise to formation of ROS and DNA adducts:

REFERENCES

1. *Carcinogenicity of 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3-Dichloropropan-1-ol (2,3-DCP)*, Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, COC/04/S2 – June 2004; http://www.iacoc.org.uk/statements/statement123dichloropropanjune2004.htm

2. De Jong, R. M., J. J. W. Tiesiga, H. J. Rozeboom, K. H. Halk, L. Tang, D. B. Janssen, B. W. Dijkstra, *Structure and Mechanism of Bacterial Haloalcohol Dehalogenase: A New Variation of the Short-Chain Dehydrogenase/Reductase Fold Without an NAD(P)H Binding Site,* The EMBO Journal **22**(19) (2003), 4933 – 4944.

3. Saha, M., A. Abushamaa, R. W. Giese, *General Method for Determining Ethylene Oxide and Related N7-Guanine DNA Adducts by Gas Chromatography-Electron Capture Mass Spectrometry*, J. Chromatogr. A **712** (1995), 345 – 354.

4. Hammond, A. H., M. J. Garie, J. R. Fry, *The Nature of Halogen Substitution Determines the Mode of Cytotoxicity of Halopropanols*, Toxicol. Appl. Pharmacol. **155**(3), 1999, 287-291.

5. Garle, M. J., C. Sinclair, P. Thurley, J. R. Fry, *Haloalcohols Deplete Glutathione When Incubated with Fortified Liver Fractions*, Xenobiotica **29**(5) (1999), 533 – 545.

C. Profiler: Protein binding alerts for Chromosomal aberration by OASIS v1.1

Mechanistic domain: S_N2

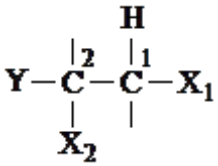
Mechanistic alert: Nucleophilic type substitution together with ring-opening of an episulfonium ion intermediate

Mechanistic domain: A_N2

Mechanistic alert: Michael type nucleophilic addition and Schiff base formation

Structural alert: Halogenated Vicinal Hydrocarbons

Halogenated chemicals have many uses in industrial processes as solvents, precursors of vinyl monomers, pesticides, gasoline additives and synthetic building blocks [1].
Dihalogenated vicinal compounds can be presented by the following general structure:



where: X_1 and X_2 = Br, Cl, I; C_1 and C_2 = Csp^3 (acy or scy); Y = H, Csp^3 (acy or scy), OH, O-P^{+5} and S^{+2}

First mechanism – GSH-dependent activation of vicinal dihaloalkanes

Mechanistic domain: S_N2

Mechanistic alert: Nucleophilic type substitution together with ring-opening of an episulfonium ion intermediate

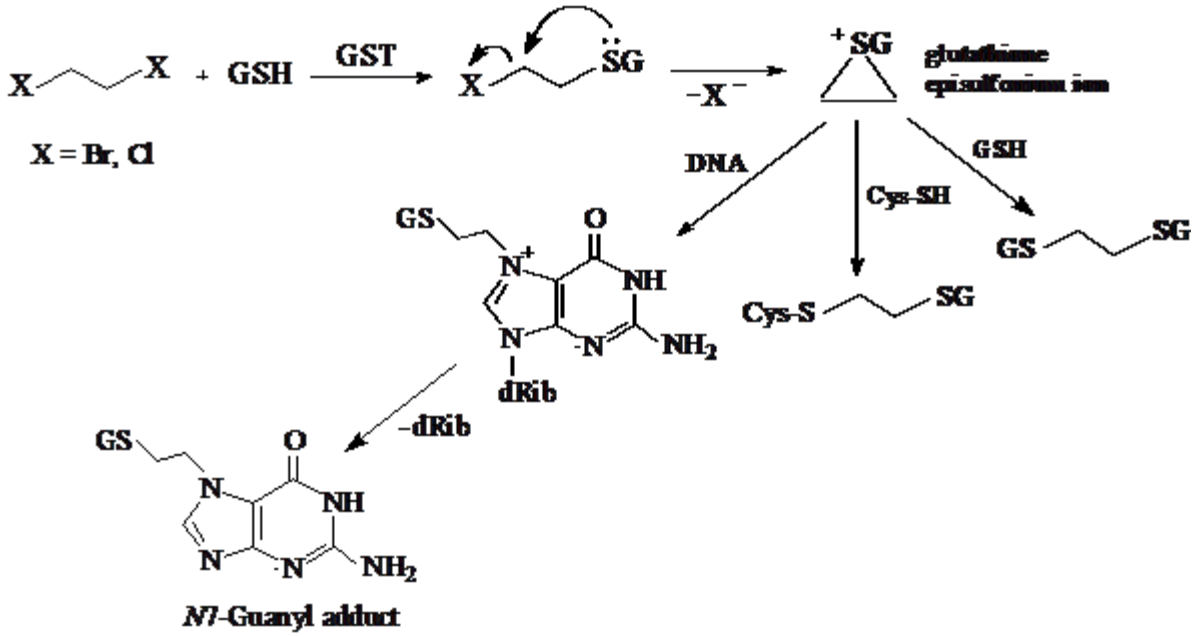
All halogenated hydrocarbons can be acutely toxic at high doses due to their general anesthetic properties. A significant and dose-dependent increase in the frequency of chromosomal aberrations was observed in the cultures treated with 1,2-dibromoethane (ethylene dibromide (EDB)), 1,2-dichloroethane (ethylene dichloride (EDC)), 1-bromo-2-chloroethane (BCE), 1,2-dibromo-3-chloroethane (DBCE), 2,3-dichloro-1-propanol (DCP-OH), 2,3-dibromo-1-propanol (DBP-OH), tris(2,3-dibromo-1-propyl)phosphate (tris-DBP-Ph), bis(2,3-dibromo-1-propyl)phosphate (bis-DBP-Ph) and captafol [2-6]. Both 1,2-dibromoethane and 1,2-dibromo-3-chloroethane induced cancer in rats and mice [2]. Moreover, a series of studies on the biological activity of 1,2-dibromoethane, 1,2-dichloroethane and 1,2-dibromo-3-chloroethane was performed and evidence was obtained that this chemicals became bound to cellular macromolecules such as DNA, RNA and

proteins [1-3].

1,2-Dichloroethane, 1,2-dibromoethane, and the mixed 1-bromo-2-chloroethane can be activated to electrophilic species by either oxidative metabolism or conjugation with

glutathione [1-3]. Although conjugation is generally a route of detoxification, in this case it leads to genetic damage. EDC has been shown to induce DNA adduct formation as a result of GSH-dependent bioactivation [7]. The major DNA adduct formed from EDB *in vitro* has been identified as *S*-[2-(N^7-guanyl)ethyl]glutathione, which is believed to arise via GSH half-mustard (GS-CH_2-CH_2-X) [1,3]. The mechanism of alkylation is associated with an episulfonium (tiiranium) ion formation involving GSH half-mustard and the subsequent binding with the *N*7-position of guanine to yield a bulky DNA adduct via the depurination reaction (Scheme 1).

Scheme 1



The other known GSH-ethylene conjugates that have been found with $BrCH_2CH_2Br$ are N^2- and O^6-guanyl derivatives. However, only with the *N*7-guanyl adduct depurination occurs, which could result in respective mutations [1]. For the series of 10 direct alkylating halogenated hydrocarbons a positive relationship between carcinogenicity and the initial ratios of O^6/N7-alkylguanine formed with double-stranded DNA was found in vitro [8].

In vitro evidence for some DNA adduct formation via the GSH-conjugation pathway could be obtained for 1,2-dibromo-3-chloropropane, 2,3-dibromo-1-propanol and tris(2,3-dibromopropyl)phosphate [1], although the contribution of oxidative pathways seems to be more important [9].

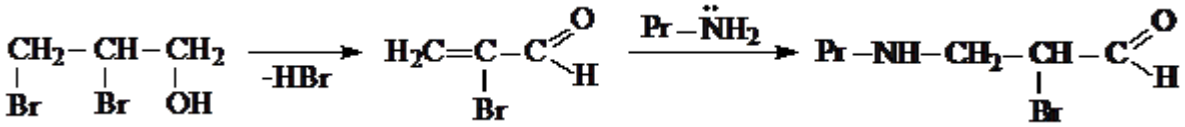
Second mechanism – Oxidative activation of vicinal dihaloalkanes

Mechanistic domain: A_N2
Mechanistic alert: Michael type nucleophilic addition and Schiff base formation

It is conceivable, that the induction of chromosomal aberrations in the superoxide-generating system may be directly or indirectly due to hydrogen peroxide formed in the cultured medium as a result of the spontaneous dismutation reaction of superoxide [10]. 2,3-Dibromo-1-propanol formed as a result of hydrolysis of tris-DBP-Ph and DBP-OH are able to undergo oxidation and oxidative dehalogenation to the corresponding unsaturated aldehyde (2-bromoacrolein) in the presence of hydrogen peroxide [10]. 2-Bromoacrolein formed, as an *α,β*-unsatured aldehyde, can participate in Michael type addition reaction as well as in the reaction of Schiff base formation. DCP-OH is able to form protein adducts in the same mechanisms as DBP-OH. All these consecutive transformations are shown in Scheme 2.

Scheme 2

Michael type addition reaction



Schiff base formation

References

1. F.P. Guengerich, Activation of dihaloalkanes by thiol-dependent mechanisms. *J. Biochem. Mol. Biol*., **2003**, 36(1), 20-27.
2. H. Tezuka, N. Ando, R. Suzuki, M. Terahata, M. Moriya, Y. Shirasu, Sister-chromatide exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. *Mutat. Res*., **1980**, 78(2), 177-191.
3. F.P. Guengerich, L.A. Peterson, J.L. Cmarik, N. Koga, P.B.Inskeep, Activation of dihaloalkanes by glutathione conjugation and formation of DNA adducts. *Environ. Health Perspect*., **1987**, 76, 15-18.
4. G.J. van Esch, Tris(2,3-dibromopropyl)phosphate and bis(2,3-dibromopropyl) phosphate. International Programme on Chemical Safety, Environmental Health Criteria 173. World Health Organization, Geneva, **1995**, pp. 18-21.
5. A. Nakamura, T. Noriyuki, S. Kojima, M. Kaniwa, T. Kawamura, The mutagenicity of halogenated alkanols and their phosphoric acid esters for *Salmonella typhimurium*. *Mutat. Res.*, **1979**, 66(4), 373-380.

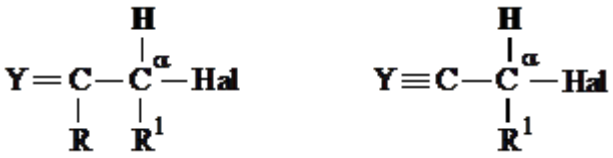
6. T. Sofuni, M. Ishidate Jr., Induction of chromosomal aberrations in cultured Chinese hamster cells in a superoxide-generating system. *Mutat*. *Res*., **1984**, 140(1), 27-31.
7. M.R. Gwinn, D.O. Johns, T.F. Bateson, K.Z. Guyton, A review of the genotoxicity of 1,2-dichloroethjane. *Mutat. Res*., **2011**, 727(1-2), 42-53.
8. H.M. Bolt, R.J. Laib, H. Peter, H. Ottenwälder, DNA adducts of halogenated hydrocarbons. *J. Cancer Res. Clin. Oncol*., **1986**, 112(), 92-96.
9. P.B. Inskeep, F.P Guengerich, Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione-S-transferases and dibromoalkane strucrures. *Carcinogenesis*, **1984**, 5(6), 805-808.
10. E.J. Søderlund, W.P. Gordon, S.D. Nelson, J.G. Omichinski, E. Dybing, Metabolism in vitro of tris(2,3-dibromopropyl) phosphate: Oxidative debromination and bis(2,3-dibromopropyl)phosphate formation as correlates to mutagenicity and covalent protein binding. *Biochem. Pharmacol.*, **1984**, 33(24), 4017-4023.

Mechanistic domain: S_N2

Mechanistic alert: Alkylation by nucleophilic substitution at sp^3-Carbon atom.

Structural alert: *alpha*-Activated Haloalkanes

alpha-Activated haloalkanes possess electron-withdrawing groups or sp^2(sp)-carbon atoms, directly bound to the *alpha*-carbon atom and can be presented with the following general structures:



where **Hal** = Cl, Br, I; **Y** = Csp^2(vinyl,acy), Csp, or Oxygen atom;
R = H, OH, Nsp^3(acy)–Csp^2(aryl); $\mathbf{R^1}$ = H, Csp^3(acy), Csp^3–Hal (Hal = F, Cl, Br, I)

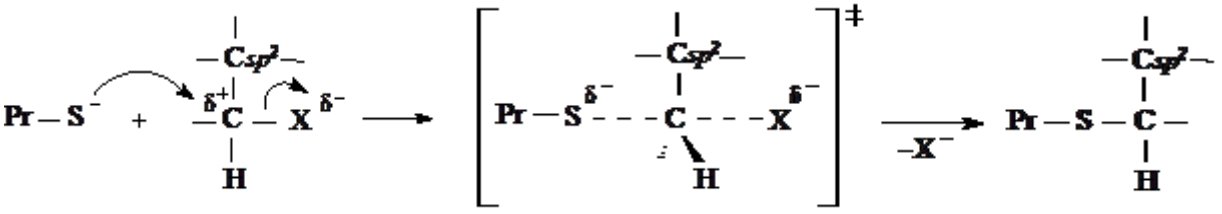
The chemicals such as 1,3-dichloropropene, 3,4-dichloro-1-butene, 2-bromopropanoic acid, alachlor and butachlor have been tested in *in vitro* chromosomal aberration assays with and without metabolic activation. Positive results have been obtained in the Chinese hamster ovary or lung cells without and in some cases with metabolic activation [1-4].

The compounds containing an allylic or propargylic moiety (such as 1,3-dichloropropene and 3,4-dichloro-1-butene, propargyl bromide) possess chemically good leaving groups (halogen atoms) and show direct mutagenic activity in the absence of S9 mix. This effect is theoretically explained by nucleophilic substitution reactions (mainly S_N2-type), leading to the alkylation of DNA and proteins [5].

The other compounds with a carbonyl group adjacent to the halogen atom (alachlor and butachlor) can also undergo displacement reactions with a strong nucleophiles [6]. It was shown that they are able to form glutathione conjugates through nucleophilic attack on the *alpha*-carbon atom [7]. In addition, alachlor *S*-cysteinyl-protein adducts were examined as potential biomarkers of alachlor exposure, a genotoxic and carcinogenic herbicide [8].

The S_N2 reaction mechanism between α-activated haloalkanes and protein thiolate ion is shown in Scheme1.

Scheme 1

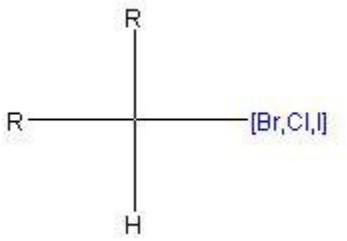


References

1. K.S. Loveday, M.H. Lugo, M.A. Resnick, B.E. Anderson, E. Zeiger, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals. *Environ. Mol. Mutagen*., **1989**, 13(1), 60-94.
2. Office of Environmental Chemicals Safety Environmental Health Bureau, Ministry of Health & Welfare in Japan, Toxicology Testing Reports of Environmental Chemicals, Chemicals Investigation Promoting Committee, Vol. 4, **1996**, p. 529.
3. 3,4-Dichlorobut-1-ene, CAS No. 760-23-6: SIDS Initial Assessment Report, OECD SIDS, **2001**.
4. M.F. Lin, C.L. Wu, T.C. Wang, Pesticide clastogenicity in Chinese hamster ovary cells. *Mutat. Res*., **1987**, 188(3), 241-250.
5. E. Eder, T. Neudecker, D. Lutz, D. Henschler, Mutagenic potential of allyl and allylic compounds. Structure-activity relationship as determined by alkylating and direct *in vitro* mutagenic properties. *Biochem*. *Pharmacol*., **1980**, 29(7), 993–998.
6. K.A. Lippa, S. Demel, I.H. Lau, A.L. Roberts, Kinetics and mechanism of the nucleophilic displacement reactions of chloroacetanilide herbicides: investigation of *alpha*-substituent effects. *J. Agric. Food Chem.*, **2004**, 52(10), 3010-3021.
7. D.M. Stamper, O.H. Tuovinen, Biodegradation of the herbicides alachlor, metolachlor, and propachlor. *Crit. Rev. Microbiol*., **1998**, 24(1), 1-22.
8. G.R. Lambert, W.T. Padgett, M.H. George, K.T. Kitchin, S. Nesnow, Quantitative analysis of alachlor protein adducts by das chromatography-mass spectrometry. *Anal. Biochem*., **1999**, 268(2), 289-296.

D. Profiler: In vitro mutagenicity (Ames test) alerts by ISS

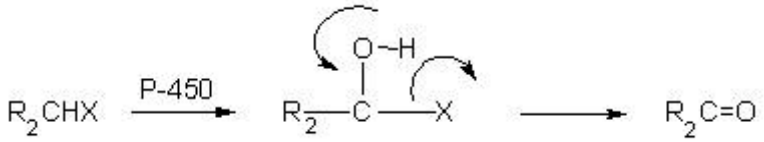
Aliphatic halogens



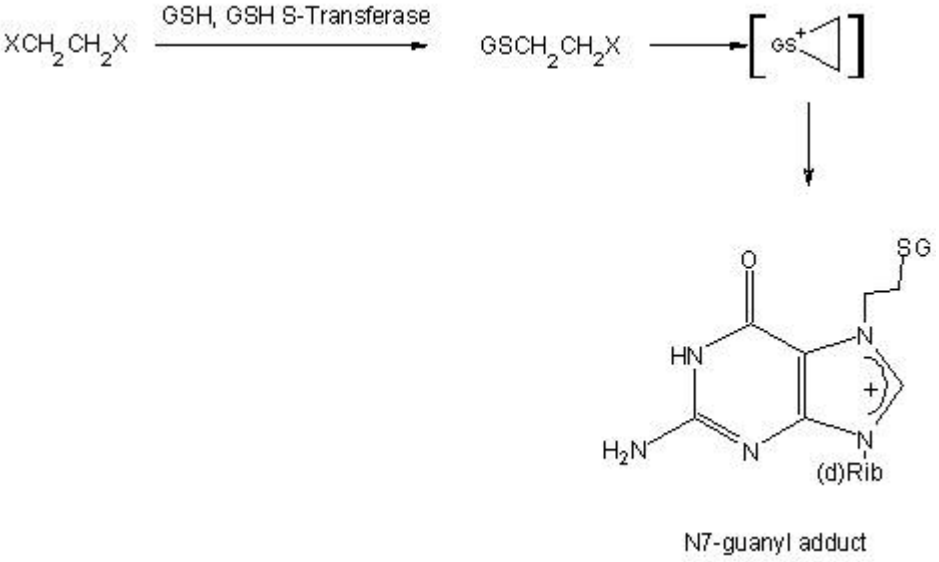
R = any atom/group

Numerous haloalkanes have been tested for carcinogenic and mutagenic activities. In general, the genotoxic potential is dependent on the nature, number, and position of halogen(s) and the molecular size of the compound (Woo *et al.* 2002). Although some aliphatic halogens have been shown to directly alkylate macromolecules (Bolt and Gansewendt 1993), biotransformation may also play an important role in their toxicity. Cytochrome P450 oxidation may produce gem-halohydrins that spontaneously dehydrohalogenate to reactive carbonyl compounds (Guengerich 1991), (see reaction 1). Alternatively, glutathione (GSH) conjugation via GSH transferases, has been proposed as an activation mechanism for several halogenated alkanes (Guengerich 2003b); (Guengerich 2003a) (as an example for dihaloethanes see Reaction 2).

1.



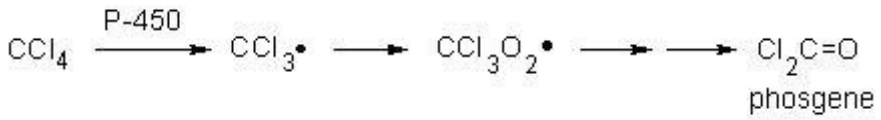
2.



N7-guanyl adduct

Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms (Woo *et al.* 2002). In the case of CCl4 (see reaction 3.), P450 reduces CCl4 to the trichloromethyl radical which can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes.

3.



Adduct formation between CCl3* and DNA is thought to function as initiator in the case of hepatic cancer. This radical can also react with oxygen to form highly reactive species, the trichloromethylperoxy radical CCl3OO*, that may initiate the chain reaction of lipid peroxidation, and ultimately generate phosgene (Guengerich 1991).

References Cited

Bolt, H. M. and Gansewendt, B. (1993). Mechanisms of Carcinogenicity of Methyl Halides. *Crit.Rev.Toxicol.* 23, 237-253.

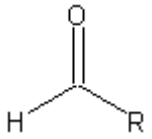
Guengerich, F. P. (1991). Oxidation of Toxic and Carcinogenic Chemicals by Human Cytochrome P-450 Enzymes. *Chem.Res.Toxicol.* 4, 391-407.

Guengerich, F. P. (2003a). Activation of Dihaloalkanes by Thiol-dependent Mechanisms. *J.Biochem.Mol.Biol.* 36, 20-27.

Guengerich, F. P. (2003b). Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. Arch.Biochem.Biophys. 409, 59-71.

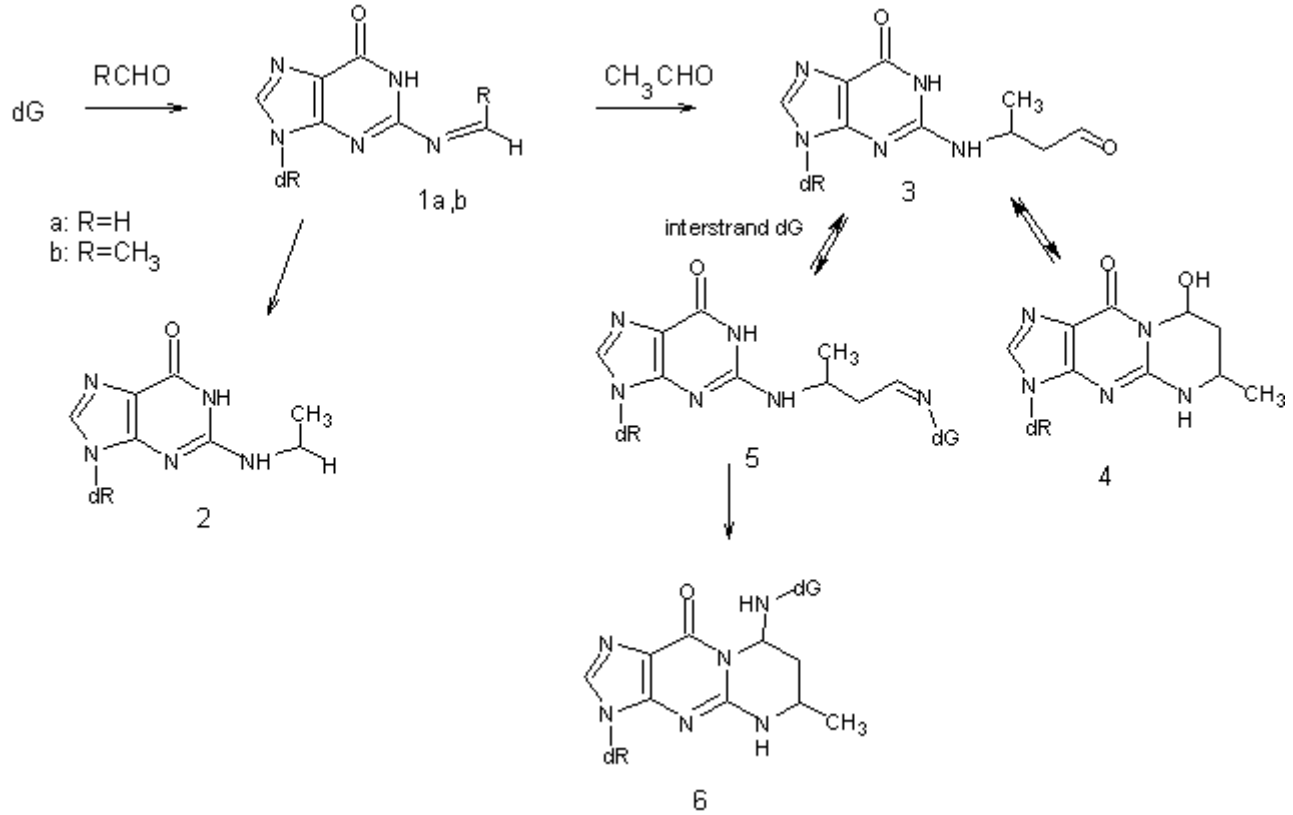
Woo, Y. T., Lai, D. Y., McLain, J. L., Ko Manibusan, M., and Dellarco, V. (2002). Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ.Health Perspect. 110, 75-87.

Simple Aldehydes



R= aliphatic or aromatic carbon
alpha, beta-unsaturated aldehydes are excluded

All compounds carrying an aldehydic group can potentially undergo Schiff base formation with a primary amine. They are to be considered potentially genotoxic, as demonstrated *in vivo* ability to react with nucleobases, without metabolic activation, forming adducts, interbase cross-links (both intra and inter-strand), and DNA-protein crosslinks The length of carbon chain for aliphatic aldehydes, and in general molecular size, can strongly modulate the formation of every type of cross-link and even the accessibility of the DNA nucleobases (Romano Zito, personal communication). DNA-protein crosslinks have been reported as the primary DNA damage induced by formaldehyde (Speit *et al.* 2007). The initial step of the reaction probably involves formation of an unstable Schiff base with the exocyclic amino group of deoxyguanosine dG (1a). In the case of acetaldehyde, this intermediate (1b) could be stabilized by reduction, producing *N*2-ethyl-dG (2), or alternatively may react with a second molecule of acetaldehyde forming a new aldehyde adduct (3) that ultimately cyclize in an 8-hydroxypropano adduct (4). The latter exists in equilibrium with its ring-opened aldehyde form, and may undergo condensation with another guanine to form imine-linked bis-nucleoside (5) which in turn cyclizes to pyrimidopurinone (6) (Wang *et al.* 2000).



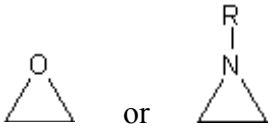
Some aldehydes may also induce hydroxyalkyl adducts in DNA, but the relevance of these DNA modifications for mutagenicity is unclear (Speit *et al.* 2007).

References Cited

Speit, G., Schütz, P., Högel, J., and Schmid, O. (2007). Characterization of the genotoxic potential of formaldehyde in V79 cells. *Mutagenesis* 22, 387-394.

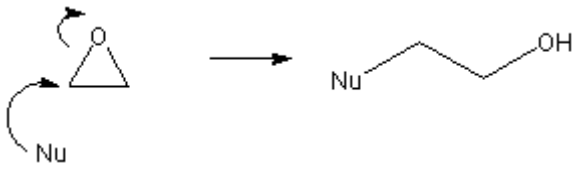
Wang, M., McIntee, E. J., Cheng, G., Shi, Y., Villalta, P. W., and Hecht, S. S. (2000). Identification of DNA Adducts of Acetaldehyde. *Chem.Res.Toxicol.* 13, 1149-1157.

Epoxides and Aziridines



or
R = any atom/group

Due to the large ring strain associated with the three-membered ring, epoxides are highly reactive molecules. They may react with nucleophilic centers of DNA molecules, giving rise to alkylated products.



Because of their reactivity they are important intermediates in chemical industry, especially in polymer production. Furthermore, epoxides may be produced endogenously by the enzymatic oxidation of other chemicals, many of which are common environmental pollutants (such as PAH, alkenes). Consequently considerable human exposure arises. The most likely route of exposure to these agents is by inhalation, although the possibility of dermal and oral absorption should also be considered. The site of alkylation of the DNA constituents is mainly determined by the ionic character of the epoxide (Koskinen and Plná 2000); (Barlow and Dipple 1998) Reactions at the ring nitrogen positions follow a bimolecular displacement mechanism whereas modification of the exocyclic groups requires some degree of substrate ionization for reaction to occur (Barlow and Dipple 1998). Simple alkyl epoxides, that are not able to stabilize an ionic charge to any great extent, react predominantly at endocyclic base nitrogens, giving rise, preferentially, to •-hydroxyethyl derivatives of cytosine-N3, adenine-N1 and N3, and guanine-N7. Molecules that are more efficient in stabilizing an ionic charge, may modify also exocyclic groups (i.e., styrene oxide, butadiene monoepoxide, PAH (Koskinen and Plná 2000); (Barlow and Dipple 1998). Aziridines are extremely reactive alkylating agents that may react by ring-opening reactions similar to those of epoxides. There are several classes of aziridine-containing natural products that exhibit potent biological activity. Among them, the mitomycins, that exhibit both anti-tumour and antibiotic activity (Sweeney 2002). Another class of naturally-occurring aziridine derivatives possessing potent cytotoxic and antitumor activities, is the Azinomycin family. The activity of these compounds lies in their ability to act as DNA cross-linking agents, *via* nucleophilic ring-opening of the aziridine and epoxide moieties by N-7 positions of purines (Zang and Gates 2000). It is not clear at present which ring opening reaction takes precedence in the cross-linking event. The PBI (pyrrolo[1,2-a]benzimidazole) class of natural products, represents another type of DNA-alkylating species containing an aziridine moiety. In these compounds, the aziridine undergoes ring-opening by nucleophilic attack of the DNA phosphate backbone, resulting in formation of a hydrolytically labile phosphotriester (that may eventually cause DNA cleavage) (Schultz *et al.* 1995).

References Cited

Barlow, T. and Dipple, A. (1998). Aralkylation of guanosine with para-substituted styrene oxides. *Chem.Res.Toxicol.* 11, 45-53.

Koskinen, M. and Plná, K. (2000). Specific DNA adducts induced by some mono-substitued epoxides in vitro and in vivo. *Chem.Biol.Interact.* 129, 209-229.

Schultz, W. G., Nieman, R. A., and Skibo, E. B. (1995). Evidence for DNA phosphate backbone alkylation and cleavage by pyrrolo[1,2-albenzimidazoles: Small molecules capable of causing base-pair-specific phosphodiester bond hydrolysis. *Proc.Natl.Acad.Sci.USA* 92, 11854-11858.

Sweeney, J. B. (2002). Aziridines: epoxides' ugly cousins? *Chem.Soc.Rev.* 31, 247-258.

Zang, H. and Gates, K. S. (2000). DNA Binding and Alkylation by the "Left Half" of Azinomycin B. *Biochemistry* 39, 14975.

E. Profiler: DNA alerts for AMES, MN and CA by OASIS v.1.3

Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, nucleophilic substitution at sp3-carbon atom

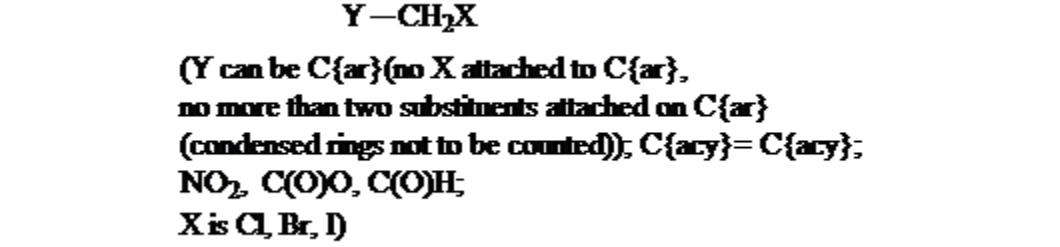
Mechanistic Domain: A_N2
Mechanistic Alert: Shiff base formation for aldehydes

Mechanistic Domain: S_N2
Mechanistic Alert: Acylation involving a leaving group

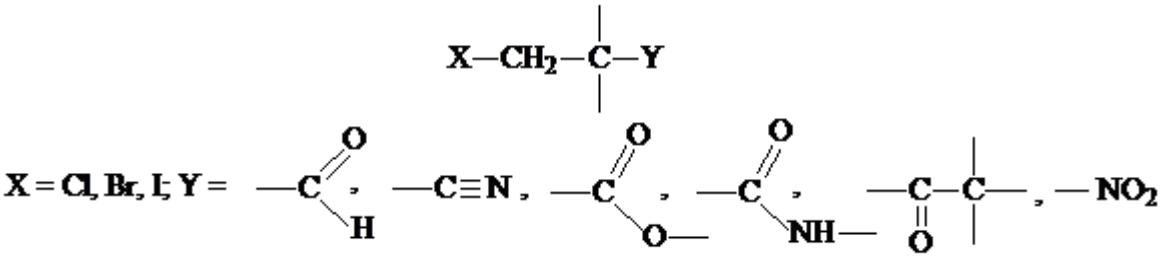
Structural alert: Haloalkane Derivatives with Labile Halogen

Principal and characteristic active structural fragments:

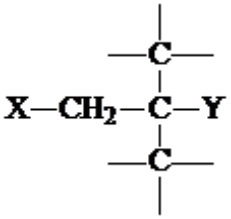
A. Primary haloalkane derivatives with labile halogen at alpha-position towards other groups:



B. Primary haloalkane derivatives with labile halogen at beta-position towards other groups:



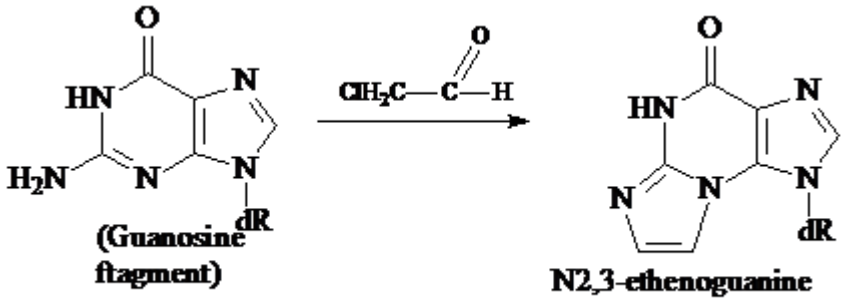
"Mask":



A. Haloalkane derivatives with labile halogen at alpha-position towards other groups

Many of these compounds represent disinfection by-products, some of which are rather toxic. They include halonitriles, some halocarbonyl- and halocarboxyl-compounds, halonitroalkanes, etc. Short-chain monohalogenated alkanes and their derivatives, containing such electron-withdrawing substituents are potential direct-acting alkylating agents, particularly if the halogen is at the terminal end of the carbon chain or at allylic (benzylic) position (primary halogenoalkane derivatives). For example, acetonitrile is not carcinogenic in rodents and is only weakly or marginally mutagenic. For halogenated acetonitriles, positive mutagenicity is expected, due to the strong –I effect of the neighboring nitrile group, however, bacterial toxicity hinders the expression of positive mutagenic potential.

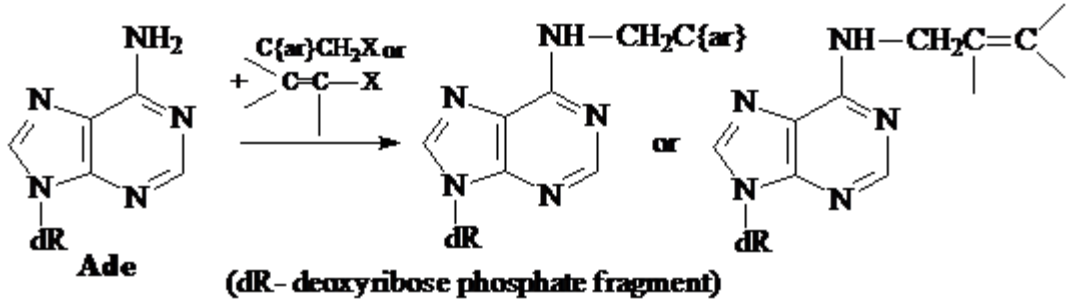
Generally, the introduction of halogen to alpha- or terminal carbon atoms with the above-mentioned structural neighbors is expected to increase the genotoxic potential by making it an alkylating and/or cross-linking agent. On the basis of the alkylating activity, the brominated compounds are expected to be more reactive than chlorinated ones, and iodoacetic acid is the most toxic and genotoxic disinfection by-product in mammalian cells reported [1, 3]. For example, bromoacetic acid is far more mutagenic than the chloroacetic acid [2]. The toxicity of brominated and chlorinated acetic acids, however, decreases as the number of halo-substituents increases [4]. Haloaldehydes with mono-substitution at the alpha- or terminal carbon atom are also expected to be potential alkylating agents [1]. For example, the reaction of the strong mutagen, chloroacetaldehyde, a reactive metabolite of the carcinogenic vinyl chloride (and bifunctional compound) with DNA produces 1,N6-ethenoadenine, 3,N4-ethenocytosine and, particularly, N2,3-ethenoguanine [5]. Therefore, if the substituent Y represents the reactive aldehyde (e.g., formyl) group), the following scheme of formation of one of the DNA adducts has been proposed [6]:



(dR– deoxyribose phosphate fragment)

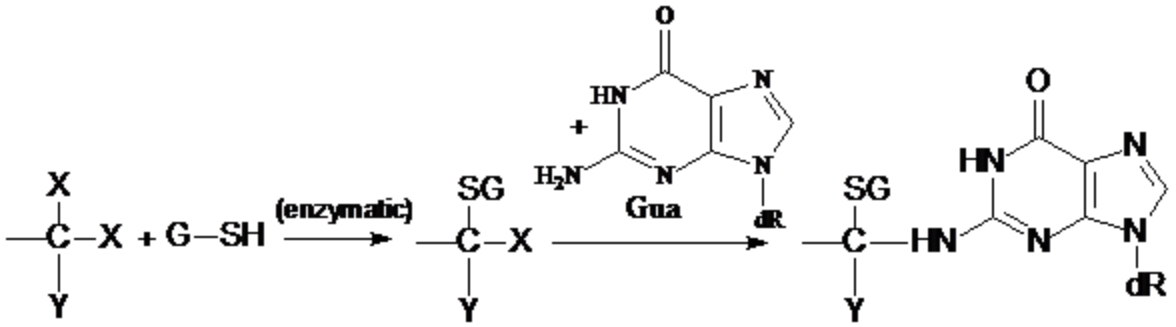
In this case, the reactivities of both the formyl and chlorine functional groups have been involved in the formation of the DNA adduct.

If Y is allylic or benzylic moiety, direct attack on DNA has also been suggested [7, 8]:



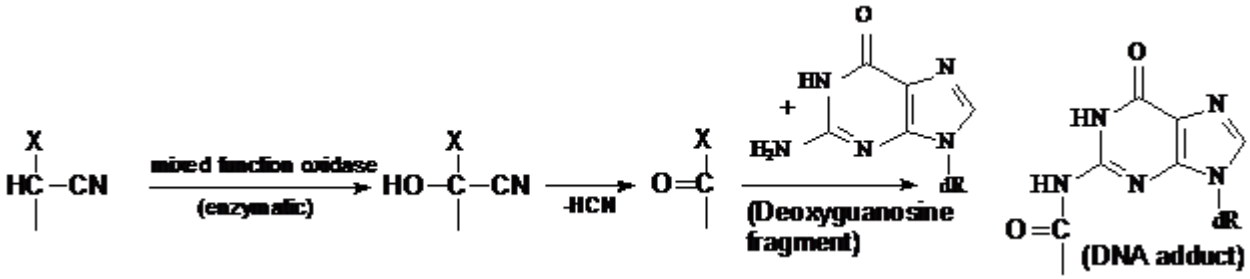
Unfortunately, little information has been available, concerning directly formed DNA adducts for the cases, where Y represent $–NO_2$, –COOH/COOR, –CN and other electron-withdrawing substituents. Chloroacetonitrile has been proposed to possess direct-acting DNA-alkylating capability, which does not manifest itself to bacterial mutagenicity, due to cytotoxicity. However, metabolic activation is also possible [9]. As far as benzyl- and allyl halides are concerned, which also belong to this class of compounds, benzyl chloride has been found to show mutagenicity in the *Salmonella*/microsome mutagenesis assay [17]. On the other hand, allyl halides such as allyl chloride and allyl bromide showed positive results in the *Ames* mutagenicity assay and have established DNA alkylating properties [18 - 20]. Similarly to the mechanistic schemes outlined above, direct attack on the purine/pyrimidine bases [18, 19] ("alkylation") has been suggested. For these compounds, minimum steric hindrance effects are important in order to show direct mutagenicity.

Bromonitromethane and chloronitromethane have been found to be mutagenic in the *Salmonella typhimurium* bioassay in the presence of S9 system. The halonitromethanes (e.g. chloropicrin) are significantly more potent mutagens and toxicants than the dihalomethanes in the *Salmonella typhimurium TA100* preincubation assay, and conjugation with glutathione is also involved [10, 11]. The following mechanism for the glutathione-dependent (enzymatic) bioactivation and attack on DNA could be suggested, by analogy with the published data [11]:

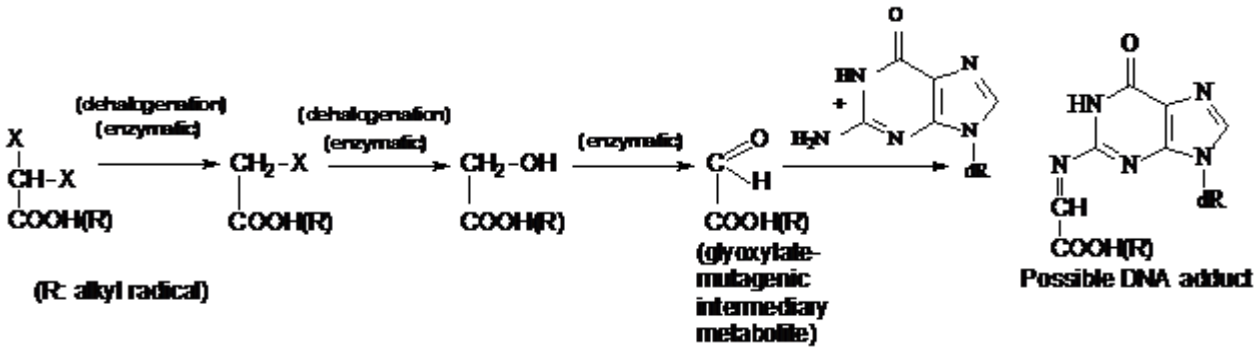


Halogenated acetonitriles, on the other hand, can be metabolized *via* oxidative dehalogenation, and this first step of the biotransformation is catalyzed by a mixed function oxidase such as cytochrome P450. It has been proposed that halocyanoalcohols formed by the

oxidative dehalogenation are converted to haloformaldehydes (formyl halides), which are mutagenic metabolites [1, 9]:

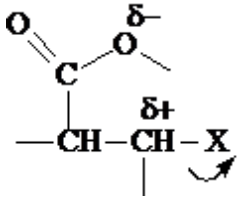


Monobromoacetic acid was evaluated as mutagenic in *Salmonella typhimurium* [12]. Possible metabolic transformation of haloacetic acids such as dichloroacetic acid takes place *via* dehalogenation and one of the intermediary metabolites is glyoxylate, which is mutagenic. The primary metabolic pathway for dichloroacetic acid involves oxidative dechlorination to form glyoxylate. This reaction, once thought to be microsomal, Cytochrome P-450 mediated, has now been shown to be NADPH- and GSH-dependent, and occurs predominantly in the cytosol [13, 14]. Based upon the above considerations, the following scheme for possible bioactivation can be expertly assumed for haloacetates:

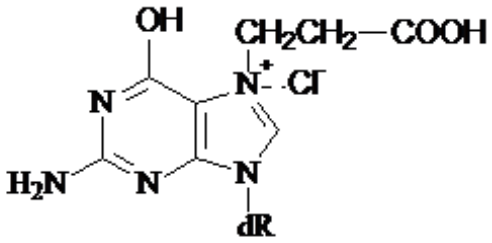


B. <u>Haloalkane derivatives with labile halogen at beta-position towards other groups</u>

These chemicals are assumed to be direct-acting mutagens, eliciting mutagenicity by alkylation of DNA fragments. For example, 3-chloropropionic acid binds DNA in mouse skin. Mutagenicity has also been suggested for methyl 3-bromopropionate, 3-chlorobutanoic acid, 3-chloropropanoic acid, etc. These compounds contain a nucleophilic center such a carboxyl ester group in beta-position with respect to the halogen. In each case, a conformation may be envisioned, in which the nucleophilic substituent stabilizes the transition state of the biological alkylating agent and the possible alkylation of DNA sub-fragments is facilitated [15]:

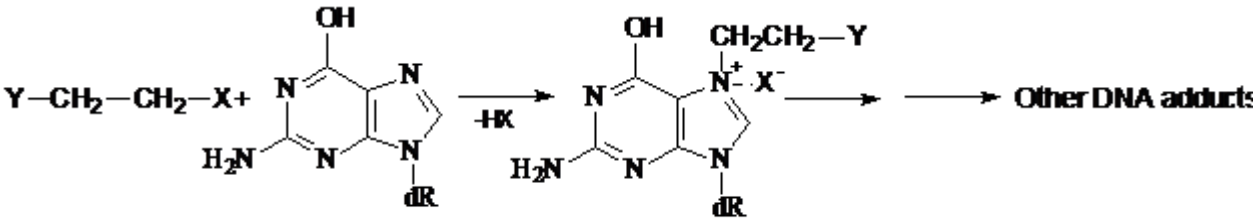


7-Carboxyethylguanine was suggested a possible DNA adduct for 3-chloropropanoic acid [16]:



(N7-Carboxyethylguanine fragment)

The following scheme for formation of DNA adducts can be expertly suggested:



(Deoxyguanosine fragment) (N7-Alkylated DNA adduct)

REFERENCES

1. Woo, Y. T., D. Lai, J. L. McLain, M. Ko Manibusan, V. Dallarco, *Use of Mechanism-Based Structure-Activity Relationships in Carcinogenic Potential Ranking for Drinking Water Disinfection By-Products*, Environ. Health Persp. **110** (2002), 75 – 87.
2. Kargalioglu, Y., B. J. McMillan, R. A. Minear, M. J. Plewa, *Analysis of the Cytotoxicity and Mutagenicity of Drinking Water Disinfection By-Products in Salmonella typhimurium*, Teratog. Carcinog. Mutag. **22**(2) (2002), 113-128; http://onlinelibrary.wiley.com/doi/10.1002/tcm.10010/abstract;jsessionid=BFC80F2B0989BAF938AE8DA5D8B15DDE.d01t01.

3. Plewa, M. J., E. D. Wagner, S. D. Richardson, A. D. Thruston, Y. T. Woo, A. B. McKague, *Chemical and Biological Characterization of Newly Discovered Iodoacid Drinking Water Disinfection By-Products*, Environ. Sci Technol. **38**(18), 2004, pp. 4713-4722; http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=86035.

4. Giller, S., F. Le Curtieux, F. Erb, D. Marzin, *Comparative Genotoxicity of Halogenated Acetic Acids Found in Drinking Water*, Mutagenesis **12**(5) (1997), 321 - 328.

5. Oesch, Fr., G. Doerjer, *Detection of N2, 3-Ethenoguanine in DNA After Treatment With Chloroacetaldehyde In Vitro*, Carcinogenesis **3** (6) (1982), 663 – 665; http://carcin.oxfordjournals.org/content/3/6/663.abstract.

6. Cheng, K. C., B. D. Preston, D. S. Cahill, M. K. Dosanjuh, B. Singer, L. A. Loeb, *The Vinyl Chloride DNA Derivative N2,3-Ethenoguanine Produces G → A Transitions in Escherichia Coli*, Proc. Natl. Acad. Sci USA **88** (1991), 9974 - 9978.

7. Fall, M., H. Haddouk, J. P. Morin, R. Forster, *Mutagenicity of Benzyl Chloride in the Salmomella/Microsome Mutagenesis Assay Depends on Exposure Conditions*, Mutat. Res. 633(1) (2007), 13 – 20; http://www.ncbi.nlm.nih.gov/pubmed/17631040

1. Eder, E., D. Henschler, T. Neudecker, *Mutagenic Properties of Allylic and Alpha,beta-Unsaturated Compounds: Consideration of Alkylating Mechanisms* Xenobiotica **12**(12), 1982, 831-848; http://pubget.com/paper/6763406.
2. Lin, E. L. C., F. B. Daniel, S. L. Herren-Freund, M. A. Pereira, *Haloacetonitriles: Metabolism, Genotoxicity, and Tumor-Initiating Activity*, Environ. Health Persp. **69** (1986), 67 – 71.

3. Kundu, B., S. D. Richardson, P. D. Swartz, P. P. Matthews, A. M. Richard, D. M. DeMarini, *Mutagenicity in Salmonella of Halonitromethanes: A Recently Recognized Class of Disinfection By-Products in Drinking Water*, Mutat. Res. **562**(1-2) (2004), 39-65.

4. Schneider, M., G. B. Quistad, J. E. Casida, *Glutathione Activation of Chloropicrin in the Salmonella Mutagenicity Test*, Mutat. Res. **439**(2) (1999), 233 - 238.

5. Brominated Acetic Acids in Drinking Water (Background Document for Development of WHO Guidelines for Drinking Water Quality, WHO/SDE/WSH/03.04/79 (2004); http://www.who.int/water_sanitation_health/dwq/chemicals/brominatedaceticacids.pdf

6. *Toxicological Review of Dichloroacetic Acid (CAS No. 79-43-6)*, In Support of Summary Information on the Integrated Risk Information Systme (IRIS), US EPA, Washington DC, August 2003; http://www.epa.gov/iris/toxreviews/0654tr.pdf.

7. *Monochloroacetic Acid in Drinking Water* (Background Document for Development of WHO Guidelines for Drinking Water Quality), WHO/SDE/WSH/03.04/85, WHO, 2004; http://www.who.int/water_sanitation_health/dwq/chemicals/monochloroaceticacid.pdf.

8. Theiss, J. C., M. B. Shimkin, L. A. Poirer, *Induction of Pulmonary Adenomas in Strain A Mice by Substituted Organohalides*, Canc. Res. **39**, 1979, 391 - 395.

9. Colburn, N. H., R. K. Boutwell, *The Binding of Beta-Propiolactone and Some Related Alkylating Agent to DNA, RNA, and Protein of Mouse Skin; Relation Between Tumor-Initiating Power of Alkylating Agents and Their Binding to DNA*, Canc. Res. **28** (1968), 653 – 660.

10. Fall, M., H. Haddouk, J. P. Morin, R. Fortun *Mutagenicity of Benzyl Chloride in the Salmonella/Microsome Mutagenesis Assay Depends on Exposure Conditions*, Mutat. Res. 633(1) (2007), 13 – 21; http://www.ncbi.nlm.nih.gov/pubmed/17631040.

11. *Allyl Bromide CAS No. 106-95-6. CSWG Evaluation* (12/16/94);
12. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/AllylBromide.pdf

13. Eder, E., D. Henschler, T. Neudecker, *Mutagenic Properties of Allylic and Alpha,beta-Unsaturated Compounds: Consideration of Alkylating Mechanisms* Xenobiotica 12(12), 1982, 831-848;
14. http://pubget.com/paper/6763406.

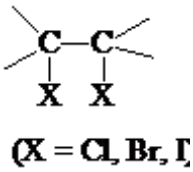
15. McCoy, E. C., L. Burrows, H. S. Rosenkranza, *Genetic Activity of Allyl Chloride*, Mutat. Res./Fund. Molec. Mechan. Mutag. 57(1) (1978), 11 – 15;
16. http://www.sciencedirect.com/science/article/pii/0027510778902294.

Mechanistic Domain: S_N2
Mechanistic Alert: Internal S_N2 reaction with aziridinium and/or cyclic sulfonium ion formation (enzymatic)

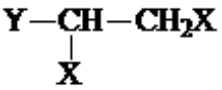
Mechanistic Domain: S_N2
Mechanistic Alert: DNA alkylation

Structural alert: Vicinal Dihaloalkanes

Principal structural fragments:



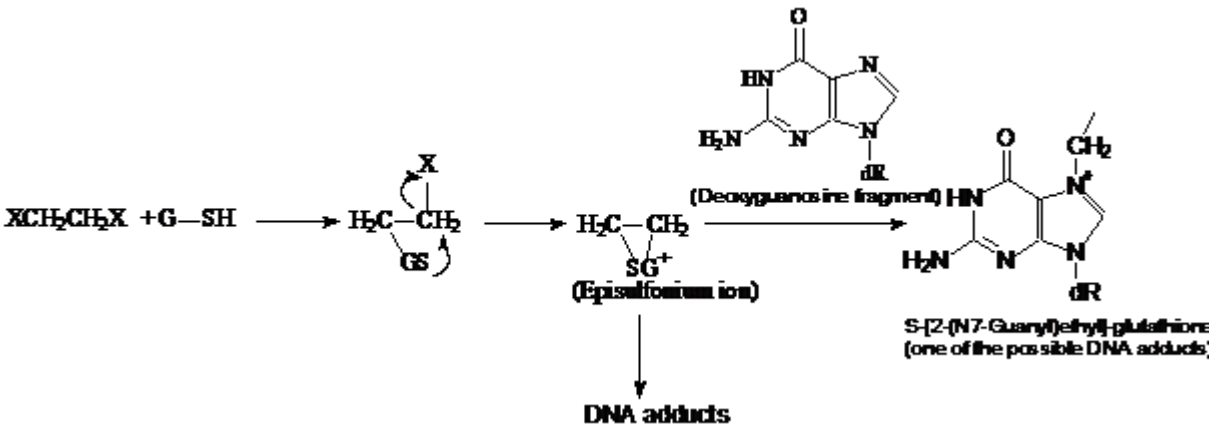
Characteristic active fragments:



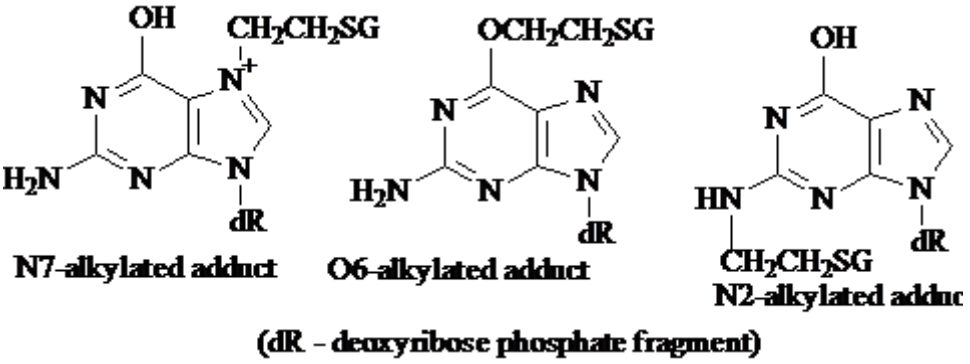
(Y is –H, $-(CH_2)_nH$ (n = 1, 2), $-O(CH_2)_nH$ (n = 0 -2), $-CH_2-O-$, C{acy}{sp2};
No other halogens bound to Y)

In vitro metabolic activation (bioactivation) is believed to be the main mutagenicity-eliciting process *via* glutathione-mediated generation of electrophilic species.

For example, 1,2-dichloroethane is reasonably anticipated to be a human carcinogen, based on sufficient evidence of carcinogenicity in experimental animals. *In vivo* and *in vitro* studies in rodents have revealed that the primary metabolic pathway for 1,2-dichloroethane probably involves conjugation with glutathione, and the compound shows bacterial mutagenicity. This is S_N2 (bimolecular nucleophilic attack) of glutathione GSH on the electron-deficient carbon of 1,2-dichloroethane (also for 1,2-dibromoethane, 1,2-dichloropropane, etc.) and S-(2-chloroethyl)-glutathione adduct is formed. One of the further possible metabolic pathways is the loss of chloride ion with the formation of *episulfonium ion*, which is highly reactive. This ion is believed to be the reactive *electrophilic* intermediate that results in covalent reaction with biopolymers such as DNA, and is believed to determine the mutagenic potential of this class of organic halides [1 – 4, 6]:

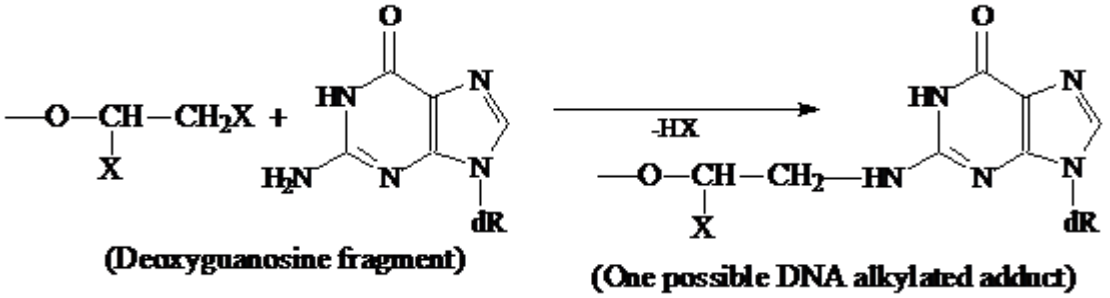


The major product of this reaction is *S*-[2-(*N*^{7}-guanyl)ethyl]glutathione, but *N*^{2}- and *O*^{6}-guanyl adducts are also formed, and all three adducts are potentially mutagenic [3]:



Similar mechanism of *in vitro* metabolic activation by forming episulfonium cation as reactive intermediate has also been suggested for structurally similar short-chain compounds such as 1,2-dibromo-3-chloropropane [5].

Beside 1,2-dichloroethane, 1,2-dibromoethane belonging to this class of compounds was also found to possess bacterial mutagenicity [7]. Short-chain vicinal dihalolakanes with halogen attached to terminal carbon atom are assumed to act by direct alkylation mechanism, too. Other short-chain vicinal haloalkane derivatives with electron-withdrawing heteroatoms adjacent to the –CHX fragment such as 1-methoxy-1,2-dichloroethane, 2,3-dibromo-propanol, etc., are believed to cause also direct mutagenicity by alkylation mechanism:



REFERENCES

1. Anders, M. W., *Glutathione-Dependent Bioactivation of Haloalkanes and Haloalkenes*, Drug Metabol. Rev. **36** (3 – 4) (2004), 583 – 594.

2. *Public Health Goal for 1,2-Dichloropropane in Drinking Water*, Office of Environmental Health Hazard Assessment, California EPA, February 1999;

   http://www.oehha.ca.gov/water/phg/pdf/12dcp_f.pdf.

3. Guengerich, F. P., L. A. Peterson, J. L. Cmarik, N. Koga, Ph. B. Inskeep, *Activation of Dihaloalkanes by Glutathione Conjugation and Formation of DNA Adducts*, Environ. Health Persp. **76** (1987), 15 – 18.

4. Liu, L., A. E. Pegg, K. M. Williams, F. P. Guengerich, *Paradoxal Enhancement of the Toxicity of 1,2-Dibromoethane by O6-Alkylguanine-DNA Alkyltransferase*, J. Biol. Chem. **277** (40) (2002), 37920 - 37928.

5. Miller, G. E., M. J. Brabec, A. P. Kulkarni, *Mutagen Activation of 1,2-Dibromo-3-Chloropropane by Cytsololic Glutathione S-Transferases and Microsomal Enzymes*, J. Toxicol. Environ. Health: Current Issues **19**(4) (1986), 503 – 518.

6. Rannug, U., A. Sundvall, C. Ramel, *The Mutagenic Effect of 1,2-Dichloroethane on Salmonella Typhimurium I. Activation Through Conjugation with Glutathion In Vitro*, Chem.-Biol. Interact. **20** (1978), 1 – 16.

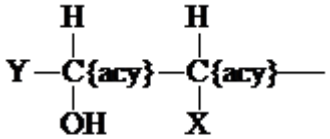
7. Strubel, K., T. Grummt, *Aliphatic and Aromatic Halocarbons as Potential Mutagens in Drinking Water. III. Halogenated Ethanes and Ethenes*, Toxicol. Environ. Chem. **15**(1-2) (1987), 101 – 128.

Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, direct-acting epoxide formed after E2 reaction

Mechanistic Domain: Radical
Mechanistic Alert: ROS formation after GSH depletion (indirect)

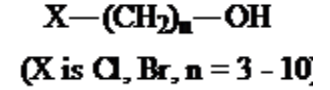
Structural alert: Haloalcohols

Principal structural fragments and alerting groups eliciting in vitro bacterial mutagenicity:

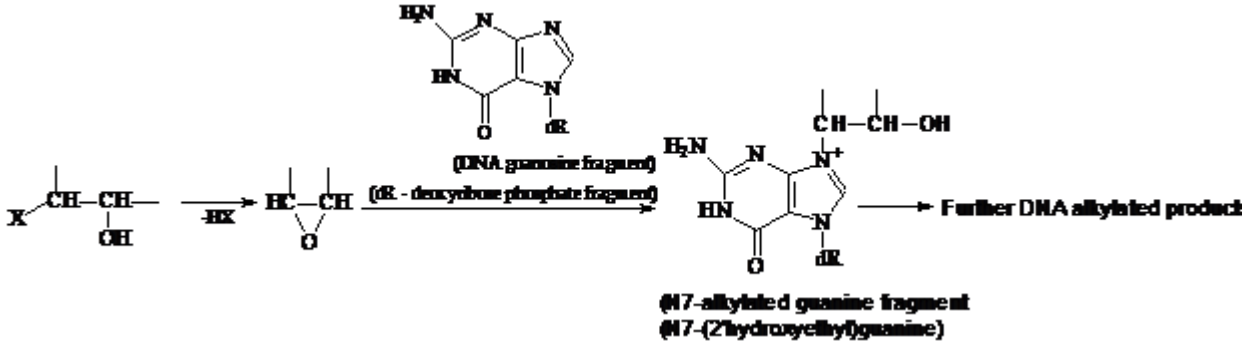


(Y can be C{sp3} or -H)

(X = Cl, Br, J)



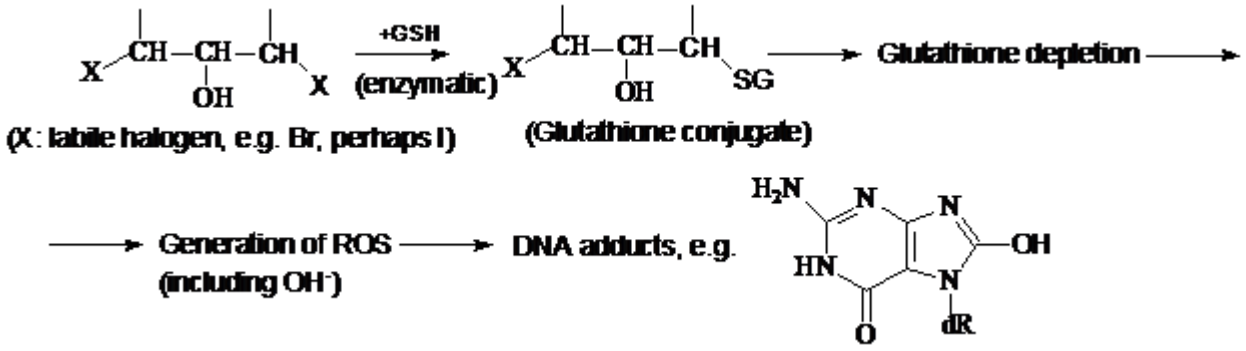
The metabolism of 1,3-dichloropropan-2-ol is likely to produce a reactive epoxide intermediate that could damage DNA, and this compound was found to be mutagenic to *Salmonella typhimurium* strains TA1535 and/or TA 100. 2,3 Dichloropropan-1-ol, on the other hand, was also mutagenic *in vitro* in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation [1]. The formation of epoxide intermediate (mutagenicity alert group) can be influenced by *haloalcohol dehalogenases* which are bacterial enzymes that catalyze the cofactor-independent dehalogenation of vicinal haloalcohols. Typical example in this respect is again the genotoxic environmental pollutant 1,3-dichloro-2-propanol, which produces epoxide, chloride ion and proton [2]. Then the epoxide is likely to exert its DNA alkylation capability [3]:



Some authors have assumed genotoxicity mechanism, associated with glutathione depletion as glutathione S-transferase was used as the enzyme source, especially with bromohydrins such as 1,3-dibromopropanol [4]. It is likely that the protection afforded by glutathione against the

toxicity of this chemical is mediated through the activity of cytosolic glutathione S-transferase. While 1,3-dichloro-2-propanol is relatively poor substrate for glutathione S-transferase, the dibromo-analogue causes extensive glutathione depletion [4]. According to another study, dichloropropanols such as 1,3-dichloropropan-2-ol, 2,3-dichloropropan-1-ol, 1,3-dibromopropan-2-ol, 1,4-dibromopropan-2-ol, 1-bromopropan-2-ol, other haloalcohols and their metabolites such as epichlorohydrin have been proved to deplete glutathione when incubated with liver fractions obtained from rats. However, difluoropropanols did not deplete glutathione [5].

It is therefore expertly assumed that glutathione depletion would further give rise to formation of ROS and DNA adducts:



Such mechanistic scheme could also apply to haloalcohols of the type:

$$X—(CH_2)_n—OH$$

(X is Cl, Br, n = 3 - 10)

REFERENCES

1. *Carcinogenicity of 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3-Dichloropropan-1-ol (2,3-DCP)*, Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, COC/04/S2 – June 2004; http://www.iacoc.org.uk/statements/statement123dichloropropanjune2004.htm

2. De Jong, R. M., J. J. W. Tiesiga, H. J. Rozeboom, K. H. Halk, L. Tang, D. B. Janssen, B. W. Dijkstra, *Structure and Mechanism of Bacterial Haloalcohol Dehalogenase: A New Variation of the Short-Chain Dehydrogenase/Reductase Fold Without an NAD(P)H Binding Site,* The EMBO Journal **22**(19) (2003), 4933 – 4944.

3. Saha, M., A. Abushamaa, R. W. Giese, *General Method for Determining Ethylene Oxide and Related N7-Guanine DNA Adducts by Gas Chromatography-Electron Capture Mass Spectrometry*, J. Chromatogr. A **712** (1995), 345 – 354.

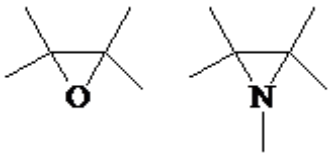
4. Hammond, A. H., M. J. Garie, J. R. Fry, *The Nature of Halogen Substitution Determines the Mode of Cytotoxicity of Halopropanols*, Toxicol. Appl. Pharmacol. **155**(3), 1999, 287-291.

5. Garle, M. J., C. Sinclair, P. Thurley, J. R. Fry, *Haloalcohols Deplete Glutathione When Incubated with Fortified Liver Fractions*, Xenobiotica **29**(5) (1999), 533 – 545.

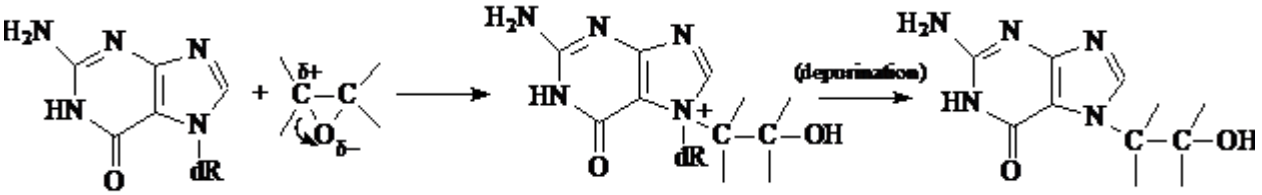
Mechanistic Domain: S_N2
Mechanistic Alert: Alkylation, direct acting epoxides and related

Structural alert: Epoxides and Aziridines

Principal structural fragments:

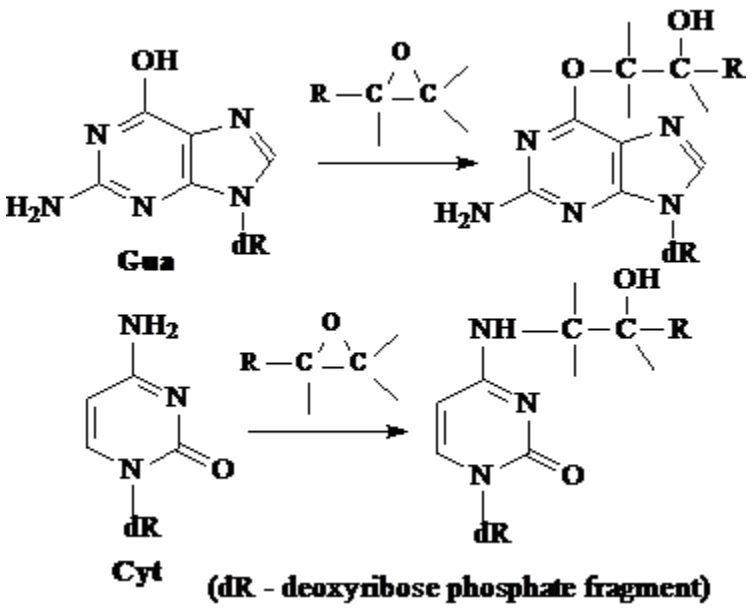


Mono-substituted epoxides have two elecrophilic carbon atoms (α- and β-carbons). The site of nucleophilic attack on alkyl epoxides under physiological conditions occurs principally at the less substituted, sterically more accessible β-carbon atom. However, epoxides with vicinal aromatic or vinyl group such as styrene epoxide and butadiene monoepoxide can react through both C-atoms, due to substituent effects leading to increase of positive charge on the α-carbon. Under physiological conditions, the main alkylation sites for simple alkyl epoxides are N7 guanine, N1-and N3-adenine, and N3-cytosine, since these heterocyclic ring nitrogens are the most nucleophilic sites for attack. Therefore, for the epoxide structural fragments, *direct alkylation* mechanisms have been proposed. Epoxides such as ethylene oxide, propylene oxide and glycydol are known carcinogens that are widely used in industrial chemistry. Mutagenic and carcinogenic epoxides can also be formed metabolically from alkenes such as ethylene, butadiene, propylene and styrene and from vinyl monomers like acrylonitrile and acrylamide. Simple epoxides react with nucleic acid bases to form 2-hydroxy-2-alkyl adducts. These are fairly unstable, due to the presence of a charged quaternary nitrogen at the site of alkylation and frequently undergo "depurination" to remove the charge. This leads to the formation of highly mutagenic sites. The final form of adduct is uncharged and stable. It is mutagenic and contributes to the toxicological hazards of exposure to simple epoxides [1]:

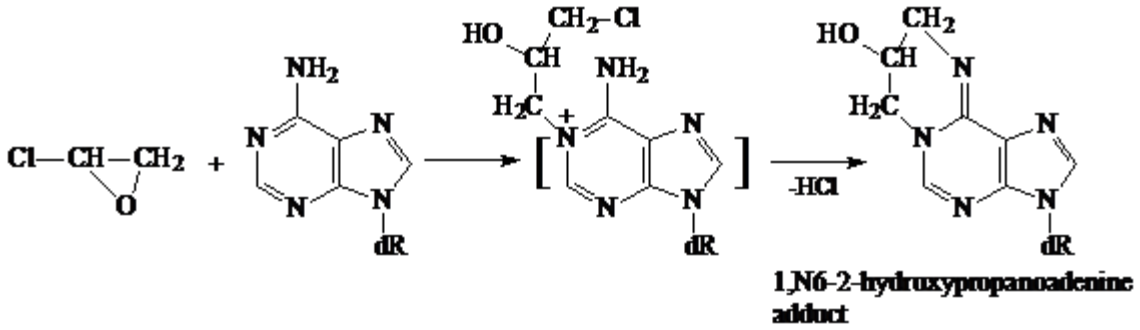


(DNA fragment)
(dR - deoxyribose phosphate fragment)

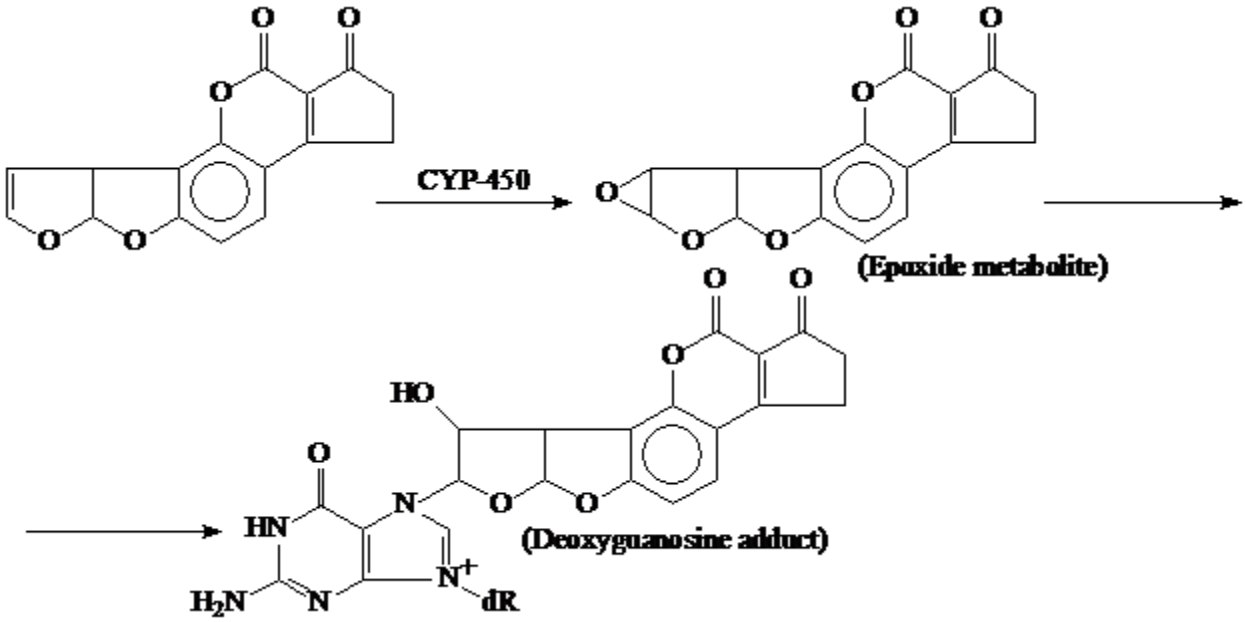
Epoxides such as styrene oxide and butadiene monoepoxide can also modify exocyclic groups as shown below [1]:



However, epichlorohydrin, unlike many other epoxides, acts as bifunctional alkylating agent. For alkylation of adenine, for example, the epoxide undergoes first ring opening; then cyclization and loss of HCl *via* the attack on N6, and the carbon, carrying chlorine contributes to the formation of 1,N6-2-hydroxypropanoadenine adduct [2]:



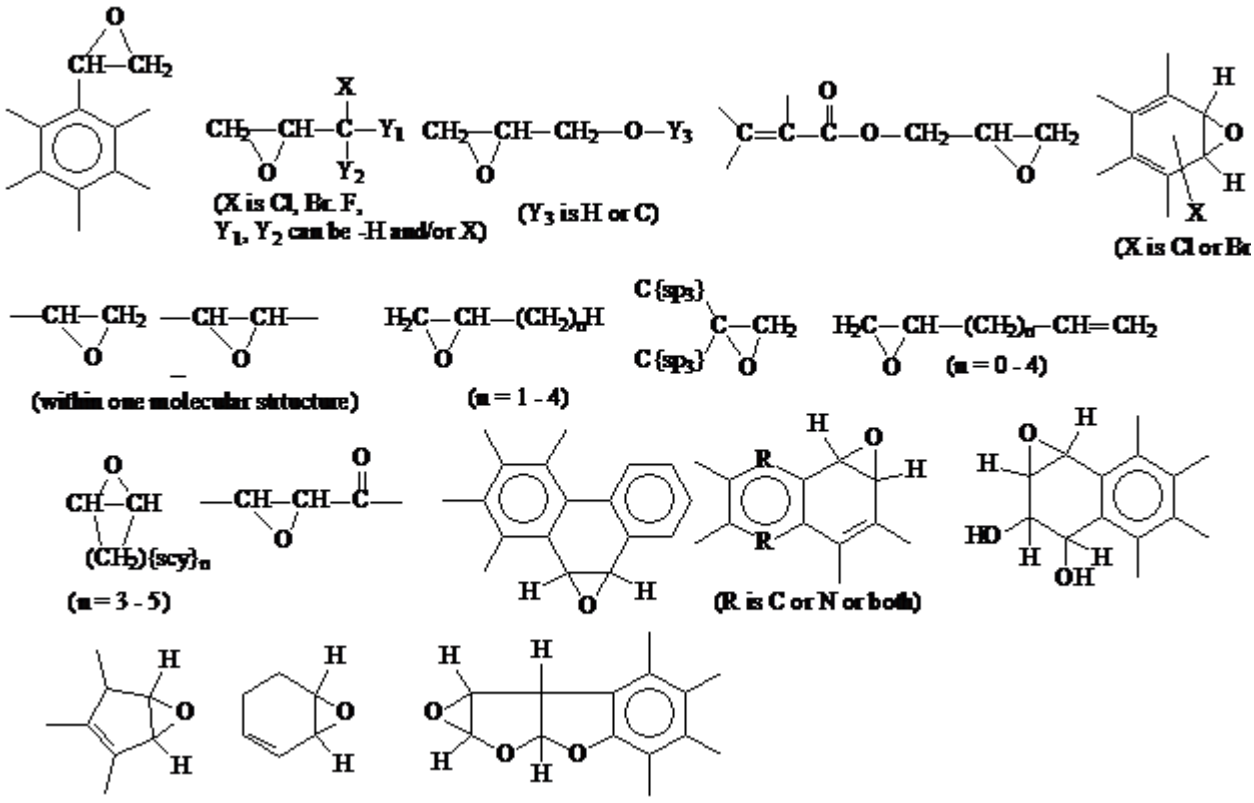
In substructures, about which more than ten test results have been reported, the epoxide fragment is most highly correlated with mutagenicity, and the percentage of positive compounds was found to be 65 – 70. Therefore, compounds that contain or are metabolically activated to products with the highly-strained epoxide fragment in the structure can be considered as suspect carcinogens [3]. For example, the well-known carcinogen (and bacterial mutagen) Aflatoxin B1 is metabolically activated to aflatoxin B1 exo-8,9-epoxide by CYP 450 monooxygenase, which then reacts with N7 of guanine to form the primary DNA adduct [4]:



The mutagenicity of a series of 13 epoxide compounds has been studied. Mono-substituted epoxides such as allyl glycidyl ether, n-butyl glycidyl ether, vinyl cyclohexene diepoxide, glycidol, glycidaldehyde, diglycidyl ether, diepoxybutane and diglycidyl ether of substituted glycerine were mutagenic in TA100 strain. The mutagenic compounds had linear structure, without any groups causing steric hindrance. On the contrary, dieldrin was inactive, due to its relatively high molecular weight, specific molecular structural envelope, and, possibly, large number of chlorine atoms causing excessive hydrophobicity. Higher-molecular weight derivatives of diglycidyl bisphenol-A were also negative in the *Ames* bacterial mutagenicity test, due to the high molecular mass and branched structure [5]. Similar results, associated with the molecular structure of epoxides were obtained, according to another publication, dealing with 45 epoxides of large structural diversity, of which mono-substituted epoxides such as epichlorohydrin, epibromohydrin, 1,2-epoxibutane, propylene oxide, glycidol, glycidaldehyde, 1,2,3,4-diepoxybutane, styrene oxide, etc. produced positive *Ames* test results [6].

According to another publication, halogenated derivatives of simple monosubstituted epoxides such as epichlorohydrin and epibromohydrin had much stronger DNA alkylating capability and bacterial mutagenicity than propylene oxide, butylene oxide, etc. The electron-withdrawing effects of the haloalkyl groups in the halogenated derivatives apparently contributes to higher epoxide reactivity [7].

The *in vitro* genotoxicity of 51 epoxides has been studied by employing the *Ames* test with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. Approximately, 75 – 80 % of all tested epoxides (mostly mono-substituted ones) were found to show positive bacterial mutagenicity [8], and their principal sub-structures are given below:

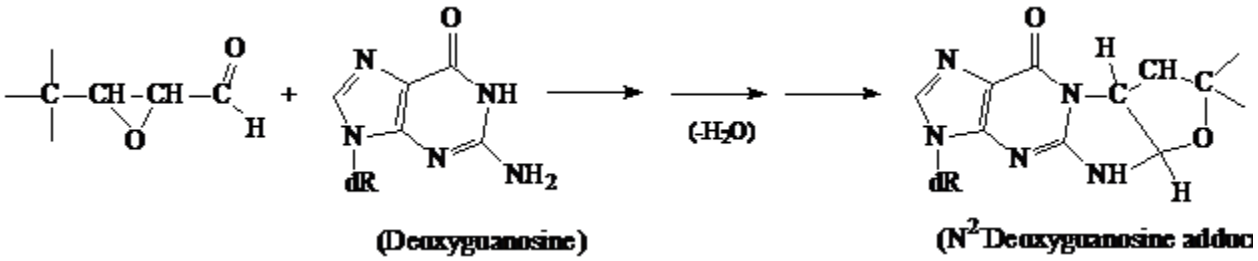


1,2-Disubstituted cycloaliphatic epoxides with one or two oxirane ring fragments such as cyclopentane- and cyclohexane oxide, diepoxycyclooctane, vinylcyclohexane dioxide, cyclopentene- and cyclohexene-3,4-epoxides, etc., were also found to show, at least, weak bacterial mutagenicity effects in the base-pair indicator strains TA100 and TA1535 [9]. Some 1,1- and 1,2-disubstituted epoxides such as 2,3-epoxybutane and oxaspiro-epoxides have also shown *Ames* mutagenicity, and the structural requirements associated with minimal steric hindrance in the vicinity of epoxide group fully apply [10, 11].

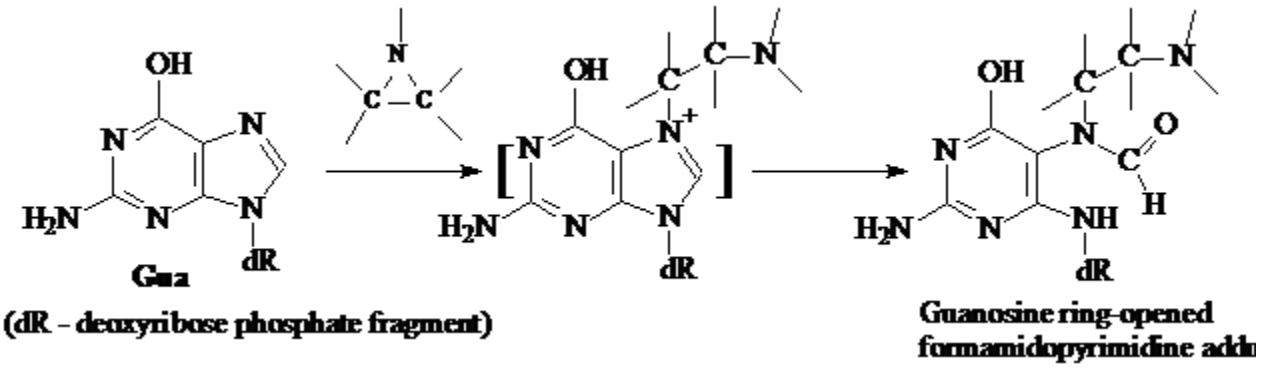
According to another publication, none of the tested chemicals such as epoxysteroids, vitamin K epoxides, pesticides (dieldrin, eldrin, heptachlor epoxides), and, also, some natural products, metabolites and antibiotics such as oleandomycin, anticapsin and asperlin, carbamazepine-9,10-oxide, diethylstilbestrol-α,β-oxide, scopolamine, etc. showed any mutagenic activity in the *Ames* test. This indicates that epoxides with complex molecular structures and more than one substituent at the epoxide moiety, which are relatively more inert and stable, and, especially, more sterically hindered, are inactive in the *Ames* mutagenicity assay. In contrast, benzo[a]pyrene 4,5-oxide, benzo[e]pyrene 4,5-oxide and 7,12-dimethylbenz[a]-anthracene 5,6-oxide, derived from polycyclic aromatic hydrocarbons (PAH) are potent mutagens [12].

Halogenated epoxides as metabolites of some haloalkenes such as vinylidene chloride and vinyl chloride, are also bacterial mutagens [13]. Non-allylic chloropropenes and their homologues, being chloro-substituted in vinyl position are mutagenic in the presence of S9 metabolic activation system. This has been explaineded by the polarizing –I and M-effects of halogen and the alkyl substituents bound to the C=C bond. The metabolic activation of such chemicals was associated with the epoxide formation [14]. In these cases, the lack of significant steric hindrance effects is essential for the mutagenic activity.

According to another publication, 2,3-epoxyaldehydes are also genotoxic chemicals. These compounds may exist as parents but, more probably, can be formed as metabolites of *alpha-beta*-unsaturated aldehydes. The structure of their adducts with guanosine can be outlined as follows [15]:



Aziridine (ethyleneimine) reacts with DNA *in vitro* mainly at the N7 position of guanine and N3 of adenine; then imidazole ring opening of the modified guanine fragment results in the formation of formamidopyrimidine adducts [16]. Possible scheme for the formation of such adducts [17] is outlined below:

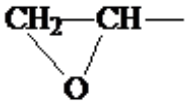


Arene imines, also containing aziridine structural fragment were shown to be very strong mutagens in bacterial and mammalian cells [18].

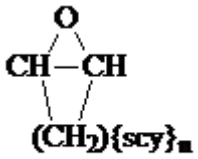
<u>Conclusions:</u>

I. **Structurally generalized active alerting groups for epoxides:**

- <u>Monosubstituted epoxides</u>:

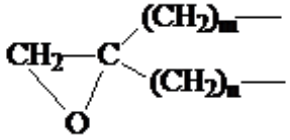


- <u>Simple cycloaliphatic epoxides:</u>

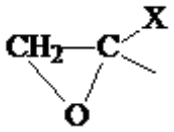


(n = 2 - 6)

- 1,1-Disubstituted epoxides and spiro-epoxides:



(m = 1 - 3; n = 1 - 3)
(If (CH_2) is acyclic, the terminal group is $-CH_3$)

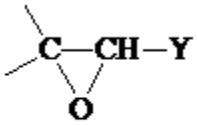


(X is Cl or Br

- 1,2-Disubstitured epoxides (including cycloaliphatic epoxides):

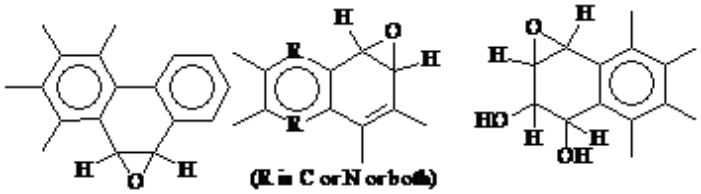
Y_1 and Y_2 can be the following structural moeties:
(a) $(-CH_2)_nH$ (n = 1 - 2)
(b) CH_2{scy} and -CH{scy}=CH{scy}-
(c) -CH{sp3}{scy} and O{scy} or -NH{scy}
(d) Y_1 is Cl or Br; Y_2 is C

- Other terminal polarized epoxides:



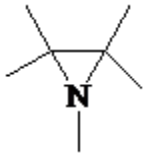
(Y can be Cl, Br or -CHO)

- Polycyclic Aromatic Hydrocarbons (PAH)-derived epoxides:



(R is C or N or both)

II. **Structurally-generalized active alerting groups for aziridines:**



REFERENCES

1. Koskinen, M., K. Plna, *Specific DNA Adducts Induced by Some Mono-Substituted Epoxides In Vitro and In Vivo*, Chem.-Biol. Interact. **129** (2000), 209 – 229.
2. Singh, U. S., K. D. Samuelian, J. J. Solomon, *Reaction of Epichlorohydrin with 2'-Deoxynucleosides: Characterization of Adducts*, Chem. Biol. Interact. **99** (1996), 109 – 128.
3. Sawatari, K., Y. Nakanishi, T. Matsushima, *Relationship Between Chemical Structures and Mutagenicity: A Preliminary Survey for a Database of Mutagenicity Test Results of New York Place Chemicals*, Industrial Health **39** (2001), 341 – 345.
4. Raney, V. M., Th. M. Harris, M. P. Stone*, DNA Conformation Mediates Aflatoxin B_1 – DNA Binding and the Formation of Guanine N_7 Adducts by Aflatoxin B1 8,9-exo-Epoxide*, Chem. Res. Toxicol. **6** (1993), 64 – 68.
5. Wade, M. J., J. W. Moyer, Ch. H. Hine, *Mutagenic Action of a Series of Epoxides*, Mutat. Res. **66** (1979), 367 – 371.
6. Voogd, C. E., J. J. van der Stel, J. J. J. A. A. Jacobs*, The Mutagenic Action of Aliphatic Epoxides*, Mutat. Res. **89** (1981), 269 – 282.
7. Hemminki, K., K. Falck, H. Vainio, *Comparison of Alkylation Rates and Mutagenicity of Directly Acting Industrial and Laboratory Chemicals: Epoxides, Glycidyl Ethers, Methylating and Ethylating Agents, Halogenated Hydrocarbons, Hydrazine Derivatives, Aldehydes, Thiuram and Dithiocarbamate Derivatives*, Arch. Toxicol. **46** (1980), 277 – 285.

8. Von der Hude, W., A. Seelbach, A. Basler, Epoxides: *Comparison of the Induction of SOS Repair in Escherichia coli PQ37 and the Bacterial Mutagenicity in the Ames Test*, Mutat. Res. **231** (1990), 205 – 218.

9. Frantz, S. W., J. E. Sinsheimer, *Bacterial Mutagenicity and Toxicity of Cycloaliphatic Epoxides*, Mutat. Res. **90** (1981), 67 – 78.

10. Meester, C. De, *Increase of 2,3-Epoxybutane Mutagenicity by Glutathione-S-Transferases*, Toxicol. Lett. **224** (1984), 255 – 262.

11. Sinsheimer, J. E., P. K. Chakraborty, E. A. Messerly, V. Gaddamidi, *Mutagenicity of Oxaspiro Compounds with Salmonella*, Mutat. Res. **224** (1989), 171 – 175.

12. Glatt, H., R. Jung, Fr. Oesch, *Bacterial Mutagenicity Investigation of Epoxides: Drugs, Drug Metabolites, Steroids and Pesticides*, Mutat. Res. **11** (1983), 99 – 118.

13. *Vinylidene Chloride*, Chemical Carcinogenesis Research Informartion System (CCRIS), US National Library of Medicine; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS.

14. Neudecker, T., W. Dekant, M. Jorns, E. Eder, D. Henschler, *Mutagenicity of Chloroolefins in the Salmonella/Mammalian Microsome Test – II. Structural Requirements for the Metabolic Activation of Non-Allylic Chloropropenes and Methylated Derivatives via Epoxide Formation*, Biochem. Pharmacol. **35**(2) (1986), 195 – 200.

15. Petrova, K. V., R. S. Jalluri, I. D. Kozekov, C. J. Rizzo, *Mechanism of 1,N^2-Etheno-2'-Deoxyguanosine Formation from Epoxyaldehydes*, Chem. Res. Toxicol. **20** (2007), 1685 – 1692.

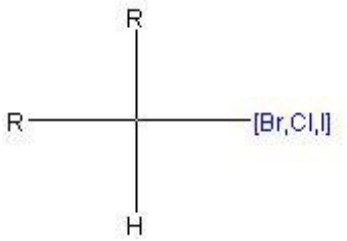
16. Cussac, C., F. Laval, *Reduction of the Toxicity and Mutagenicity of Aziridine in Mammalian Cells Harboring the Eschericia coli frg Gene*, Nucleic Acids Res. **24**(9) (1996), 1742 -1746.

17. Tudek, B., *Imidazole Ring-Opened DNA Purines and Their Biological Significance*, J. Biochem. Molec. Biol. **36**(1) (2003), 12 – 19.

18. Glatt, H., G. Ludewig, K. L. Platt, et al., *Arene Imines, a New Class of Exceptionally Potent Mutagens in Bacterial and Mammalian Cells*, Canc. Res. **45** (1985), 2600 – 2607.

F. Profiler: In vivo mutagenicity (Micronucleus) alerts by ISS

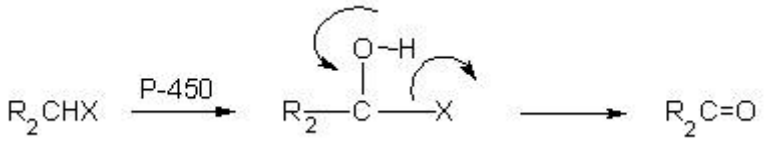
Aliphatic halogens



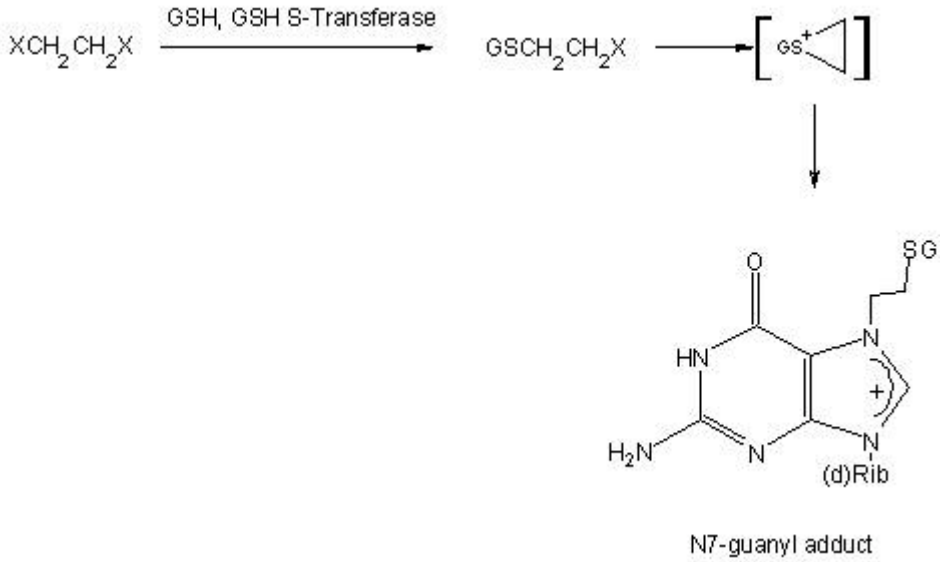
R = any atom/group

Numerous haloalkanes have been tested for carcinogenic and mutagenic activities. In general, the genotoxic potential is dependent on the nature, number, and position of halogen(s) and the molecular size of the compound (Woo *et al.* 2002). Although some aliphatic halogens have been shown to directly alkylate macromolecules (Bolt and Gansewendt 1993), biotransformation may also play an important role in their toxicity. Cytochrome P450 oxidation may produce gem-halohydrins that spontaneously dehydrohalogenate to reactive carbonyl compounds (Guengerich 1991), (see reaction 1). Alternatively, glutathione (GSH) conjugation via GSH transferases, has been proposed as an activation mechanism for several halogenated alkanes (Guengerich 2003b); (Guengerich 2003a) (as an example for dihaloethanes see Reaction 2).

1.



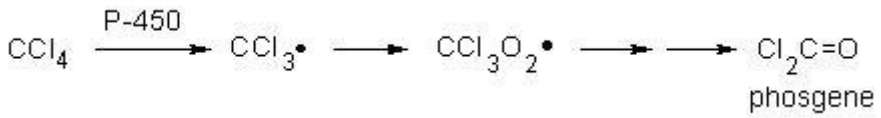
2.



N7-guanyl adduct

Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms (Woo *et al.* 2002). In the case of CCl4 (see reaction 3.), P450 reduces CCl4 to the trichloromethyl radical which can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes.

3.



Adduct formation between CCl3* and DNA is thought to function as initiator in the case of hepatic cancer. This radical can also react with oxygen to form highly reactive species, the trichloromethylperoxy radical CCl3OO*, that may initiate the chain reaction of lipid peroxidation, and ultimately generate phosgene (Guengerich 1991).

References Cited

Bolt, H. M. and Gansewendt, B. (1993). Mechanisms of Carcinogenicity of Methyl Halides. *Crit.Rev.Toxicol.* 23, 237-253.

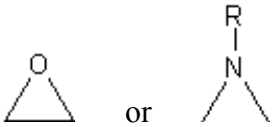
Guengerich, F. P. (1991). Oxidation of Toxic and Carcinogenic Chemicals by Human Cytochrome P-450 Enzymes. *Chem.Res.Toxicol.* 4, 391-407.

Guengerich, F. P. (2003a). Activation of Dihaloalkanes by Thiol-dependent Mechanisms. *J.Biochem.Mol.Biol.* 36, 20-27.

Guengerich, F. P. (2003b). Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. Arch.Biochem.Biophys. 409, 59-71.

Woo, Y. T., Lai, D. Y., McLain, J. L., Ko Manibusan, M., and Dellarco, V. (2002). Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ.Health Perspect. 110, 75-87.

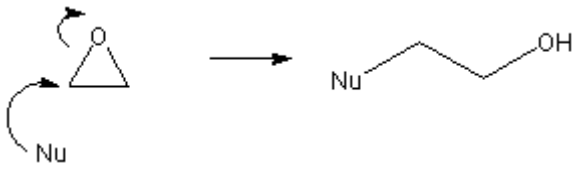
Epoxides and Aziridines



or

R = any atom/group

Due to the large ring strain associated with the three-membered ring, epoxides are highly reactive molecules. They may react with nucleophilic centers of DNA molecules, giving rise to alkylated products.



Because of their reactivity they are important intermediates in chemical industry, especially in polymer production. Furthermore, epoxides may be produced endogenously by the enzymatic oxidation of other chemicals, many of which are common environmental pollutants (such as PAH, alkenes). Consequently considerable human exposure arises. The most likely route of exposure to these agents is by inhalation, although the possibility of dermal and oral absorption should also be considered. The site of alkylation of the DNA constituents is mainly determined by the ionic character of the epoxide (Koskinen and Plná 2000); (Barlow and Dipple 1998) Reactions at the ring nitrogen positions follow a bimolecular displacement mechanism whereas modification of the exocyclic groups requires some degree of substrate ionization for reaction to occur (Barlow and Dipple 1998). Simple alkyl epoxides, that are not able to stabilize an ionic charge to any great extent, react predominantly at endocyclic base nitrogens, giving rise, preferentially, to •-hydroxyethyl derivatives of cytosine-N3, adenine-N1 and N3, and guanine-N7. Molecules that are more efficient in stabilizing an ionic charge, may modify also exocyclic groups (i.e., styrene oxide, butadiene monoepoxide, PAH (Koskinen and Plná 2000); (Barlow and Dipple 1998). Aziridines are extremely reactive alkylating agents that may react by ring-opening reactions similar to those of epoxides. There are several classes of aziridine-containing natural products that exhibit potent biological activity. Among them, the mitomycins, that exhibit both anti-tumour and antibiotic activity (Sweeney 2002). Another class of naturally-occurring aziridine derivatives possessing potent cytotoxic and antitumor activities, is the Azinomycin family. The activity of these compounds lies in their ability to act as DNA cross-linking agents, *via* nucleophilic ring-opening of the aziridine and epoxide moieties by N-7 positions of purines (Zang and Gates 2000). It is not clear at present which ring opening reaction takes precedence in the cross-linking event. The PBI (pyrrolo[1,2-a]benzimidazole) class of natural products, represents another type of DNA-alkylating species containing an aziridine moiety. In these compounds, the aziridine undergoes ring-opening by nucleophilic attack of the DNA phosphate backbone, resulting in formation of a hydrolytically labile phosphotriester (that may eventually cause DNA cleavage) (Schultz *et al.* 1995).

References Cited

Barlow, T. and Dipple, A. (1998). Aralkylation of guanosine with para-substituted styrene oxides. *Chem.Res.Toxicol.* 11, 45-53.

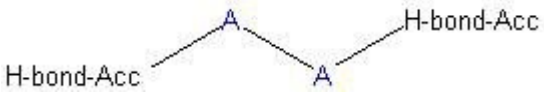
Koskinen, M. and Plná, K. (2000). Specific DNA adducts induced by some mono-substitued epoxides in vitro and in vivo. *Chem.Biol.Interact.* 129, 209-229.

Schultz, W. G., Nieman, R. A., and Skibo, E. B. (1995). Evidence for DNA phosphate backbone alkylation and cleavage by pyrrolo[1,2-albenzimidazoles: Small molecules capable of causing base-pair-specific phosphodiester bond hydrolysis. *Proc.Natl.Acad.Sci.USA* 92, 11854-11858.

Sweeney, J. B. (2002). Aziridines: epoxides' ugly cousins? *Chem.Soc.Rev.* 31, 247-258.

Zang, H. and Gates, K. S. (2000). DNA Binding and Alkylation by the "Left Half" of Azinomycin B. *Biochemistry* 39, 14975.

Hacceptor-path3-Hacceptor



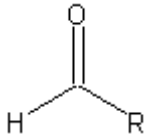
A= Any atom, except Hydrogen
H-bond-Acc= Any atom that is a potential Hydrogen bond acceptor

This alert explores the possibility that a chemical interacts with DNA and/or proteins *via* non-covalent binding, such as DNA intercalation or groove-binding (Snyder et al. 2006). Among the descriptors potentially accounting for non-covalent interactions, the present molecular framework representing two bonded atoms connecting two H bond acceptors (calculated with software Leadscope Enteprise 2.4.15-6) resulted in an increased sensitivity/specificity for what concerns the Micronucleus training set.

References Cited

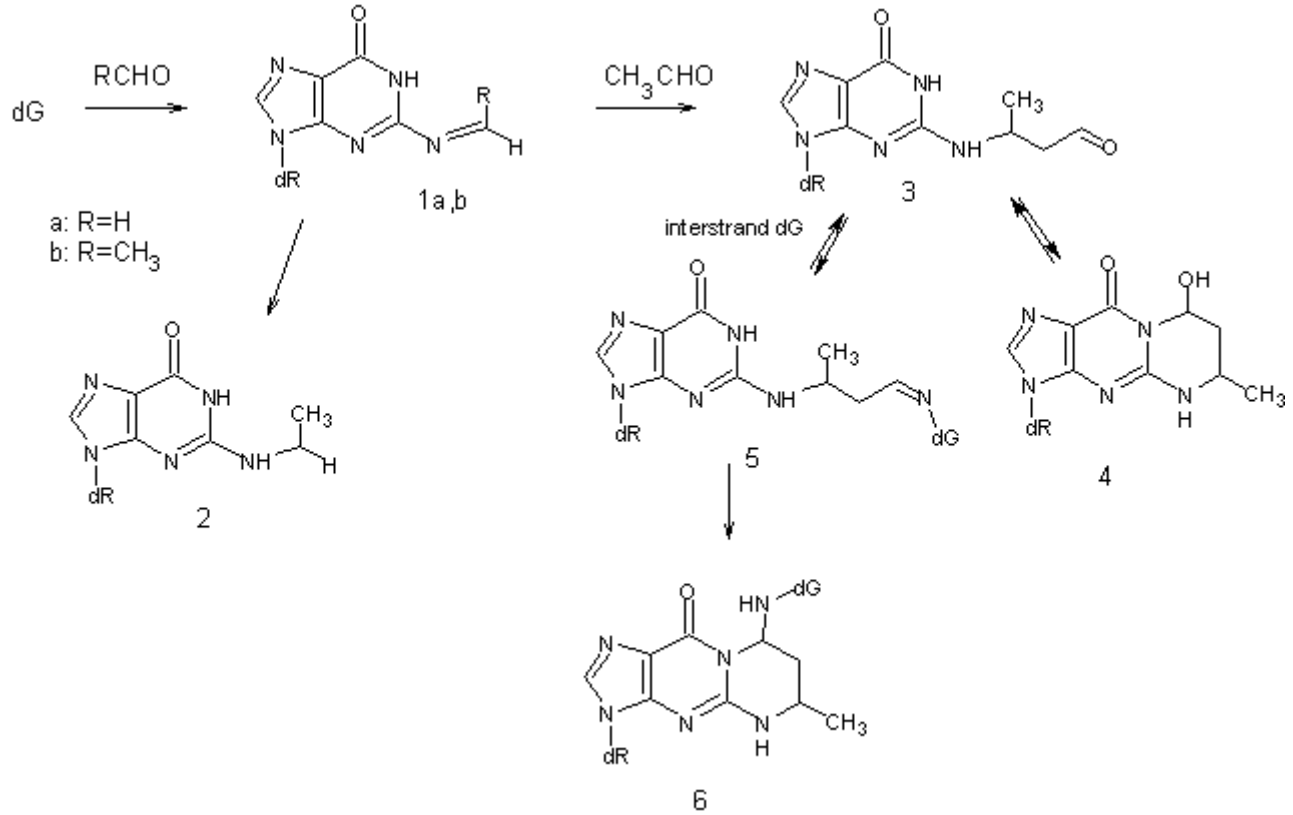
Snyder, R. D., Ewing, D. and Hendry, L. B. 2006. DNA intercalative potential of marketed drugs testing positive in *in vitro* cytogenetics assays. *Mutat. Res.* 609, 47-59.

Simple Aldehydes



R= aliphatic or aromatic carbon
alpha, beta-unsaturated aldehydes are excluded

All compounds carrying an aldehydic group can potentially undergo Schiff base formation with a primary amine. They are to be considered potentially genotoxic, as demonstrated *in vivo* ability to react with nucleobases, without metabolic activation, forming adducts, interbase cross-links (both intra and inter-strand), and DNA-protein crosslinks The length of carbon chain for aliphatic aldehydes, and in general molecular size, can strongly modulate the formation of every type of cross-link and even the accessibility of the DNA nucleobases (Romano Zito, personal communication). DNA-protein crosslinks have been reported as the primary DNA damage induced by formaldehyde (Speit *et al.* 2007). The initial step of the reaction probably involves formation of an unstable Schiff base with the exocyclic amino group of deoxyguanosine dG (1a). In the case of acetaldehyde, this intermediate (1b) could be stabilized by reduction, producing *N*2-ethyl-dG (2), or alternatively may react with a second molecule of acetaldehyde forming a new aldehyde adduct (3) that ultimately cyclize in an 8-hydroxypropano adduct (4). The latter exists in equilibrium with its ring-opened aldehyde form, and may undergo condensation with another guanine to form imine-linked bis-nucleoside (5) which in turn cyclizes to pyrimidopurinone (6) (Wang *et al.* 2000).



Some aldehydes may also induce hydroxyalkyl adducts in DNA, but the relevance of these DNA modifications for mutagenicity is unclear (Speit *et al.* 2007).

References Cited

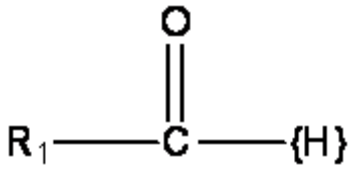
Speit, G., Schütz, P., Högel, J., and Schmid, O. (2007). Characterization of the genotoxic potential of formaldehyde in V79 cells. *Mutagenesis* 22, 387-394.

Wang, M., McIntee, E. J., Cheng, G., Shi, Y., Villalta, P. W., and Hecht, S. S. (2000). Identification of DNA Adducts of Acetaldehyde. *Chem.Res.Toxicol.* 13, 1149-1157.

G. Profiler: Oncologic Primary Classification

Category: Aldehyde Type Compounds

Component: Aldehyde



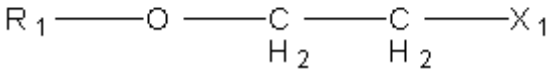
R_1: aliphatic (alkyl chain);
alicyclic (cycloaliphatic);
aromatic (aromatic ring system 1-2 rings);
other types (H, benzyl, phenylethyl, COOH, COO-, C(O)Cn, etc.).

Substituents: The following substituents may be placed on R1 groups: hydroxyl (OH), carboxylic acid (COOH), sulfonic acid (SO3H), halogens (Cl, Br, I, F), Other, and, additionally, alkyl (Cn) groups on aromatic rings.

Exceptions: Heteroatoms can not replace carbon atoms in the alkyl chain nor can oxo groups be added. The degradation products of these compounds should be considered separately. If the R group contains heteroatoms and degradation products are not known, replace the heteroatoms with carbon atoms.

Category: Alpha, beta-Haloether Reactive Functional Groups

Component: : alpha-beta-Haloether (direct-acting alkylating agent)



R_1 : aliphatic (alkyl chain);
alicyclic (cycloaliphatic);
aromatic (aromatic ring system 1-4 rings);
other types (H, benzyl, phenylethyl)

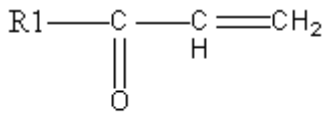
X_1 : F, Cl, Br, I

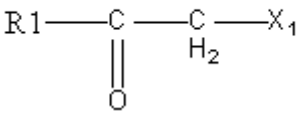
Substituents: The following substituents may be placed on alkyl R groups : hydroxyl (OH), carboxylic acid (COOH), sulfonic acid (SO3H), halogens (Cl, Br, I, F), and Other. In addition to these, aryl R group my have vinyl and allyl groups as well as alkyl (Cn) on aromatic rings. The methylene/ethylene moiety (C-X/C-C-X) may be substituted with the following: OH, COOH, SO3H, Cl, Br, I, F, Cn.

Exceptions: Additional substituents, may not be added to alkyl chains of the methylene/ethylene moiety. Heteroatoms can not be replace the carbon atoms in the R1 alkyl chain nor can keto groups be added

Category: Reactive Ketone Reactive Functional Groups

Component: Reactive Ketones (direct-acting alkylating agent)





R_1: aliphatic (alkyl chain)
alicyclic (cycloaliphatic)
aromatic (aromatic ring system 1-4 rings)
other types (benzyl, phenylethyl)

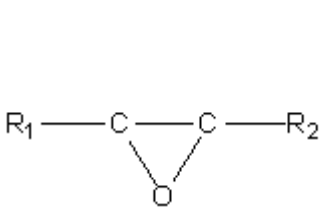
X_1: Must be replaced with a halogen

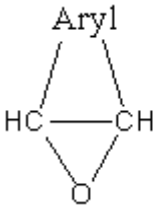
Substituents: The following substituents may be placed on alkyl R groups: hydroxyl (OH), carboxylic acid (COOH), sulfonic acid (SO3H), halogens (Cl, Br, I, F), and Other. In addition to these, aryl R group my have vinyl and allyl groups as well as alkyl (Cn) on aromatic rings. The methylene/ethylene moiety (C-X/C=C) may be substituted with the following: OH, COOH, SO3H, Cl, Br, I, F, Cn, and Other.

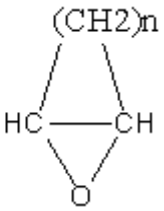
Exceptions: Unsaturated alkyl chains, other than vinyl and allyl, are treated as saturated alkyl groups. Additional substituents, may not be added to alkyl chains of the methylene/ethylene moiety. Heteroatoms can not be replace the carbon atoms in the R1 alkyl chain nor can keto groups be added.

Category: Epoxide Reactive Functional Groups

Component: Epoxides (direct-acting alkylating agent)







R_1/R_2: aliphatic (alkyl chain);
alicyclic (cycloaliphatic);
aromatic (aromatic ring system 1-4 rings);
other types (H, benzyl, phenylethyl)

Substituents: The following substituents may be placed on alkyl R groups : hydroxyl (OH), carboxylic acid (COOH), sulfonic acid (SO3H), halogens (Cl, Br, I, F), and Other. In addition to these, aryl R group may have vinyl and allyl groups as well as alkyl (Cn) on aromatic rings. The epoxide carbons may be substituted with the following: OH, COOH, SO3H, Cl, Br, I, F, alkyl (Cn), alkoxy (OCn) and acyloxy (O(O)Cn).

H. Profiler: Carcinogenicity (genotox and nongenotox) alerts by ISS

(Poly) Halogenated Cycloalkanes (nongenotoxic)

Any cycloalkane skeleton with three or more halogens directly bound to the same ring
The mechanisms of carcinogenic action of this class of compouds is unclear. Several possible epigenetic mechanisms have been proposed which include (i) inhibition of intercellular communication, (ii) degranulation of the rough endoplasmic reticulum, and (iii) hormonal imbalance (Woo and Lai 2005);(Woo *et al.* 1995).
Production of reactive oxygen species by organochlorine pesticides has been also implicated in the toxicity and carcinogenicity of these compounds; however, the mechanism by which these agents stimulate the production of oxygen radicals is unknown (Tithof *et al.* 2000). Among them, Dieldrin, an organochlorine insecticide, has also been demonstrated to be genotoxic and evidences of relationships between genotoxicity and oxidative stress have been reported (Cicchetti and Argentin 2003).

References Cited

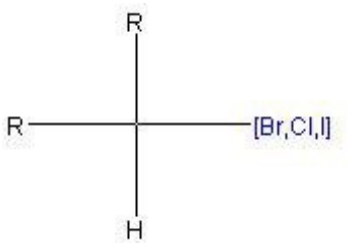
Cicchetti, R. and Argentin, G. (2003). The role of oxidative stress in the *in vitro* induction of micronuclei by pesticides in mouse lung fibroblasts. *Mutagenesis* **18**, 127-132.

Tithof, P. K., Olivero, J., Ruehle, K., and Ganey, P. E. (2000). Activation of Neutrophil Calcium-Dependent and -Independent Phospholipases A2 by Organochlorine Compounds. *Toxicol.Sci.* **53**, 40-47.

Woo, Y. T. and Lai, D. Y. (2005). Oncologic: a mechanism based expert system for predicting the carcinogenic potential of chemicals. In 'Predictive toxicology.' (Ed C. Helma.) pp. 385-413. (Taylor and Francis: Boca Raton.)

Woo, Y. T., Lai, D. Y., Argus, M. F., and Arcos, J. C. (1995). Development of structure-activity relationship rules for predicting carcinogenic potential of chemicals. *Toxicol.Lett.* **79**, 219-228.

Aliphatic halogens (genotoxic)

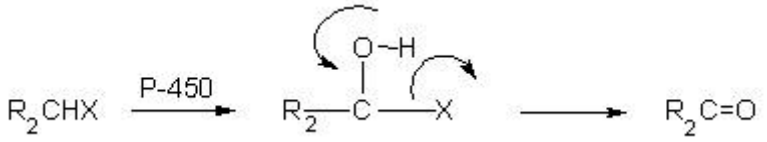


R = any atom/group

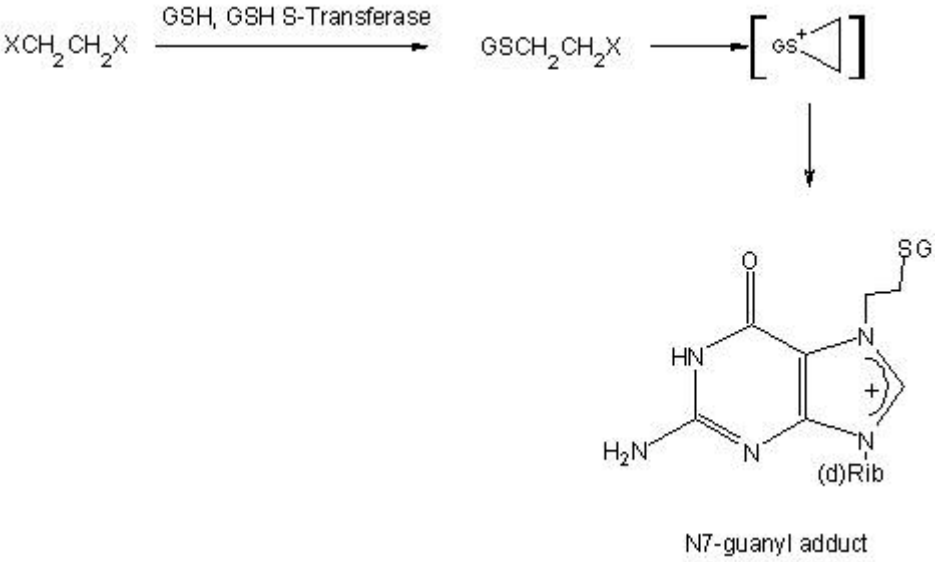
Numerous haloalkanes have been tested for carcinogenic and mutagenic activities. In general, the genotoxic potential is dependent on the nature, number, and position of halogen(s) and the molecular size of the compound (Woo *et al.* 2002). Although some aliphatic halogens have been shown to directly alkylate macromolecules (Bolt and Gansewendt 1993), biotransformation may also play an important role in their toxicity. Cytochrome P450 oxidation may produce gem-halohydrins that spontaneously dehydrohalogenate to reactive carbonyl compounds (Guengerich 1991), (see reaction 1). Alternatively, glutathione (GSH) conjugation via GSH transferases, has been proposed as an activation mechanism for several halogenated alkanes (Guengerich 2003b); (Guengerich 2003a) (as an example for

dihaloethanes see Reaction 2).

1.



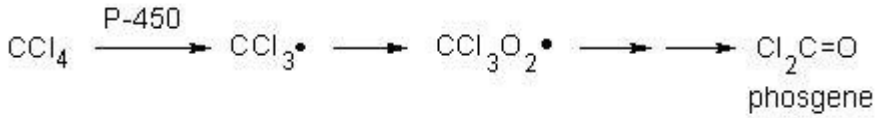
2.



N7-guanyl adduct

Fully halogenated haloalkanes tend to act by free radical or nongenotoxic mechanisms (Woo *et al.* 2002). In the case of CCl4 (see reaction 3.), P450 reduces CCl4 to the trichloromethyl radical which can bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes.

3.



Adduct formation between CCl3* and DNA is thought to function as initiator in the case of hepatic cancer. This radical can also react with oxygen to form highly reactive species, the trichloromethylperoxy radical CCl3OO*, that may initiate the chain reaction of lipid peroxidation, and ultimately generate phosgene (Guengerich 1991).

References Cited

Bolt, H. M. and Gansewendt, B. (1993). Mechanisms of Carcinogenicity of Methyl Halides. *Crit.Rev.Toxicol.* 23, 237-253.

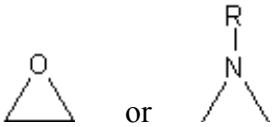
Guengerich, F. P. (1991). Oxidation of Toxic and Carcinogenic Chemicals by Human Cytochrome P-450 Enzymes. *Chem.Res.Toxicol.* 4, 391-407.

Guengerich, F. P. (2003a). Activation of Dihaloalkanes by Thiol-dependent Mechanisms. *J.Biochem.Mol.Biol.* 36, 20-27.

Guengerich, F. P. (2003b). Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. Arch.Biochem.Biophys. 409, 59-71.

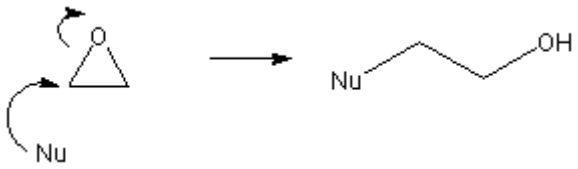
Woo, Y. T., Lai, D. Y., McLain, J. L., Ko Manibusan, M., and Dellarco, V. (2002). Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ.Health Perspect. 110, 75-87.

Epoxides and Aziridines (genotoxic)



or
R = any atom/group

Due to the large ring strain associated with the three-membered ring, epoxides are highly reactive molecules. They may react with nucleophilic centers of DNA molecules, giving rise to alkylated products.



Because of their reactivity they are important intermediates in chemical industry, especially in polymer production. Furthermore, epoxides may be produced endogenously by the enzymatic oxidation of other chemicals, many of which are common environmental pollutants (such as PAH, alkenes). Consequently considerable human exposure arises. The most likely route of exposure to these agents is by inhalation, although the possibility of dermal and oral absorption should also be considered. The site of alkylation of the DNA constituents is mainly determined by the ionic character of the epoxide (Koskinen and Plná 2000); (Barlow and Dipple 1998) Reactions at the ring nitrogen positions follow a bimolecular displacement mechanism whereas modification of the exocyclic groups requires some degree of substrate ionization for reaction to occur (Barlow and Dipple 1998). Simple alkyl epoxides, that are not able to stabilize an ionic charge to any great extent, react predominantly at endocyclic base nitrogens, giving rise, preferentially, to •-hydroxyethyl derivatives of cytosine-N3, adenine-N1 and N3, and guanine-N7. Molecules that are more efficient in stabilizing an ionic charge, may modify also exocyclic groups (i.e., styrene oxide, butadiene monoepoxide, PAH (Koskinen and Plná 2000); (Barlow and Dipple 1998). Aziridines are extremely reactive alkylating agents that may react by ring-opening reactions similar to those of epoxides. There are several classes of aziridine-containing natural products that exhibit potent biological activity. Among them, the mitomycins, that exhibit both anti-tumour and antibiotic activity (Sweeney 2002). Another class of naturally-occurring aziridine derivatives possessing potent cytotoxic and antitumor activities, is the Azinomycin family. The activity of these compounds lies in their ability to act as DNA cross-linking agents, *via* nucleophilic ring-opening of the aziridine and epoxide moieties by N-7 positions of purines (Zang and Gates 2000). It is not clear at present which ring opening reaction takes precedence in the cross-linking event. The PBI (pyrrolo[1,2-a]benzimidazole) class of natural products, represents another type of DNA-alkylating species containing an aziridine moiety. In these compounds, the aziridine undergoes ring-opening by nucleophilic attack of the DNA phosphate backbone, resulting in formation of a hydrolytically labile phosphotriester (that may eventually cause DNA cleavage) (Schultz *et al.* 1995).

References Cited

Barlow, T. and Dipple, A. (1998). Aralkylation of guanosine with para-substituted styrene oxides. *Chem.Res.Toxicol.* 11, 45-53.

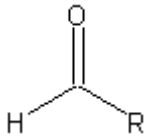
Koskinen, M. and Plná, K. (2000). Specific DNA adducts induced by some mono-substitued epoxides in vitro and in vivo. *Chem.Biol.Interact.* 129, 209-229.

Schultz, W. G., Nieman, R. A., and Skibo, E. B. (1995). Evidence for DNA phosphate backbone alkylation and cleavage by pyrrolo[1,2-albenzimidazoles: Small molecules capable of causing base-pair-specific phosphodiester bond hydrolysis. *Proc.Natl.Acad.Sci.USA* 92, 11854-11858.

Sweeney, J. B. (2002). Aziridines: epoxides' ugly cousins? *Chem.Soc.Rev.* 31, 247-258.

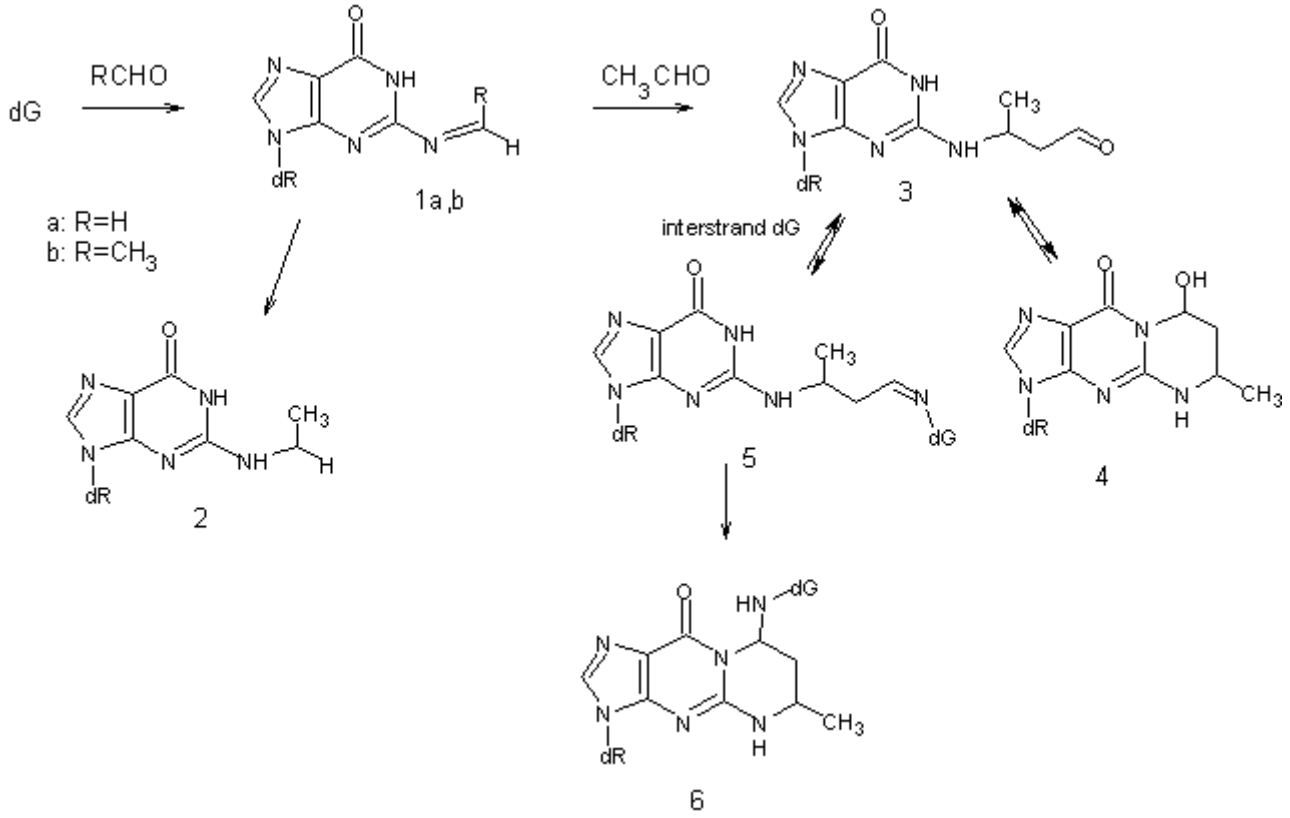
Zang, H. and Gates, K. S. (2000). DNA Binding and Alkylation by the "Left Half" of Azinomycin B. *Biochemistry* 39, 14975.

Simple Aldehydes (genotoxic)



R= aliphatic or aromatic carbon
alpha, beta-unsaturated aldehydes are excluded

All compounds carrying an aldehydic group can potentially undergo Schiff base formation with a primary amine. They are to be considered potentially genotoxic, as demonstrated *in vivo* ability to react with nucleobases, without metabolic activation, forming adducts, interbase cross-links (both intra and inter-strand), and DNA-protein crosslinks The length of carbon chain for aliphatic aldehydes, and in general molecular size, can strongly modulate the formation of every type of cross-link and even the accessibility of the DNA nucleobases (Romano Zito, personal communication). DNA-protein crosslinks have been reported as the primary DNA damage induced by formaldehyde (Speit *et al.* 2007). The initial step of the reaction probably involves formation of an unstable Schiff base with the exocyclic amino group of deoxyguanosine dG (1a). In the case of acetaldehyde, this intermediate (1b) could be stabilized by reduction, producing *N*2-ethyl-dG (2), or alternatively may react with a second molecule of acetaldehyde forming a new aldehyde adduct (3) that ultimately cyclize in an 8-hydroxypropano adduct (4). The latter exists in equilibrium with its ring-opened aldehyde form, and may undergo condensation with another guanine to form imine-linked bis-nucleoside (5) which in turn cyclizes to pyrimidopurinone (6) (Wang *et al.* 2000).



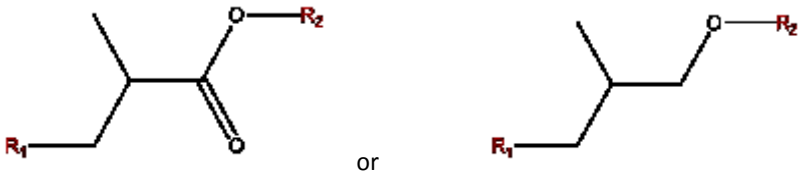
Some aldehydes may also induce hydroxyalkyl adducts in DNA, but the relevance of these DNA modifications for mutagenicity is unclear (Speit *et al.* 2007).

References Cited

Speit, G., Schütz, P., Högel, J., and Schmid, O. (2007). Characterization of the genotoxic potential of formaldehyde in V79 cells. *Mutagenesis* 22, 387-394.

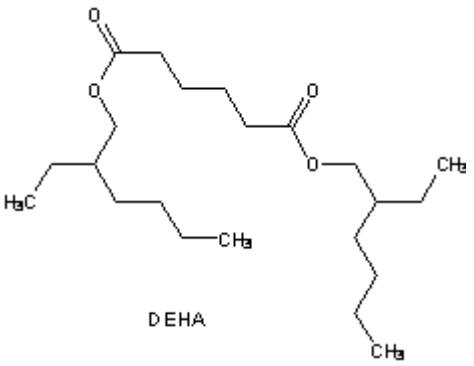
Wang, M., McIntee, E. J., Cheng, G., Shi, Y., Villalta, P. W., and Hecht, S. S. (2000). Identification of DNA Adducts of Acetaldehyde. *Chem.Res.Toxicol.* 13, 1149-1157.

Substituted n-Alkylcarboxylic Acids (nongenotoxic)



R1 – at least one aliphatic carbon, which is not in ring, not bonded to any O and except alkyl chains with C>8
R2 = Any atom/group
All carbons or oxygen between R1 and R2 are not in ring

Substances belonging to this chemical class are potentially reactive as peroxisome proliferators (PPs). PPs are a diverse group of chemicals, including hypolipidemic drugs, plasticizers and herbicides, that were found to cause liver cancer when chronically administered to rats and mice (Reddy et al., 1979). These chemicals are considered nongenotoxic agents, given generally negative results in genotoxicity assays. Even if the mechanism by which these chemicals cause tumors is not fully understood, peroxisome proliferator-activated receptor alpha (PPAR a) is thought to mediate most of the PP effects in the rodent liver (Gonzalez et al., 1998). Two hypotheses have been proposed to account for PP induced hepatocarcinogenesis in rodents: (i) increase in DNA damage through induction of oxidative stress (Reddy and Rao, 1989) and (ii) alteration of hepatocyte growth control by enhanced cell proliferation or decreased apoptosis (Corton et al., 2000).
Among alkylcarboxilic acids, 2-ethylhexanoic acid have shown to be an active PP. 2-ethylhexanoic acid was also identified as the proximate PP of several 2-ethylhexyl-containing compounds, such as di-(2-ethylhexyl)adipate (DEHA, see figure) (Cornu et al., 1992).



DEHA

References Cited

Cornu,MC, J C Lhuguenot, A M Brady, R Moore, C R Elcombe, 1992, Identification of the proximate peroxisome proliferator(s) derived from di (2-ethylhexyl) adipate and species differences in response.: Biochem.Pharmacol., v. 43, p. 2129-2134.

Corton,JC, P Lapinskas, F J Gonzalez, 2000, Central role of PPARa in the mechanism of action of hepatocarcinogenic peroxisome proliferators: Mutat.Res., v. 448, p. 139-151.

Gonzalez,FJ, J M Peters, R C Cattley, 1998, Mechanism of Action of the Nongenotoxic Peroxisome Proliferators: Role of the Peroxisome Proliferator-Activated Receptor α: J.Natl.Cancer Inst., v. 90, p. 1702-1709.

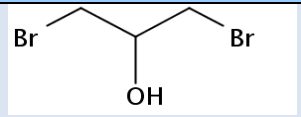
Reddy,JK, M S Rao, 1989, Oxidative DNA damage caused by persistent peroxisome proliferation: its role in hepatocarcinogenesis: Mutat.Res., v. 214, p. 63-68.

Reddy,JK, M S Rao, D L Azarnoff, S Sell, 1979, Mitogenic and carcinogenic effects of a hypolipidemic peroxisome proliferator, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14, 643), in rat and mouse liver: Cancer Res., v. 39, p. 152-161.

Appendix 8: Literature search document

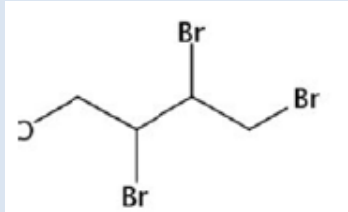
Literature search template

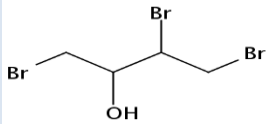
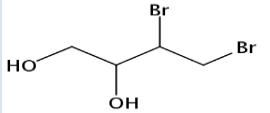
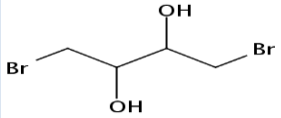
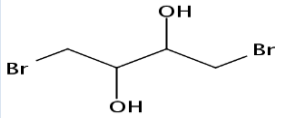
Literature search

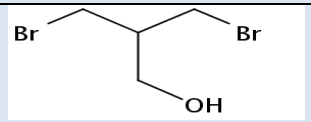
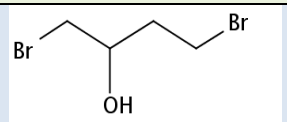
Literature search for toxicological relevant data on theoretical bromated flame retardants in the databases: SciFinder, PubMed and Scopus								
Date	**Substance name and common synonyms**	**Databases & Search Engines**	**Search terms** (e.g. substance name, CAS No and combinations.etc)	**Limitations applied to search**	**No. of 'hits'**	**No. of (potentially) relevant hits**	**Structure**	**Comments & follow-up actions**
21 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	SciFinder	96-21-9		385	See below		
21 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	SciFinder	96-21-9	Exclude Patents	221	15		

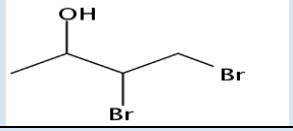
21 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	PubMed	96-21-9		2	0		
21 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	PubMed	Propanol AND dibromo		32	14		
28 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	Scopus	Propanol AND dibromo		91	3		

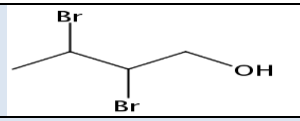
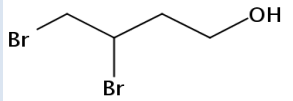
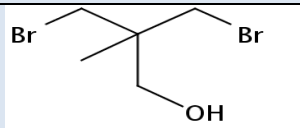
21 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	PubMed	hydroxypropane AND dibromo OR dibromohydrin OR dibromopropanol OR hydroxy AND dibromopropane OR dibromohydrin		4	0		
28 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	Scopus	hydroxypropane AND dibromo		3	1		
28 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	Scopus	dibromohydrin OR dibromopropanol		72	11		

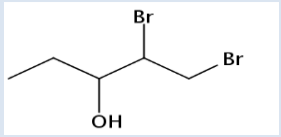
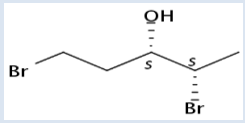
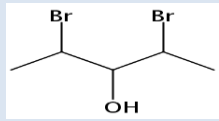
28 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	Scopus	hydroxy AND dibromopropane		8	0		
28 July 2015	**2-Propanol, 1,3-dibromo- 1,3-Dibromo-2-hydroxypropane; 1,3-Dibromo-2-propanol; 1,3-Dibromohydrin; 1,3-Dibromopropanol; 2-Hydroxy-1,3-dibromopropane; Glycerol 1,3-dibromohydrin; Glycerol α,γ-dibromohydrin; α,γ-Dibromohydrin; α-Dibromohydrin**	Scopus	dibromohydrin		17	0		
21 July 2015	**1-Butanol, 2,3,4-tribromo-**	SciFinder	855236-37-2		1	0		
23 July 2015	**1-Butanol, 2,3,4-tribromo-**	PubMed	855236-37-2		0	0		
23	**1-Butanol, 2,3,4-tribromo-**	PubMed	1-butanol, 2,3,4-		0	0		

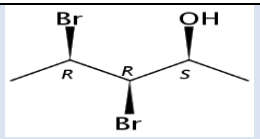
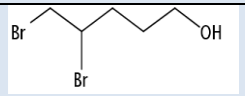
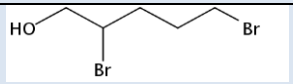
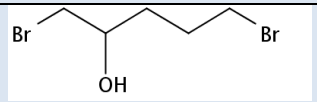
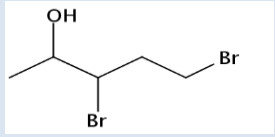
July 2015			tribromo- OR 2,3,4-tribromo t-butanol					
23 July 2015	**1-Butanol, 2,3,4-tribromo-**	PubMed	Butanol tribromo		0	0		
28 July 2015	**1-Butanol, 2,3,4-tribromo-**	Scopus	Butanol AND tribromo		1	0		
21 July 2015	**2-Butanol, 1,3,4-tribromo-; 1,2,4-Tribromo-3-butanol**	SciFinder	87018-38-0		3	0		
21 July 2015	**1,2-Butanediol, 3,4-dibromo-; 3,4-Dibromo-1,2-butanediol**	SciFinder	35330-59-7		4	0		
27 July 2015	**1,2-Butanediol, 3,4-dibromo-; 3,4-Dibromo-1,2-butanediol**	PubMed	Butanediol AND dibromo		7	0		
28 July 2015	**1,2-Butanediol, 3,4-dibromo-; 3,4-Dibromo-1,2-butanediol**	Scopus	Butanediol AND dibromo		7	0		
22 July 2015	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	14396-65-7		60	See below		
22 July 2015	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	14396-65-7	Exclude patents	30	1		
22 July 2015	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	299-70-7		18	See below		Alternate CAS RN to 14396-65-7
22 July	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	299-70-7	NOT 14396-65-7	11	2		Alternate CAS RN to 14396-65-7

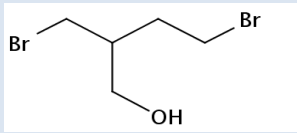
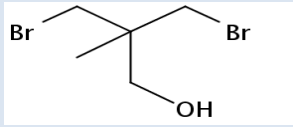
2015				AND Exclude Patents				
22 July 2015	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	1947-59-7		9	See below		Alternate CAS RN to 14396-65-7
22 July 2015	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	1947-59-7	NOT 14396-65-7 NOT 299-70-7 AND Exclude Patents	4	0		Alternate CAS RN to 14396-65-7
22 July 2015	**2,3-Butanediol, 1,4-dibromo-; 1,4-Dibromo-2,3-butanediol**	SciFinder	15410-44-3		6	1		Alternate CAS RN to 14396-65-7
22 July 2015	**1-propanol, 3-bromo-2-(bromomethyl)-; 3-Bromo-2-(bromomethyl)-1-propanol**	SciFinder	106023-63-6		16	See below		
22 July 2015	**1-propanol, 3-bromo-2-(bromomethyl)-; 3-Bromo-2-(bromomethyl)-1-propanol**	SciFinder	106023-63-6	Exclude Patents	11	0		
27 July 2015	**1-propanol, 3-bromo-2-(bromomethyl)-; 3-Bromo-2-(bromomethyl)-1-propanol**	PubMed	Propanol AND bromo		184	3		
28 July 2015	**1-propanol, 3-bromo-2-(bromomethyl)-; 3-Bromo-2-(bromomethyl)-1-propanol**	Scopus	Propanol AND bromo		246	8		
22 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	SciFinder	19398-47-1		87	See below		
22	**2-Butanol, 1,4-dibromo; 1,4-**	SciFinder	19398-47-1	Exclude	38	2		

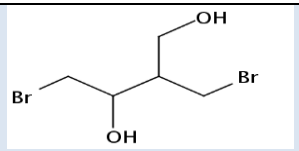
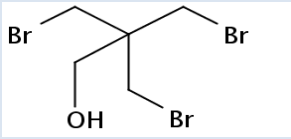
July 2015	**Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**			Patents				
22 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	SciFinder	64028-90-6		9	See below		Alternate CAS RN to 19398-47-1
22 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	SciFinder	64028-90-6	Exclude Patents	5	0		Alternate CAS RN to 19398-47-1
22 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	SciFinder	1360729-08-3		5	See below		Alternate CAS RN to 19398-47-1
22 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	SciFinder	1360729-08-3	Exclude Patents	2	0		Alternate CAS RN to 19398-47-1
27 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	PubMed	Butanol AND dibromo		5	1		
28 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	Scopus	Butanol AND dibromo		23	0		
28 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	PubMed	Hydroxy AND dibromobutane		2	0		
28 July 2015	**2-Butanol, 1,4-dibromo; 1,4-Dibromo-2-butanol; 2-Hydroxy-1,4-dibromobutane**	Scopus	Hydroxy AND dibromobutane		10	0		
22 July 2015	**2-Butanol, 3,4-dibromo; 3,4-Dibromo-2-butanol**	SciFinder	79033-40-2		7	See below		
22 July 2015	**2-Butanol, 3,4-dibromo; 3,4-Dibromo-2-butanol**	SciFinder	79033-40-2	Exclude Patents	6	1		

22 July 2015	**1-Butanol, 2,3-dibromo; 2,3-Dibromo-1-butanol; 2,3-Dibromobutanol**	SciFinder	4021-75-4		19	See below		
22 July 2015	**1-Butanol, 2,3-dibromo; 2,3-Dibromo-1-butanol; 2,3-Dibromobutanol**	SciFinder	4021-75-4	Exclude Patents	10	0		
22 July 2015	**1-Butanol, 2,3-dibromo; 2,3-Dibromo-1-butanol; 2,3-Dibromobutanol**	SciFinder	54899-03-5		8	0		Alternate CAS RN to 4021-75-4
22 July 2015	**1-Butanol, 2,3-dibromo; 2,3-Dibromo-1-butanol; 2,3-Dibromobutanol**	SciFinder	70528-70-0		3	0		Alternate CAS RN to 4021-75-4
22 July 2015	**1-Butanol, 2,3-dibromo; 2,3-Dibromo-1-butanol; 2,3-Dibromobutanol**	SciFinder	70528-70-0	Exclude Patents	1	0		Alternate CAS RN to 4021-75-4
22 July 2015	**1-Butanol, 3,4-dibromo-; 3,4- Dibromobutanol**	SciFinder	87018-30-2		12	See below		
22 July 2015	**1-Butanol, 3,4-dibromo-; 3,4- Dibromobutanol**	SciFinder	87018-30-2	Exclude Patents	9	0		
22 July 2015	**1-Propanol, 3-bromo-2-(bromomethyl-2-methyl-; 2,2-Bis(bromomethyl)propanol**	SciFinder	105100-80-9	Exclude Patents	0	0		
28 July 2015	**1-Propanol, 3-bromo-2-(bromomethyl-2-methyl-; 2,2-Bis(bromomethyl)propanol**	PubMed	Dibromobutanoyl OR bis (bromomethyl)propanol		5	0		
28 July 2015	**1-Propanol, 3-bromo-2-(bromomethyl-2-methyl-; 2,2-Bis(bromomethyl)propanol**	Scopus	Dibromobutanol OR Bis(bromomethyl) propanol		0	0		

22 July 2015	**2-Pentanol, 4,5-dibromo-**	SciFinder	213821-22-8	Exclude Patents	2	0		
27 July 2015	**2-Pentanol, 4,5-dibromo-**	PubMed	Pentanol AND dibromo		1	0		
28 July 2015	**2-Pentanol, 4,5-dibromo-**	Scopus	Pentanol AND dibromo		6	0		
22 July 2015	**3-Pentanol, 1,2-dibromo-**	SciFinder	408319-76-6	Exclude Patents	3	0		
22 July 2015	**3-Pentanol, 1,4-dibromo-, (R*,R*)-(9Cl)**	SciFinder	159475-15-7	Exclude Patents	1	0		
22 July 2015	**3-Pentanol, 1,4-dibromo-, (R*,R*)-(9Cl)**	SciFinder	159475-16-8	Exclude Patents	1	0		Alternate CAS RN to 159475-15-7
22 July 2015	**3-Pentanol, 2,4-dibromo-; 2,4-Dibromo-3-pentanol**	SciFinder	343268-04-2	Exclude Patents	1	0		
22 July 2015	**3-Pentanol, 2,4-dibromo-; 2,4-Dibromo-3-pentanol**	SciFinder	72770-99-1	Exclude Patents	1	0		Alternate CAS RN to 343268-04-2
23 July 2015	**2-Pentanol, 3,4-dibromo-, (2R, 3S,4S)-rel-; 2-Pentanol, 3,4-dibromo-,**	SciFinder	76377-07-6	Exclude Patents	2	0		

	(2R*,3S*,4S*)- (9Cl); 2-Pentanol, 3,4-dibromo-, (2R*,3S*,4S*)-(±)-							
23 July 2015	**2-Pentanol, 3,4-dibromo-, (2R, 3S,4S)-rel-; 2-Pentanol, 3,4-dibromo-, (2R*,3S*,4S*)- (9Cl); 2-Pentanol, 3,4-dibromo-, (2R*,3S*,4S*)-(±)-**	SciFinder	76420-11-6	Exclude Patents	1	0		Alternate CAS RN to 76377-07-6
23 July 2015	**1-Pentanol, 4,5-dibromo-; 1,2-Dibromo-5-pentanol; 4,5-Dibromo-1-pentanol**	SciFinder	59287-66-0	Exclude Patents	8	0		
23 July 2015	**1-Pentanol, 2,5-dibromo-**	SciFinder	856991-78-1	Exclude Patents	0	0		
23 July 2015	**2-Pentanol, 1,5-dibromo-; 1,5-Dibromo-2-pentanol**	SciFinder	100606-66-4	Exclude Patents	8	1		
23 July 2015	**2-Pentanol, 1,5-dibromo-; 1,5-Dibromo-2-pentanol**	SciFinder	1092554-97-6	Exclude Patents	1	0		Alternate CAS RN to 100606-66-4
23 July 2015	**2-Pentanol, 2,5-dibromo-**	SciFinder	213821-20-6	Exclude Patents	1	0		

23 July 2015	**2-Pentanol, 2,5-dibromo-**	SciFinder	159475-17-9	Exclude Patents	1	0		Alternate CAS RN to 213821-20-6
23 July 2015	**2-Pentanol, 2,5-dibromo-**	SciFinder	159475-18-0	Exclude Patents	1	0		Alternate CAS RN to 213821-20-6
23 July 2015	**1-Butanol, 4-bromo-2-(bromomethyl)-**	SciFinder	98069-26-2	Exclude Patents	1	0		
27 July 2015	**1-Butanol, 4-bromo-2-(bromomethyl)-**	PubMed	Butanol AND bromo AND bromomethyl		0	0		
28 July 2015	**1-Butanol, 4-bromo-2-(bromomethyl)-**	Scopus	Butanol AND bromo AND bromomethyl		3	0		
23 July 2015	**1-Propanol, 3-bromo-2-(bromomethyl)-2-methyl-; 2,2-Bis(bromomethyl)propanol**	SciFinder	105100-80-9	Exclude Patents	0	0		
27 July 2015	**1-Propanol, 3-bromo-2-(bromomethyl)-2-methyl-; 2,2-Bis(bromomethyl)propanol**	PubMed	Propanol AND bromo AND bromomethyl		4	0		
28 July 2015	**1-Propanol, 3-bromo-2-(bromomethyl)-2-methyl-; 2,2-Bis(bromomethyl)propanol**	Scopus	Propanol AND bromo AND bromomethyl		15	0		
23 July 2015	**1,3-Butanediol, 4-bromo-2-(bromomethyl)-**	SciFinder	44804-46-8	Exclude Patents	0	0		

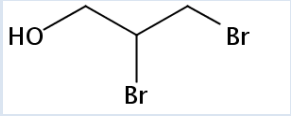
								
27 July 2015	**1,3-Butanediol, 4-bromo-2-(bromomethyl)-**	PubMed	Butanediol AND bromo		6	0		
28 July 2015	**1,3-Butanediol, 4-bromo-2-(bromomethyl)-**	Scopus	Butanediol AND bromo		29	2		
23 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol; 3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol**	SciFinder	1522-92-5	Exclude Patents	109	5		
23 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol;**	SciFinder	36483-57-5	Exclude Patents NOT 1522-92-5	0	0		Alternate CAS RN to 1522-92-5

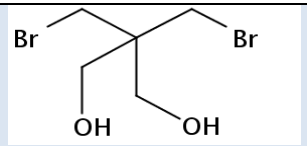
	3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol							
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol; 3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol**	PubMed	Tris(bromomethyl) ethanol OR bis(bromomethyl)propanol OR bis(bromomethyl)propyl OR Pentaerythritol OR Tribromoneopentanol OR Tribromoneopentyl		983	See below		Tribromopentanol not found in PubMed
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol;**	PubMed	Tris(bromomethyl) ethanol (1) OR bis(bromomethyl)propanol AND bromo (3) OR bis(bromomethyl)p		509	See below		Numbers in (x) equals number of hits for each serach term

	3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol		ropyl AND alcohol (0) OR Pentaerythritol (978) OR Tribromoneopentyl AND alcohol (4)					
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol; 3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol**	PubMed	tris (bromomethyl) ethanol OR bis (bromomethyl) propanol AND bromo OR bis (bromomethyl) propyl AND alcohol OR pentaerythritol AND tribromide OR pentaerythritol AND bromohydrin OR tribromoneopentyl AND alcohol		4	0		
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol;**	Scopus	~~tris (bromomethyl) ethanol~~ OR ~~bis (bromomethyl) propanol~~ AND bromo		Not valid	–		Scopus rejects tris(bromomethyl)ethanol and bis(bromomethyl)propanol Leaving out the brackets

	3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol							does not yield any hits
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol; 3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol**	Scopus	bisbromomethyl propyl AND alcohol		0	0		
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol;**	Scopus	pentaerythritol AND tribromide		4	0		

	3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol							
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol; 3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol**	Scopus	pentaerythritol AND bromohydrin		0	0		
28 July 2015	**1-Propanol, 3-bromo-2,2-bis(bromomethyl)-; 2,2,2-Tris(bromomethyl)ethanol; 2,2-Bis(bromomethyl)-3-bromo-1-propanol;**	Scopus	tribromoneopentyl AND alcohol		0	0		

	3-Bromo-2,2-bis(bromomethyl)-1-propanol; 3-Bromo-2,2-bis(bromomethyl)propanol; 3-Bromo-2,2-bis(bromomethyl)propyl alcohol; Pentaerythritol tribromide; Pentaerythritol tribromohydrin; Tribromoneopentanol; Tribromoneopentyl alcohol							
23 July 2015	**1-Propanol, 2,3-dibromo-; 1,2-Dibromopropan-3-ol; 2,3-Dibromo-1-propanol; 2,3-Dibromopropyl alcohol**	SciFinder	96-13-9	Exclude Patents	327	92		
23 July 2015	**1-Propanol, 2,3-dibromo-; 1,2-Dibromopropan-3-ol; 2,3-Dibromo-1-propanol; 2,3-Dibromopropyl alcohol**	SciFinder	83165-36-0	Exclude Patents	9	1		Alternate CAS RN to 96-13-9
23 July 2015	**1-Propanol, 2,3-dibromo-; 1,2-Dibromopropan-3-ol; 2,3-Dibromo-1-propanol; 2,3-Dibromopropyl alcohol**	SciFinder	83165-35-9	Exclude Patents	10	0		Alternate CAS RN to 96-13-9
28 July 2015	**1-Propanol, 2,3-dibromo-; 1,2-Dibromopropan-3-ol; 2,3-Dibromo-1-propanol; 2,3-Dibromopropyl alcohol**	PubMed	dibromopropan OR dibromopropyl AND alcohol		18	0		
28 July 2015	**1-Propanol, 2,3-dibromo-; 1,2-Dibromopropan-3-ol; 2,3-Dibromo-1-propanol; 2,3-Dibromopropyl alcohol**	Scopus	dibromopropan OR (dibromopropyl AND alcohol)		53	8		
23 July	**1,3-Propanediol, 2,2-bis(bromomethyl)-;**	SciFinder	3296-90-0	Exclude Patents	284	53		

2015	**1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin**							
27 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin**	PubMed	Propanediol AND bromomethyl		26	10		Belongs to CAS RN 3296-90-0
28 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane;**	Scopus	Propanediol AND bromomethyl		47	4		Belongs to CAS RN 3296-90-0

	2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin							
27 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin**	PubMed	Bis(hydroxymethyl)propane OR Dihydroxymethylpropane OR Dimethylolpropane AND Dibromo OR Bis(bromomethyl) AND Propanediol OR Dibromomethyl AND Propanediol OR Dibromoneopentyl glycol		28	14		Belongs to CAS RN 3296-90-0
29 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide;**	Scopus	Bishydroxymethylpropane OR Dihydroxymethylpropane		1	0		Belongs to CAS RN 3296-90-0

	Pentaerythritol dibromohydrin							
29 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin**	Scopus	Dimethylolpropane AND Dibromo		0	0		Belongs to CAS RN 3296-90-0
29 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin**	Scopus	Bis(bromomethyl) AND Propanediol		0	0		Belongs to CAS RN 3296-90-0
29 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-**	Scopus	Dibromomethyl AND Propanediol		7	0		Belongs to CAS RN 3296-90-0

	dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin							
29 July 2015	**1,3-Propanediol, 2,2-bis(bromomethyl)-; 1,3-Dibromo-2,2-bis(hydroxymethyl)propane; 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-Dibromo-2,2-dimethylolpropane; 2,2-Bis(bromomethyl)-1,3-propanediol; 2,2-Dibromomethyl-1,3-propanediol; Dibromoneopentyl glycol; Pentaerythritol dibromide; Pentaerythritol dibromohydrin**	Scopus	Dibromoneopentyl AND glycol		41	3		Belongs to CAS RN 3296-90-0

Summary of literature search

	Literature Search Results
Search Term	Dibromoneopentyl AND glycol
Database	Scopus
Limitation(s)	
Relevant Papers	3
Date	July 29, 2015
Comments	Belongs to CAS RN 3296-90-0

Bach, P. H., & Nguyen, T. K. T. (1998). Renal papillary necrosis - 40 years on. *Toxicologic Pathology, 26*(1), 73-91.

Felter, S. P., Conolly, R. B., Bercu, J. P., Bolger, P. M., Boobis, A. R., Bos, P. M. J., Carthew, P., Doerrer, N. G., Goodman, J. I., Harrouk, W. A., Kirkland, D. J., Lau, S. S., Llewellyn, G. C., Preston, R. J., Schoeny, R., Schnatter, A. R., Tritscher, A., Van Velsen, F., & Williams, G. M. (2011). A proposed framework for assessing risk from less-than-lifetime exposures to carcinogens. *Critical Reviews in Toxicology, 41*(6), 507-544.

Thomas, R., Thomas, R. S., Auerbach, S. S., & Portier, C. J. (2013). Biological Networks for Predicting Chemical Hepatocarcinogenicity Using Gene Expression Data from Treated Mice and Relevance across Human and Rat Species. *PLoS ONE, 8*(5).

	Literature Search Results
Search Term	dibromopropan OR (dibromopropyl AND alcohol)
Database	Scopus
Limitation(s)	
Relevant Papers	8
Date	July 28, 2015
Comments	Belongs to CAS RN 96-13-9

2,3-Dibromopropan-1-ol. In. (2000) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 77 (pp. 439-453).

Summary of final evaluations. In. (2000) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 77 (pp. 529).

Allison, L. F., Yeh, K., & Smith, B. F. (1979). Flame-retardant children's sleepwear. I. Comparison of home and laboratory laundering.

Beckwith, O. P. (1980). Tris: was it really a cancer risk in sleepwear?

Blum, A., & Ames, B. N. (1980). Tris: was it really a cancer risk in sleepwear?

Lock, E. A., & Hard, G. C. (2004). Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of

renal carcinogens based on mechanistic information. *Critical Reviews in Toxicology, 34*(3), 211-299.

Mann, T., & Lutwak Mann, C. (1982). Passage of chemicals into human and animal semen: Mechanisms and significance. *Critical Reviews in Toxicology, 11*(1), 1-14.

Perocco, P., Bolognesi, S., & Alberghini, W. (1983). Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro. *Toxicology Letters, 16*(1-2), 69-75.

	Literature Search Results
Search Term	Propanediol AND bromomethyl
Database	Scopus
Limitation(s)	
Relevant Papers	4
Date	July 28, 2015
Comments	Belongs to CAS RN 3296-90-0

2,2-Bis(bromomethyl)propane-1,3-diol. In. (2000) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, vol. 77 (pp. 455-468).

Addition of National Toxicology Program Carcinogens; Community right-to-know toxic chemical release reporting. (2010). *Federal Register, 75*(227), 72727-72734.

Maronpot, R. R., Zeiger, E., McConnell, E. E., Kolenda-Roberts, H., Wall, H., & Friedman, M. A. (2009). Induction of tunica vaginalis mesotheliomas in rats by xenobiotics. *Critical Reviews in Toxicology, 39*(6), 512-537.

Thomas, J., Haseman, J. K., Goodman, J. I., Ward, J. M., Loughran Jr, T. P., & Spencer, P. J. (2007). A review of large granular lymphocytic leukemia in Fischer 344 rats as an initial step toward evaluating the implication of the endpoint to human cancer risk assessment. *Toxicological Sciences, 99*(1), 3-19.

	Literature Search Results
Search Term	Butanediol AND bromo
Database	Scopus
Limitation(s)	
Relevant Papers	2
Date	July 28, 2015
Comments	Belongs to CAS RN 44804-46-8

Goossens, A. (2007). Cosmetic allergens. *Annali Italiani di Dermatologia Allergologica Clinica e Sperimentale, 61*(3), 86-90.

Mason, P., Greer, V. P., Kirby, A. J., Simons, C., Nicholls, P. J., & Smith, H. J. (2003). Some aryl substituted 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoates and 3-(4-nitrophenyl)-1-phenyl-1,4-butanediols and related compounds as inhibitors of rat liver microsomal retinoic acid metabolising enzymes. *Journal of Enzyme Inhibition and Medicinal Chemistry, 18*(6), 511-528.

	Literature Search Results
Search Term	Propanol AND bromo
Database	Scopus
Limitation(s)	
Relevant Papers	8
Date	July 28, 2015
Comments	Belongs to CAS RN 106023-63-6

Hou, F., Xing, C., Li, B., Cheng, J., Chen, W., & Zhang, M. (2015). Application of BALB/c mouse in the local lymph node assay: BrdU-ELISA for the prediction of the skin sensitizing potential of chemicals. *Journal of Pharmacological and Toxicological Methods, 72*, 53-58.

Hyman, J., Leifer, Z., & Rosenkranz, H. S. (1980). The E. coli pol A1 - assay A quantitative procedure for diffusible and non-diffusible chemicals. *Mutation Research/Environmental Mutagenesis and Related Subjects, 74*(2), 107-111.

Jones, A. R. (1975). The metabolism of 3-chloro-, 3-bromo- and 3-iodopropan-1,2-diol in rats and mice. *Xenobiotica, 5*(3), 155-165

Jones, A. R., & Walsh, D. A. (1979). The oxidative metabolism of 1-bromopropane in the rat. *Xenobiotica, 9*(12), 763-772.

Katoh, M., Hamajima, F., Ogasawara, T., & Hata, K. I. (2009). Assessment of human epidermal model LabCyte EPI-MODEL for in vitro skin irritation testing according to European Centre for the Validation of Alternative Methods (ECVAM)-validated protocol. *Journal of Toxicological Sciences, 34*(3), 327-334.

Kim, Y. J., & Ryu, J. C. (2010). Evaluation of the genetic toxicity of synthetic chemicals (XIX) -in vivo peripheral blood reticulocytes micronucleus assay with four chemicals. *Toxicology and Environmental Health Sciences, 2*(2), 104-109.

Pfeiffer, E. H., & Dunkelberg, H. (1980). Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. *Food and Cosmetics Toxicology, 18*(2), 115-118.

Theiss, J. C., Shimkin, M. B., & Poirier, L. A. (1979). Induction of pulmonary adenomas in strain A mice by substituted organohalides. *Cancer Research, 39*(2 I), 391-395.

	Literature Search Results
Search Term	dibromohydrin OR dibromopropanol
Database	Scopus
Limitation(s)	
Relevant Papers	11
Date	July 28, 2015
Comments	Belongs to (CAS RN 96-21-9)

Erratum: Children absorb tris-BP flame retardant from sleepwear: Urine contains the mutagenic metabolite, 2-3-dibromopropanol (Science (1978) (1020)). (1978). *Science, 202*(4370), 857.

Ames, B. N. (1979). Identifying environmental chemicals causing mutations and cancer. *Science, 204*(4393), 587-593.

Carr, H. S., & Rosenkranz, H. S. (1978). Mutagenicity of derivatives of the flame retardant tris(2,3-dibromopropyl) phosphate: Halogenated propanols. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis, 57*(3), 381-384.

Eldefrawi, A. T., Mansour, N. A., Brattsten, L. B., Ahrens, V. D., & Lisk, D. J. (1977). Further toxicologic studies with commercial and candidate flame retardant chemicals. Part II. *Bull Environ Contam Toxicol, 17*(6), 720-726.

Elliott, W. C., Lynn, R. K., Houghton, D. C., Kennish, J. M., & Bennett, W. M. (1982). Nephrotoxicity of the flame retardant, tris(2,3-dibromopropyl) phosphate, and its metabolites. *Toxicology and Applied Pharmacology, 62*(1), 179-182.

Fishbein, L. (1984). Toxicity of the components of styrene polymers: polystyrene, acrylonitrile-butadiene-styrene (ABS) and styrene-butadiene-rubber (SBR). Reactants and additives. *Progress in clinical and biological research, 141*, 239-262.

Liepins, R., & Pearce, E. M. (1977). Chemistry and toxicity of flame retardants for plastics. *Environmental Health Perspectives, Vol. 17*, 55-63.

Osterberg, R. E., Bierbower, G. W., & Ulsamer, A. G. (1978). The comparative toxicity of the flame retardant tris(2,3-dibromopropyl) phosphate and the metabolite 2,3-dibromopropanol in the rat. *Toxicology and Applied Pharmacology, 45*(1).

Soderlund, E., Dybing, E., & Nelson, S. D. (1980). Neprotoxicity and hepatotoxicity of tris(2,3-dibromopropyl) phosphate in the rat. *Toxicology and Applied Pharmacology, 56*(2), 171-181.

Soderlund, E. J., Nelson, S. D., & Dybing, E. (1979). Mutagenic activation of tri(2,3-dibromopropyl)phosphate: The role of microsomal oxidative metabolism. *Acta Pharmacologica et Toxicologica, 45*(2), 112-121.

Söderlund, E. J., Nelson, S. D., & Dybing, E. (1981). In vitro and in vivo covalent binding of the kidney toxicant and carcinogen tris(2,3-dibromopropyl)-phosphate. *Toxicology, 21*(4), 291-304.

	Literature Search Results
Search Term	hydroxypropane AND dibromo
Database	Scopus
Limitation(s)	
Relevant Papers	1

Date	July 28 2015
Comments	Belongs to CAS RN 96-21-9

Uhnáková, B., Ludwig, R., Pěknicová, J., Homolka, L., Lisá, L., Šulc, M., Petříčková, A., Elzeinová, F., Pelantová, H., Monti, D., Křen, V., Haltrich, D., & Martínková, L. (2011). Biodegradation of tetrabromobisphenol A by oxidases in basidiomycetous fungi and estrogenic activity of the biotransformation products. *Bioresource Technology, 102*(20), 9409-9415.

	Literature Search Results
Search Term	Propanol AND dibromo
Database	Scopus
Limitation(s)	
Relevant Papers	3
Date	July 28 2015
Comments	Belongs to CAS RN 96-21-9

Blum, A., & Ames, B. N. (1977). Flame retardant additives as possible cancer hazards. The main flame retardant in children's pajamas is a mutagen and should not be used. *Science, 195*(4273), 17-23.

Danni, O., Brossa, O., & Burdino, E. (1981). Toxicity of halogenated hydrocarbons in pretreated rats - an experimental model for the study of Integrated permissible limits of environmental poisons. *International Archives of Occupational and Environmental Health, 49*(2), 165-176.

Jones, A. R., Porter, K., & Stevenson, D. (1981). The renal toxicity of some halogenated derivatives of propane in the rat. *Naturwissenschaften, 68*(2), 98-99.

	Literature Search Results
Search Term	Bis(hydroxymethyl)propane OR Dihydroxymethylpropane OR Dimethylolpropane AND Dibromo OR Bis(bromomethyl) AND Propanediol OR Dibromomethyl AND Propanediol OR Dibromoneopentyl glycol
Database	PubMed
Limitation(s)	
Relevant Papers	14
Date	July 28 2015
Comments	Belongs to CAS RN 3296-90-0

Environ Health Perspect. 1997 Feb;105 Suppl 1:271-2.

Reproductive toxicology. 2,2-bis(bromomethyl)-1,3-propanediol.

[No authors listed]

Toxicol Pathol. 1997 Nov-Dec;25(6):541-8.

Carcinogenic activity of the flame retardant, 2,2-bis(bromomethyl)-1,3-propanediol in rodents, and comparison with the carcinogenicity of other NTP brominated chemicals.

Dunnick JK[1], Heath JE, Farnell DR, Prejean JD, Haseman JK, Elwell MR.

Author information

- [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.

Abstract

Several brominated chemicals have been shown to be multisite-multispecies carcinogens in laboratory animals, and in this paper we report that the flame retardant, 2,2-bis(bromomethyl)-1,3-propanediol (BMP) is also a multisite carcinogen in both sexes of Fischer 344 rats and B6C3F1 mice. BMP was administered continuously in the diet for up to 2 yr to rats at doses of 0, 2,500, 5,000, or 10,000 ppm and to mice at doses of 0, 312, 625, or 1,250 ppm. Interim groups of rats were examined at 15 mo. An additional recovery group of male rats received the chemical for 3 mo at 20,000 ppm in the feed, and then the control diet for the remainder of the study. Chemical exposure caused neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal's gland, oral cavity, esophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas in the male rat; mammary gland, oral cavity, esophagus, and thyroid gland in the female rat; lung, kidney, and Harderian gland in male mice; and subcutaneous tissue, lung, and Harderian gland in the female mouse. The recovery group of male rats presented with the same spectrum of treatment-related neoplasms as in the core study. In this recovery group, BMP (at 20,000 ppm) caused irreversible effects at numerous sites after 90 days of exposure that was not detectable by histologic examination, but without further exposure resulted in carcinogenic responses at 2 yr. BMP is mutagenic in the salmonella test, but it was not determined if the BMP-induced effects that eventually lead to development of neoplasms at multiple sites are the same in both species and in all organ systems affected.

IARC Monogr Eval Carcinog Risks Hum. 2000;77:455-68.

2,2-Bis(bromomethyl)propane-1,3-diol

[No authors listed]

Toxicol Pathol. 2003 Jan-Feb;31 Suppl:88-91.

Use of the Japanese medaka (Oryzias latipes) and guppy (Poecilia reticulata) in carcinogenesis testing under national toxicology program protocols.

Hawkins WE[1], Walker WW, Fournie JW, Manning CS, Krol RM.

Author information

- [1]Gulf Coast Research Laboratory, The University of Southern Mississippi, Ocean Springs, Mississippi 39564, USA. william.hawkins@usm.edu

Abstract

A need exists for whole animal toxicity, mutagenesis, and carcinogenesis models that are alternative to the traditional rodent test models and that are economical, sensitive, and scientifically acceptable. Among small fish models, the Japanese medaka (Oryzias latipes) is preeminent for investigating effects of carcinogenic and/or toxic waterborne hazards to humans. The guppy (Poecilia reticulata), although less widely used, is valuable as a comparison species. Both species are easy to maintain and handle in the laboratory and there is a large body of background information on their responsiveness to a range of classes of carcinogens. There are considerable data on the occurrence of background diseases and on spontaneous neoplastic lesions, both of which occur relatively rarely. With few modifications, the medaka and guppy are amenable to carcinogenicity testing under the rigid standards established by the National Toxicology Program (NTP) for rodent tests. The advantages of the small fish in carcinogenesis studies are best realized in long-term studies that involve environmentally realistic exposures. Studies to identify chronic effects can be conducted in about 12 months, near the life span of medaka in our laboratory. Practically, 9-month studies are optimal but shorter study cycles and a variety of exposure/growout and initiation/promotion scenarios are available. Studies on 3 compounds tested in medaka under NTP protocols are under review and preliminary analysis indicates that chronic carcinogenicity bioassays with medaka, guppy, and potentially with other small fish species are feasible and scientifically valid.

Rep Carcinog. 2004;11:III36-7.

2,2-bis(Bromomethyl)-1,3-propanediol (technical grade).

[No authors listed]

Update of

- 2,2-bis(Bromomethyl)-1,3-propanediol (technical grade). [Rep Carcinog. 2002]

Rep Carcinog. 2002;10:35-6.

2,2-bis(Bromomethyl)-1,3-propanediol (technical grade).

National Toxicology Program.

Update in

- 2,2-bis(Bromomethyl)-1,3-propanediol (technical grade). [Rep Carcinog. 2004]

Natl Toxicol Program Tech Rep Ser. 2005 Oct;(528):1-190.

NTP carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol, nitromethane, and 1,2,3-trichloropropane (cas nos. 3296-90-0, 75-52-5, and 96-18-4) in guppies (Poecilia reticulata) and medaka (Oryzias latipes) (Waterborne Studies).

National Toxicology Program[1].

Author information

- [1]National Toxicology Program, PO Box 12233, Research Triangle Park, NC 27709, USA.

Abstract

The NTP chose to initiate studies in fish as an exploration of alternate or additional models for examining chemical toxicity and carcinogenicity. The use of small fish species in carcinogenicity testing offered potential advantages as a bioassay test system, including significant savings in cost and time over rodent studies. Large numbers of small fish could be easily maintained in a limited area. The two species chosen for study were guppy (Poecilia reticulata) and medaka (Oryzias latipes), both of which are hardy, easily maintained, and have a low occurrence of background lesions. The three chemicals chosen for study in fish had already been studied by the NTP in rodents, permitting a comparison of results between the two models. Two of the chemicals used (2,2-bis(bromomethyl)-1,3-propanediol and 1,2,3-trichloropropane) were mutagenic and multisite carcinogens in rats and mice. The third chemical, nitromethane, was nonmutagenic with a more modest carcinogenic response in rodents. Male and female guppies and medaka were exposed to 2,2-bis(bromomethyl)- 1,3-propanediol (greater than 99% pure), nitromethane, (greater than 99% pure), or 1,2,3-trichloropropane (99% pure) in aquaria water for up to 16 months. OVERALL STUDY DESIGN: Groups of approximately 220 guppies (two replicates of 110) were maintained in aquaria water containing nominal concentrations of 0, 24, 60, or 150 mg/L 2,2-bis(bromomethyl)-1,3-propanediol; 0, 10, 30, or 70 mg/L nitromethane; or 0, 4.5, 9.0, or 18.0 mg/L 1,2,3-trichloropropane. Groups of approximately 340 medaka (two replicates of 170) were maintained in aquaria water containing 0, 24, 60, or 150 mg/L 2,2-bis(bromomethyl)-1,3-propanediol; 0, 10, 20, or 40 mg/L nitromethane; or 0, 4.5, 9.0, or 18.0 mg/L 1,2,3-

trichloropropane. The overall study durations were 16 months for all guppy studies, 14 months for 2,2-bis(bromomethyl)-1,3-propanediol-exposed medaka, and 13 months for nitromethane- and 1,2,3-trichloropropane-exposed medaka. Ten guppies and 10 medaka from each group replicate were sacrificed at 9 months for histopathologic analysis. Approximately one third of the remaining fish from each group were placed in chemical-free water at 9 months and constituted a stop-exposure study component. The remainder of the fish were exposed for the duration of the study and constituted the core study component. A stop-exposure component was added to determine if stopping the exposure at 9 months and transferring to chemical-free aquaria might allow for better survival and tumor development. The sex of guppies and medaka was determined at histopathologic analysis. 2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL - 16-MONTH STUDY IN GUPPIES: 2,2-Bis(bromomethyl)-1,3-propanediol was chronically toxic to guppies in the 60 and 150 mg/L core and stop-exposure groups. Due to mortality, exposure of core study animals in the 150 mg/L group was terminated on day 443, after approximately 64 weeks on study, and fish were maintained in 2,2-bis(bromomethyl)- 1,3-propanediol-free water in the exposure system until the end of the study at 69 weeks. Nominal exposure concentrations of 24, 60, and 150 mg/L provided actual aquaria water exposure concentrations of 20.0, 53.5, and 139.0 mg/L 2,2-bis(bromomethyl)- 1,3-propanediol, respectively. There were no treatment-related differences between the control and exposed groups in body weights or lengths. At 9 months, hepatocellular adenomas occurred in one 24 mg/L male and in one 150 mg/L male. In the core study, the incidence of hepatocellular adenoma or carcinoma (combined) in 150 mg/L males was greater than that in the controls; multiple adenomas occurred in two 150 mg/L males and in one 150 mg/L female. Cholangioma occurred in a small number of exposed males and females. In the stop-exposure study, incidences of hepatocellular adenoma (including multiple) and of hepatocellular carcinoma were greater in 150 mg/L males than in controls. One cholangioma and one cholangiocarcinoma occurred in the 150 mg/L female group. 14-MONTH STUDY IN MEDAKA: Exposure to 2,2-bis(bromomethyl)-1,3-propanediol did not result in any significant reduction in survival, although the mortality of fish was somewhat greater in the 60 and 150 mg/L core study groups than in the control and 24 mg/L groups. After reallocation, mortality of medaka in the 60 and 150 mg/L core groups was slightly increased over the corresponding stop-exposure groups. Nominal exposure concentrations of 24, 60, and 150 mg/L provided actual exposure concentrations of 19.4, 56.9, and 137.8 mg/L 2,2-bis(bromomethyl)- 1,3-propanediol, respectively. Core study animals in the 60 and 150 mg/L groups were significantly larger, in both body length and weight, than control group fish. In the core study, the incidence of hepatocellular adenoma or carcinoma (combined) was increased in 150 mg/L males. Cholangiocarcinomas occurred in a few exposed males and females, with all but one occurring in 150 mg/L fish. One cholangioma occurred in a 150 mg/L female, and one occurred in a control female. In the stop-exposure study, incidences of hepatocellular adenoma or carcinoma (combined) were marginally increased in the 150 mg/L group of males and in the 60 and 150 mg/L groups of females as compared with controls. Cholangiocarcinoma occurred in one male and one female in the 150 mg/L groups and in one control female. NITROMETHANE - 16-MONTH STUDY IN GUPPIES: Although the cause of death could not be confirmed in many cases, mortality in the 70 mg/L groups appeared to indicate that this level of nitromethane exposure was chronically toxic. This is confirmed by the similar survival rate of guppies from all treatments following removal from treatment aquaria and placement in stop-exposure. Due to the high mortality of fish in the 70 mg/L core study groups, these fish were removed from treatment (day 396) and fixed for histological analyses after approximately 57 weeks on

study. The controls and other exposed groups were sacrificed at 70 weeks. Nominal exposure concentrations of 10, 30, and 70 mg/L provided actual exposure concentrations of 9.9, 28.7, and 66.4 mg/L nitromethane, respectively. There were no treatment-related differences between the control and exposed groups in body lengths or weights. 13-MONTH STUDY IN MEDAKA: Nitromethane in the aquaria supported a substantial microfaunal growth which, without frequent cleaning, affected water quality and treatment concentrations. To maintain acceptable water quality and treatment concentrations potentially affected by the rapid microfaunal growth, the study aquaria were brushed once and siphoned three times each day. Due to this frequent activity, a number of fish probably died due to mechanical injury. Unfortunately, the cause of death could not be confirmed in many cases; the mortality from this activity is believed to have been approximately uniform among treatments and should not have affected the comparison of survival between treatments. Based on mortality in this study and the previous life-span evaluation, the life phase of this study was terminated approximately 13.5 months after hatching. Nominal exposure concentrations of 10, 20, and 40 mg/L resulted in actual exposure concentrations of 9.3, 20.8, and 41.7 mg/L nitromethane, respectively. No differences between control and exposed groups were found in body lengths or weights at the 9-month interim evaluation. Due to mortality, unequal numbers of fish were distributed among the core study and stop-exposure aquaria at 9 months. Differences in lengths and weights were found at 13 months. The biological significance of this finding is unknown. At 9 months, a single cholangiocarcinoma occurred in a 40 mg/L male. Hepatocellular adenomas occurred in two 20 mg/L males and in one 40 mg/L female. In the core study, one cholangioma occurred in a 20 mg/L male, and cholangiocarcinomas were seen in a few exposed males, but none occurred in control males. 1,2,3-TRICHLOROPROPANE - 16-MONTH STUDY IN GUPPIES: The survival of exposed guppies was less than that of the control group at 9 months. Reduced survival was evident at 6 months in the 18.0 mg/L groups and at 7 months in the 4.5 and 9.0 mg/L groups. Survival was significantly reduced in the 18.0 mg/L core study group within 1 month of the 9-month interim evaluation, and mortality in this group was 42.6% between 9 months and study termination. Nominal exposure concentrations of 4.5, 9.0, and 18.0 mg/L resulted in actual exposure concentrations of 4.4, 8.8, and 18.2 mg/L 1,2,3-trichloropropane, respectively. Guppies in the 18.0 mg/L core study group were significantly longer and weighed more than the controls. Fish in the 18.0 mg/L stop-exposure group also weighed more than the controls. Mortality of fish during the study resulted in unequal numbers of individuals distributed to core study and stop-exposure aquaria at 9 months. This appears to have influenced the length and weight of fish measured at study termination (i.e., the smaller tank population allowed the fish to grow more). Observed differences in weight and length between controls and 18.0 mg/L fish was most likely an artifact of the reduced numbers of fish in the 18.0 mg/L aquaria. At 9 months, multiple hepatocellular adenomas occurred in one 4.5 mg/L male, and one hepatocellular adenoma occurred in a control male. In the core study, increased incidences of cholangiocellular (bile duct) and hepatocellular neoplasms occurred in exposed groups of males and females. Cholangioma and cholangiocarcinoma were seen in several exposed males and females. In the stop-exposure study, increased incidences of hepatocellular neoplasms occurred in 18.0 mg/L males and increased incidences of cholangiocellular (bile duct) neoplasms occurred in 18.0 mg/L females. (ABSTRACT TRUNCATED)

Toxicol Sci. 2006 Jul;92(1):143-56. Epub 2006 Mar 31.

The utility of the guppy (Poecilia reticulata) and medaka (Oryzias latipes) in evaluation of chemicals for carcinogenicity.

Kissling GE[1], Bernheim NJ, Hawkins WE, Wolfe MJ, Jokinen MP, Smith CS, Herbert RA, Boorman GA.

Author information

- [1]Environmental Medicine and Diseases Program and Environmental Toxicology Program, National Institute of Environmental Health Sciences, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA. kissling@niehs.nih.gov

Abstract

There has been considerable interest in the use of small fish models for detecting potential environmental carcinogens. In this study, both guppies (Poecilia reticulata) and medaka (Oryzias latipes) were exposed in the aquaria water to three known rodent carcinogens for up to 16 months. Nitromethane, which caused mammary gland tumors by inhalation exposure in female rats, harderian gland and lung tumors in male and female mice, and liver tumors in female mice by inhalation, failed to increase tumors in either guppies or medaka. Propanediol, which when given in the feed was a multisite carcinogen in both sexes of rats and mice, caused increased liver tumors in male guppies and male medaka. There was reduced survival in female guppies and no increased tumors in female medaka. 1,2,3-Trichloropropane, which when administered by oral gavage was a multisite carcinogen in both sexes of rats and mice, caused an increased incidence of tumors in the liver of both male and female guppies and medaka and in the gallbladder of male and female medaka. The results of this study demonstrate that for these three chemicals, under these specific exposure conditions, the fish appear less sensitive and have a narrower spectrum of tissues affected than rodents. These results suggest that fish models are of limited utility in screening unknown chemicals for potential carcinogenicity.

Drug Metab Dispos. 2009 Feb;37(2):408-16. doi: 10.1124/dmd.108.023937. Epub 2008 Nov 24.

Absorption, distribution, metabolism, and excretion of 2,2-bis(bromomethyl)-1,3-propanediol in male fischer-344 rats.

Hoehle SI[1], Knudsen GA, Sanders JM, Sipes IG.

Author information

- [1]Department of Pharmacology, College of Medicine, The University of Arizona, P.O. Box 245050, Tucson, AZ 85724-5050, USA.

Abstract

2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant, previously shown to be a multisite carcinogen in experimental animals. Studies were performed to characterize the dispositional and metabolic fate of BMP after oral or intravenous administration to male Fischer-344 rats. After a single oral administration of [(14)C]BMP (10 or 100 mg/kg) >80% of the low dose and 48% of the high dose were excreted by 12 h in the urine predominantly as a glucuronide metabolite. After repeated daily oral doses for 5 or 10 days, route and rate of elimination were similar to those obtained after single administrations of BMP. In all studies, the radioactivity recovered in feces was low (<15%). The total amount of radioactivity remaining in tissues at 72 h after a single oral administration of BMP (100 mg/kg) was less than 1% of the dose, and repeated daily dosing did not lead to retention in tissues. After intravenous administration, the radiolabel found in blood decreased rapidly. Excretion profiles were similar to those after oral administration. Parent BMP and BMP glucuronide were present in blood plasma after oral or intravenous dosing. After an intravenous dose of BMP (15 mg/kg) the hepatic BMP glucuronide was primarily exported into the bile (>50% within 6 h), but it underwent enterohepatic recycling with subsequent elimination in the urine. These data indicate that the extensive extraction and rapid glucuronidation by the liver limits exposure of internal tissues to BMP by greatly reducing its systemic bioavailability after oral exposure.

Drug Metab Dispos. 2010 Jun;38(6):957-62. doi: 10.1124/dmd.110.032110. Epub 2010 Mar 3.

In vitro glucuronidation of 2,2-bis(bromomethyl)-1,3-propanediol by microsomes and hepatocytes from rats and humans.

Rad G[1], Hoehle SI, Kuester RK, Sipes IG.

Author information

- [1]Department of Clinical Pharmacology, College of Medicine, The University of Arizona, P.O. Box 245050, Tucson, AZ 85724-5050, USA.

Abstract

2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant used in unsaturated polyester resins. In a 2-year bioassay BMP was shown to be a multisite carcinogen in rats and mice. Because glucuronidation is the key metabolic transformation of BMP by rats, in this study the in vitro hepatic glucuronidation of BMP was compared across several species. In addition, the glucuronidation activities of human intestinal microsomes and specific human hepatic UDP-glucuronosyltransferase (UGT) enzymes for BMP were determined. To explore other possible routes of metabolism for BMP, studies were conducted with rat and human hepatocytes. Incubation of hepatic microsomes with BMP in the presence of UDP-glucuronic acid resulted in the formation of a BMP monoglucuronide. The order of

hepatic microsomal glucuronidation activity of BMP was rats, mice >> hamsters > monkeys >>> humans. The rate of glucuronidation by rat hepatic microsomes was 90-fold greater than that of human hepatic microsomes. Human intestinal microsomes converted BMP to BMP glucuronide at a rate even lower than that of human hepatic microsomes. Among the human UGT enzymes tested, only UGT2B7 had detectable glucuronidation activity for BMP. BMP monoglucuronide was the only metabolite formed when BMP was incubated with suspensions of freshly isolated hepatocytes from male F-344 rats or with cryopreserved human hepatocytes. Glucuronidation of BMP in human hepatocytes was extremely low. Overall, the results support in vivo studies in rats in which BMP glucuronide was the only metabolite found. The poor glucuronidation capacity of humans for BMP suggests that the pharmacokinetic profile of BMP in humans will be dramatically different from that of rodents.

Rep Carcinog. 2011;12:70-1.

2,2-Bis(bromomethyl)-1,3-propanediol (technical grade).

National Toxicology Program.

Toxicology. 2011 Dec 18;290(2-3):271-7. doi: 10.1016/j.tox.2011.10.006. Epub 2011 Oct 14.

Induction of DNA damage in human urothelial cells by the brominated flame retardant 2,2-bis(bromomethyl)-1,3-propanediol: role of oxidative stress.

Kong W[1], Kuester RK, Gallegos A, Sipes IG.

Author information

- [1]Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, AZ 85724, USA.

Abstract

2,2-bis(bromomethyl)-1,3-propanediol (BMP) is an extensively used brominated flame retardant found in urethane foams and polyester resins. In a 2-year dietary study conducted by the National Toxicology Program, BMP caused neoplastic lesions at multiple sites including the urinary bladder in both rats and mice. The mechanism of its carcinogenic effect is unknown. In the present study, using SV-40 immortalized human urothelial cells (UROtsa), endpoints associated with BMP induced DNA damage and oxidative stress were investigated. The effects of time (1-24h) and concentration (5-100 μM) on BMP induced DNA strand breaks were assessed via the alkaline comet assay. The results revealed evidence of DNA strand breaks at 1 and 3h following incubation of cells with non-cytotoxic concentrations of BMP. Strand breaks were not present after 6h of incubation. Evidences for BMP associated oxidative stress include: an elevation of intracellular ROS formation as well

as induction of Nrf2 and HSP70 protein levels. In addition, DNA strand breaks were attenuated when cells were pre-treated with N-acetyl-l-cysteine (NAC) and oxidative base modifications were revealed when a lesion specific endonuclease, human 8-hydroxyguanine DNA glycosylase 1 (hOGG1) was introduced into the comet assay. In conclusion, these results demonstrate that BMP induces DNA strand breaks and oxidative base damage in UROtsa cells. Oxidative stress is a significant, determinant factor in mediating these DNA lesions. These early genotoxic events may, in part, contribute to BMP-induced carcinogenesis observed in rodents.

Reprod Toxicol. 2012 Jun;33(3):269-79. doi: 10.1016/j.reprotox.2012.01.002. Epub 2012 Jan 18.

Occupational exposures to chemicals as a possible etiology in premature ovarian failure: a critical analysis of the literature.

Béranger R[1], Hoffmann P, Christin-Maitre S, Bonneterre V.

Author information

- [1]UJF-Grenoble 1/CNRS/TIMC-IMAG UMR 5525 (EPSP Team: Environnement et Prédiction de la Santé des Populations), Grenoble F-38041, France. r.beranger26@gmail.com

Abstract

Premature ovarian failure (POF) is a cause of infertility that affects about 1% of women under 40, and is considered as idiopathic in 75% of cases. An occupational chemical origin has been identified at least once with 2-bromopropane, but human studies are rare and experimental data are sparse. This review aims to carry out a critical synthesis of knowledge of the chemical agents likely to affect follicular stock in humans and/or animals, by direct toxicity to follicles, or by increasing their recruitments. Of 140 chemical agents (or groups) studied, 20 have been identified as potentially damaging to the ovarian reserve. For the majority of toxic agents, only experimental data are currently available. At least four of these agents are likely to lead to POF in descendents (ethylene glycol methyl ether; 2,2-bis(bromomethyl)-1,3-propanediol; benzo[a]pyrene; dimethylbenzantracene). We propose a strategy aiming to encourage progress in identifying occupational factors responsible for POF.

Toxicol Lett. 2013 Oct 9;222(3):273-9. doi: 10.1016/j.toxlet.2013.07.026. Epub 2013 Aug 13.

Comparison of 2,2-bis(bromomethyl)-1,3-propanediol induced genotoxicity in UROtsa cells and primary rat hepatocytes: relevance of metabolism and oxidative stress.

Kong W[1], Gu P, Knudsen GA, Sipes IG.

Author information

- [1]Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, AZ 85724, United States. Electronic address: weixik@uci.edu.

Abstract

2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant used in urethane foams and polyester resins. In a two year dietary study, BMP caused neoplastic lesions at multiple sites including the urinary bladder of both rats and mice. However, liver was not a target tissue. We previously reported that BMP elicited oxidative DNA damage in a human uroepithelial cell line (UROtsa). The present in vitro study investigated the susceptibility of target (UROtsa cells) and non-target cells (primary rat hepatocytes) to BMP-induced genotoxicity. In contrast to hepatocytes, BMP exhibited greater genotoxic potential in UROtsa cells as evidenced by the concentration dependent increase in DNA strand breaks and DNA binding. Total content of intracellular GSH quantified in UROtsa cells (2.7±1.0nmol/mg protein) was 4 fold lower than that in hepatocytes (10.7±0.3nmol/mg protein). HPLC analysis indicated BMP was not metabolized and/or consumed in UROtsa cells at any of the concentrations tested (10-250μM) but was extensively converted to a mono-glucuronide in hepatocytes. These results demonstrate that a target cell line such as UROtsa cells are more susceptible to BMP-induced DNA damage when compared to non-target cells. This increased susceptibility may relate to the deficiency of antioxidant and/or metabolic capabilities in UROtsa cells.

Mutat Res Genet Toxicol Environ Mutagen. 2014 Jul 15;769:1-6. doi: 10.1016/j.mrgentox.2014.04.015. Epub 2014 May 10.

Effects of seven chemicals on DNA damage in the rat urinary bladder: a comet assay study.

Wada K[1], Yoshida T[2], Takahashi N[2], Matsumoto K[2].

Author information

- [1]Toxicology Division, The Institute of Environmental Toxicology, 4321, Uchimoriya-machi, Joso-shi, Ibaraki 303-0043, Japan. Electronic address: wada@iet.or.jp.
- [2]Toxicology Division, The Institute of Environmental Toxicology, 4321, Uchimoriya-machi, Joso-shi, Ibaraki 303-0043, Japan.

Abstract

The in vivo comet assay has been used for the evaluation of DNA damage and repair in various tissues of rodents. However, it can give false-positive results due to non-specific DNA damage associated with cell death. In this study, we examined whether the in vivo comet assay can distinguish between genotoxic and non-genotoxic DNA damage in urinary bladder cells, by using the following seven chemicals related to urinary bladder carcinogenesis in rodents: N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), glycidol, 2,2-bis(bromomethyl)-1,3-propanediol (BMP), 2-nitroanisole (2-NA), benzyl isothiocyanate (BITC), uracil, and melamine. BBN, glycidol, BMP, and 2-NA are known to be Ames test-positive and they are expected to produce DNA damage in the absence of cytotoxicity. BITC, uracil, and melamine are Ames test-negative with metabolic activation but have the potential to induce non-specific DNA damage due to cytotoxicity. The test chemicals were administered orally to male Sprague-Dawley rats (five per group) for each of two consecutive days. Urinary bladders were sampled 3h after the second administration and urothelial cells were analyzed by the comet assay and subjected to histopathological examination to evaluate cytotoxicity. In the urinary bladders of rats treated with BBN, glycidol, and BMP, DNA damage was detected. In contrast, 2-NA induced neither DNA damage nor cytotoxicity. The non-genotoxic chemicals (BITC, uracil, and melamine) did not induce DNA damage in the urinary bladders under conditions where some histopathological changes were observed. The results indicate that the comet assay could distinguish between genotoxic and non-genotoxic chemicals and that no false-positive responses were obtained.

	Literature Search Results
Search Term	Propanediol AND bromomethyl
Database	PubMed
Limitation(s)	
Relevant Papers	10
Date	July 27 2015
Comments	Belongs to CAS RN 3296-90-0

Reproductive toxicology. 2,2-bis(bromomethyl)-1,3-propanediol. (1997). *Environ Health Perspect, 105 Suppl 1*, 271-272.

2,2-Bis(bromomethyl)propane-1,3-diol. (2000). *IARC Monogr Eval Carcinog Risks Hum, 77*, 455-468

2,2-bis(Bromomethyl)-1,3-propanediol (technical grade). (2004). *Rep Carcinog, 11*, III36-37.

Beranger, R., Hoffmann, P., Christin-Maitre, S., & Bonneterre, V. (2012). Occupational exposures to chemicals as a possible etiology in premature ovarian failure: a critical analysis of the literature. *Reprod Toxicol, 33*(3), 269-279.

National Toxicology, P. (1996). NTP Toxicology and Carcinogenesis Studies of 2,2-Bis(Bromomethyl)-1,3-Propanediol (FR-1138(R)) (CAS No. 3296-90-0) in F344 Rats and B6C3F1 Mice (Feed Studies). *Natl Toxicol Program Tech Rep Ser, 452*, 1-465.

National Toxicology, P. (2002). 2,2-bis(Bromomethyl)-1,3-propanediol (technical grade). *Rep Carcinog, 10*, 35-36.

National Toxicology, P. (2005). NTP carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol, nitromethane, and 1,2,3-trichloropropane (cas nos. 3296-90-0, 75-52-5, and 96-18-4) in guppies (Poecilia reticulata) and medaka (Oryzias latipes) (Waterborne Studies). *Natl Toxicol Program Tech Rep Ser*(528), 1-190.

National Toxicology, P. (2011). 2,2-Bis(bromomethyl)-1,3-propanediol (technical grade). *Rep Carcinog, 12*, 70-71.

Ton, T. V., Hong, H. H., Anna, C. H., Dunnick, J. K., Devereux, T. R., Sills, R. C., & Kim, Y. (2004). Predominant K-ras codon 12 G --> A transition in chemically induced lung neoplasms in B6C3F1 mice. *Toxicol Pathol, 32*(1), 16-21

Treinen, K. A., Chapin, R. E., Gulati, D. K., Mounce, R., Morris, L. Z., & Lamb, J. C. t. (1989). Reproductive toxicity of 2,2-bis(bromomethyl)-1,3-propanediol in a continuous breeding protocol in Swiss (CD-1) mice. *Fundam Appl Toxicol, 13*(2), 245-255.

	Literature Search Results
Search Term	butanol AND dibromo
Database	PubMed
Limitation(s)	
Relevant Papers	1
Date	July 27 2015
Comments	Belongs to CAS RN 19398-47-1

Arch Environ Contam Toxicol. 2007 Jul;53(1):134-9. Epub 2007 Mar 29.

Automated solid phase extraction and quantitative measurement of 2,3-dibromo-1-propanol in urine using gas chromatography-mass spectrometry.

De Alwis GK[1], Needham LL, Barr DB.

Author information

- [1]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mailstop F 17, Atlanta, GA 30341, USA.

Abstract

2,3-Dibromo-1-propanol (DBP) was used as an active flame retardant in the 1970s. It was also used as an intermediate in the preparation of insecticide formulations, pharmaceuticals and the flame

retardants tris(2,3-dibromopropyl) phosphate (Tris-BP) and tetrabromobisphenol A bis (2,3-dibromopropyl ether). DBP is also produced in vivo as a metabolic product of Tris-BP in humans. In 1977, sleepwear containing DBP and Tri-BP was banned because of evidence of carcinogenicity animal studies. Although the production of DBP was reduced after 1977, studies show that DBP is still detected in indoor air and dust; hence, the U.S. population may be exposed potentially to DBP. Only a few methods have been reported in the literature for assessing exposure to DBP or Tris-BP by measuring DBP in urine. These methods are based on a labor-intensive and time-consuming liquid-liquid extraction for the isolation of DBP from the urine matrix. To measure urinary DBP in humans, a fast, accurate, and sensitive method was developed with a limit of detection of 0.1 ng/mL and extraction recovery of 96%. This method involves enzymatic cleavage of the DBP-glucuronide or sulfate conjugate, automated solid phase extraction, and analysis by gas chromatography-mass spectrometry using 1,4-dibromo-2-butanol as the internal standard.

	Literature Search Results
Search Term	Propanol AND bromo
Database	PubMed
Limitation(s)	
Relevant Papers	3
Date	July 27 2015
Comments	Belongs to CAS RN 106023-63-6

Biol Reprod. 2007 Mar;76(3):496-505. Epub 2006 Nov 8.

CYP2E1-catalyzed oxidation contributes to the sperm toxicity of 1-bromopropane in mice.

Garner CE[1], Sloan C, Sumner SC, Burgess J, Davis J, Etheridge A, Parham A, Ghanayem BI.

Author information

- [1]RTI International, Research Triangle Park, North Carolina 27709, USA. cegarner@rti.org

Abstract

1-bromopropane (1-BrP) induces dose- and time-dependent reproductive organ toxicity and reduced sperm motility in rodents. The contribution of cytochrome P4502E1 (CYP2E1) to both 1-BrP metabolism and the induction of male reproductive toxicity was investigated using wild-type (WT) and Cyp2e1-/- mice. In gas uptake inhalation studies, the elimination half-life of [1,2,3-(13)C]-1-BrP was longer in Cyp2e1-/- mice relative to WT (3.2 vs. 1.3 h). Urinary metabolites were identified by 13C nuclear magnetic resonance. The mercapturic acid of 1-bromo-2-hydroxypropane (2OHBrP) was the major urinary metabolite in WT mice, and products of conjugation of 1-BrP with glutathione (GSH) were insignificant. The ratio of GSH conjugation to 2-hydroxylation increased 5-fold in Cyp2e1-/- mice relative to WT. After 1-BrP exposure, hepatic GSH was decreased by 76% in WT mice vs. 47% in Cyp2e1-/- mice. Despite a 170% increase in 1-BrP exposure in Cyp2e1-/- vs. WT mice, sperm motility in exposed Cyp2e1-/- mice did not change relative to unexposed matched controls. This suggests that metabolites produced through CYP2E1-mediated oxidation may be responsible for 1-BrP-induced sperm toxicity. Both 1-BrP and 2OHBrP inhibited the motility of sperm

obtained from WT mice in vitro. However, only 2OHBrP reduced the motility of sperm obtained from Cyp2e1-/- mice in vitro, suggesting that conversion of parent compound to 2OHBrP within the spermatozoa may contribute, at least in part, to reduced motility. Overall, these data suggest that metabolism of 1-BrP is mediated in part by CYP2E1, and activation of 1BrP via this enzyme may contribute to the male reproductive toxicity of this chemical.

Toxicol Appl Pharmacol. 2006 Aug 15;215(1):23-36. Epub 2006 Mar 2.

Metabolism and disposition of 1-bromopropane in rats and mice following inhalation or intravenous administration.

Garner CE[1], Sumner SC, Davis JG, Burgess JP, Yueh Y, Demeter J, Zhan Q, Valentine J, Jeffcoat AR, Burka LT, Mathews JM.

Author information

- [1]Department of Drug Metabolism and Disposition, RTI International, Research Triangle Park, NC 27709, USA. cegarner@rti.org

Abstract

Workplace exposure to 1-bromopropane (1-BrP) can potentially occur during its use in spray adhesives, fats, waxes, and resins. 1-BrP may be used to replace ozone depleting solvents, resulting in an increase in its annual production in the US, which currently exceeds 1 million pounds. The potential for human exposure to 1-BrP and the reports of adverse effects associated with potential occupational exposure to high levels of 1-BrP have increased the need for the development of biomarkers of exposure and an improved understanding of 1-BrP metabolism and disposition. In this study, the factors influencing the disposition and biotransformation of 1-BrP were examined in male F344 rats and B6C3F1 mice following inhalation exposure (800 ppm) or intravenous administration (5, 20, and 100 mg/kg). [1,2,3-(13)C]1-BrP and [1-(14)C]1-BrP were administered to enable characterization of urinary metabolites using NMR spectroscopy, LC-MS/MS, and HPLC coupled radiochromatography. Exhaled breath volatile organic chemicals (VOC), exhaled CO(2), urine, feces, and tissues were collected for up to 48 h post-administration for determination of radioactivity distribution. Rats and mice exhaled a majority of the administered dose as either VOC (40-72%) or (14)CO(2) (10-30%). For rats, but not mice, the percentage of the dose exhaled as VOC increased between the mid (approximately 50%) and high (approximately 71%) dose groups; while the percentage of the dose exhaled as (14)CO(2) decreased (19 to 10%). The molar ratio of exhaled (14)CO(2) to total released bromide, which decreased as dose increased, demonstrated that the proportion of 1-BrP metabolized via oxidation relative to pathways dependent on glutathione conjugation is inversely proportional to dose in the rat. [(14)C]1-BrP equivalents were recovered in urine (13-17%, rats; 14-23% mice), feces (<2%), or retained in the tissues and carcass (<6%) of rats and mice administered i.v. 5 to 100 mg/kg [(14)C]1-BrP. Metabolites characterized in urine of rats and mice include N-acetyl-S-propylcysteine, N-acetyl-3-(propylsulfinyl)alanine, N-acetyl-S-(2-hydroxypropyl)cysteine, 1-bromo-2-hydroxypropane-O-glucuronide, N-acetyl-S-(2-oxopropyl)cysteine, and N-acetyl-3-[(2-oxopropyl)sulfinyl]alanine. These metabolites may be formed following oxidation of 1-bromopropane to 1-bromo-2-propanol and bromoacetone and following subsequent

glutathione conjugation with either of these compounds. Rats pretreated with 1-aminobenzotriazole (ABT), a potent inhibitor of P450 excreted less in urine (down 30%), exhaled as (14)CO2 (down 80%), or retained in liver (down 90%), with a concomitant increase in radioactivity expired as VOC (up 52%). Following ABT pretreatment, rat urinary metabolites were reduced in number from 10 to 1, N-acetyl-S-propylcysteine, which accounted for >90% of the total urinary radioactivity in ABT pretreated rats. Together, these data demonstrate a role for cytochrome P450 and glutathione in the dose-dependent metabolism and disposition of 1-BrP in the rat.

Experientia. 1974 Nov 15;30(11):1238-9.

The comparative metabolism of 3-bromo-propane-1,2-diol and 3-bromopropanol in the rat.

Jones AR, Bashir AA, Low SJ.

	Literature Search Results
Search Term	Propanol AND dibromo
Database	PubMed
Limitation(s)	
Relevant Papers	14
Date	July 27 2015
Comments	Belongs to CAS RN 96-21-9

2,3-Dibromo-1-propanol. (2004). *Rep Carcinog, 11*, III84.

Eustis, S. L., Haseman, J. K., Mackenzie, W. F., & Abdo, K. M. (1995). Toxicity and carcinogenicity of 2,3-dibromo-1-propanol in F344/N rats and B6C3F1 mice. *Fundam Appl Toxicol, 26*(1), 41-50.

Fishbein, L. (1979). Potential halogenated industrial carcinogenic and mutagenic chemicals. III. Alkane halides, alkanols and ethers. *Sci Total Environ, 11*(3), 223-257

Haseman, J. K., & Hailey, J. R. (1997). An update of the National Toxicology Program database on nasal carcinogens. *Mutat Res, 380*(1-2), 3-11.

Lewis, D. F., Ioannides, C., & Parke, D. V. (1990). A prospective toxicity evaluation (COMPACT) on 40 chemicals currently being tested by the National Toxicology Program. *Mutagenesis, 5*(5), 433-435.

Marsden, P. J., & Casida, J. E. (1982). 2-Haloacrylic acids as indicators of mutagenic 2-haloacrolein intermediates in mammalian metabolism of selected promutagens and carcinogens. *J Agric Food Chem, 30*(4), 627-631.

National Toxicology, P. (1993). NTP Toxicology and Carcinogenesis Studies of 2,3-Dibromo-1-Propanol (CAS No. 96-13-9) in F344/N Rats and B6C3F1 Mice (Dermal Studies). *Natl Toxicol Program Tech Rep Ser, 400*, 1-202.

National Toxicology, P. (2002). 2,3-Dibromo-1-propanol. *Rep Carcinog, 10*, 82-83.

National Toxicology, P. (2011). 2,3-Dibromo-1-propanol. *Rep Carcinog, 12*, 138-139.

Okumura, H., Nakazawa, J., Tsuganezawa, K., Usui, T., Osada, H., Matsumoto, T., Tanaka, A., & Yokoyama, S. (2006). Phenothiazine and carbazole-related compounds inhibit mitotic kinesin Eg5 and trigger apoptosis in transformed culture cells. *Toxicol Lett, 166*(1), 44-52.

Omura, M., Hirata, M., Zhao, M., Tanaka, A., & Inoue, N. (1995). Comparative testicular toxicities of two isomers of dichloropropanol, 2,3-dichloro-1-propanol, and 1,3-dichloro-2-propanol, and their metabolites alpha-chlorohydrin and epichlorohydrin, and the potent testicular toxicant 1,2-dibromo-3-chloropropane. *Bull Environ Contam Toxicol, 55*(1), 1-7.

Shirai, T., Ohshima, M., Masuda, A., Tamano, S., & Ito, N. (1984). Promotion of 2-(ethylnitrosamino)ethanol-induced renal carcinogenesis in rats by nephrotoxic compounds: positive responses with folic acid, basic lead acetate, and N-(3,5-dichlorophenyl)succinimide but not with 2,3-dibromo-1-propanol phosphate. *J Natl Cancer Inst, 72*(2), 477-482.

Stolzenberg, S. J., & Hine, C. H. (1979). Mutagenicity of halogenated and oxygenated three-carbon compounds. *J Toxicol Environ Health, 5*(6), 1149-1158.

Yoon, J. S., Mason, J. M., Valencia, R., Woodruff, R. C., & Zimmering, S. (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mutagen, 7*(3), 349-367.

	Literature Search Results
Search Term	3296-90-0
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	53
Date	July 23 2015
Comments	

Use of historical controls in time-adjusted trend tests for carcinogenicity
Quick ViewOther Sources

- By Ibrahim J G; Ryan L M
- From Biometrics (1996), 52(4), 1478-85. | Language: English, Database: MEDLINE
- We develop a method for incorporating historical control information into time-adjusted tests for dose effects in carcinogenicity studies. After discretizing the time scale, we use a multinomial distribution to model the number of animals dying with tumor in each interval. Data from past studies are used to estimate the parameters characterizing the prior. A score test derived from the resulting Dirichlet-multinomial generalizes the test of Tarone (1982, Biometrics

38, 215-220) and reduces, in the limit, to the log-rank test in the case of a diffuse prior. The methodology is illustrated with data from a study of the fire retardant 2,2-Bis(bromomethyl)-1,3-propanediol.

Reproductive toxicology. 2,2-bis(bromomethyl)-1,3-propanediol
Quick ViewOther Sources

- By Anonymous
- From Environmental health perspectives (1997), 105 Suppl 1, 271-2. | Language: English, Database: MEDLINE

2,2-Bis(bromomethyl)propane-1,3-diol
Quick ViewOther Sources

- By Anonymous
- From IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer (2000), 77, 455-68. | Language: English, Database: MEDLINE

On the use of historical control data for trend test in carcinogenicity studies
Quick ViewOther Sources

- By Sun J
- From Biometrics (1999), 55(4), 1273-6. | Language: English, Database: MEDLINE
- Historical control data are often available in carcinogenicity studies and are included for testing dose effects in current studies. A new method is developed for incorporating the historical control information into a dose effect test. The method generalizes the test procedures proposed by Tarone (1982, Biometrics 38, 215-220) and Ibrahim and Ryan (1996, Biometrics 52, 1478-1485) by taking into account the variation resulting from parameter estimation based on historical data. Two examples are discussed for illustrating the proposed method.

Use of the Japanese medaka (Oryzias latipes) and guppy (Poecilia reticulata) in carcinogenesis testing under national toxicology program protocols
Quick ViewOther Sources

- By Hawkins William E; Walker William W; Fournie John W; Manning C Steve; Krol Rena M
- From Toxicologic pathology (2003), 31 Suppl, 88-91. | Language: English, Database: MEDLINE
- A need exists for whole animal toxicity, mutagenesis, and carcinogenesis models that are alternative to the traditional rodent test models and that are economical, sensitive, and scientifically acceptable. Among small fish models, the Japanese medaka (Oryzias latipes) is preeminent for investigating effects of carcinogenic and/or toxic waterborne hazards to humans. The guppy (Poecilia reticulata), although less widely used, is valuable as a comparison species. Both species are easy to maintain and handle in the laboratory and there is a large body of background information on their responsiveness to a range of classes of carcinogens. There are considerable data on the occurrence of background diseases and on spontaneous neoplastic lesions, both of which occur relatively rarely. With few modifications, the medaka and guppy are amenable to carcinogenicity testing under the rigid standards established by the National Toxicology Program (NTP) for rodent tests. The advantages of the small fish in carcinogenesis studies are best realized in long-term studies that involve environmentally realistic exposures. Studies to identify chronic effects can be conducted in about 12 months, near the life span of medaka in our laboratory. Practically, 9-month studies are optimal but shorter study cycles and a variety of exposure/growout and initiation/promotion scenarios are available. Studies on 3 compounds tested in medaka under NTP protocols are under review and preliminary analysis indicates that chronic carcinogenicity bioassays with medaka, guppy, and potentially with other small fish species are feasible and scientifically valid.

2,2-bis(Bromomethyl)-1,3-propanediol (technical grade)
Quick ViewOther Sources

- No Author and Editor data available

- From Report on carcinogens : carcinogen profiles / U.S. Dept. of Health and Human Services, Public Health Service, National Toxicology Program (2002), 10, 35-6. | Language: English, Database: MEDLINE

NTP carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol, nitromethane, and 1,2,3-trichloropropane (cas nos. 3296-90-0, 75-52-5, and 96-18-4) in guppies (Poecilia reticulata) and medaka (Oryzias latipes) (Waterborne Studies)

Quick ViewOther Sources

- No Author and Editor data available
- From National Toxicology Program technical report series (2005), (528), 1-190. | Language: English, Database: MEDLINE

- The NTP chose to initiate studies in fish as an exploration of alternate or additional models for examining chemical toxicity and carcinogenicity. The use of small fish species in carcinogenicity testing offered potential advantages as a bioassay test system, including significant savings in cost and time over rodent studies. Large numbers of small fish could be easily maintained in a limited area. The two species chosen for study were guppy (Poecilia reticulata) and medaka (Oryzias latipes), both of which are hardy, easily maintained, and have a low occurrence of background lesions. The three chemicals chosen for study in fish had already been studied by the NTP in rodents, permitting a comparison of results between the two models. Two of the chemicals used (2,2-bis(bromomethyl)-1,3-propanediol and 1,2,3-trichloropropane) were mutagenic and multisite carcinogens in rats and mice. The third chemical, nitromethane, was nonmutagenic with a more modest carcinogenic response in rodents. Male and female guppies and medaka were exposed to 2,2-bis(bromomethyl)- 1,3-propanediol (greater than 99% pure), nitromethane, (greater than 99% pure), or 1,2,3-trichloropropane (99% pure) in aquaria water for up to 16 months. OVERALL STUDY DESIGN: Groups of approximately 220 guppies (two replicates of 110) were maintained in aquaria water containing nominal concentrations of 0, 24, 60, or 150 mg/L 2,2-bis(bromomethyl)-1,3-propanediol; 0, 10, 30, or 70 mg/L nitromethane; or 0, 4.5, 9.0, or 18.0 mg/L 1,2,3-trichloropropane. Groups of approximately 340 medaka (two replicates of 170) were maintained in aquaria water containing 0, 24, 60, or 150 mg/L 2,2-bis(bromomethyl)-1,3-propanediol; 0, 10, 20, or 40 mg/L nitromethane; or 0, 4.5, 9.0, or 18.0 mg/L 1,2,3-trichloropropane. The overall study durations were 16 months for all guppy studies, 14 months for 2,2-bis(bromomethyl)-1,3-propanediol-exposed medaka, and 13 months for nitromethane- and 1,2,3-trichloropropane-exposed medaka. Ten guppies and 10 medaka from each group replicate were sacrificed at 9 months for histopathologic analysis. Approximately one third of the remaining fish from each group were placed in chemical-free water at 9 months and constituted a stop-exposure study component. The remainder of the fish were exposed for the duration of the study and constituted the core study component. A stop-exposure component was added to determine if stopping the exposure at 9 months and transferring to chemical-free aquaria might allow for better survival and tumor development. The sex of guppies and medaka was determined at histopathologic analysis. 2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL - 16-MONTH STUDY IN GUPPIES: 2,2-Bis(bromomethyl)-1,3-propanediol was chronically toxic to guppies in the 60 and 150 mg/L core and stop-exposure groups. Due to mortality, exposure of core study animals in the 150 mg/L group was terminated on day 443, after approximately 64 weeks on study, and fish were maintained in 2,2-bis(bromomethyl)- 1,3-propanediol-free water in the exposure system until the end of the study at 69 weeks. Nominal exposure concentrations of 24, 60, and 150 mg/L provided actual aquaria water exposure concentrations of 20.0, 53.5, and 139.0 mg/L 2,2-bis(bromomethyl)- 1,3-propanediol, respectively. There were no treatment-related differences between the control and exposed groups in body weights or lengths. At 9 months, hepatocellular adenomas occurred in one 24 mg/L male and in one 150 mg/L male. In the core study, the incidence of hepatocellular adenoma or carcinoma (combined) in 150 mg/L males was greater than that in the controls; multiple adenomas occurred in two 150 mg/L males and in one 150 mg/L female. Cholangioma occurred in a small number of exposed males and females. In the stop-exposure study, incidences of hepatocellular adenoma (including multiple) and of hepatocellular carcinoma were greater in 150 mg/L males than in controls. One cholangioma and one cholangiocarcinoma occurred in the 150 mg/L female group. 14-MONTH STUDY IN MEDAKA: Exposure to 2,2-bis(bromomethyl)-1,3-propanediol did not result in any

significant reduction in survival, although the mortality of fish was somewhat greater in the 60 and 150 mg/L core study groups than in the control and 24 mg/L groups. After reallocation, mortality of medaka in the 60 and 150 mg/L core groups was slightly increased over the corresponding stop-exposure groups. Nominal exposure concentrations of 24, 60, and 150 mg/L provided actual exposure concentrations of 19.4, 56.9, and 137.8 mg/L 2,2-bis(bromomethyl)- 1, 3-propanediol, respectively. Core study animals in the 60 and 150 mg/L groups were significantly larger, in both body length and weight, than control group fish. In the core study, the incidence of hepatocellular adenoma or carcinoma (combined) was increased in 150 mg/L males. Cholangiocarcinomas occurred in a few exposed males and females, with all but one occurring in 150 mg/L fish. One cholangioma occurred in a 150 mg/L female, and one occurred in a control female. In the stop-exposure study, incidences of hepatocellular adenoma or carcinoma (combined) were marginally increased in the 150 mg/L group of males and in the 60 and 150 mg /L groups of females as compared with controls. Cholangiocarcinoma occurred in one male and one female in the 150 mg/L groups and in one control female. NITROMETHANE - 16-MONTH STUDY IN GUPPIES: Although the cause of death could not be confirmed in many cases, mortality in the 70 mg/L groups appeared to indicate that this level of nitromethane exposure was chronically toxic. This is confirmed by the similar survival rate of guppies from all treatments following removal from treatment aquaria and placement in stop-exposure. Due to the high mortality of fish in the 70 mg/L core study groups, these fish were removed from treatment (day 396) and fixed for histological analyses after approximately 57 weeks on study. The controls and other exposed groups were sacrificed at 70 weeks. Nominal exposure concentrations of 10, 30, and 70 mg/L provided actual exposure concentrations of 9.9, 28.7, and 66.4 mg/L nitromethane, respectively. There were no treatment-related differences between the control and exposed groups in body lengths or weights. 13-MONTH STUDY IN MEDAKA: Nitromethane in the aquaria supported a substantial microfaunal growth which, without frequent cleaning, affected water quality and treatment concentrations. To maintain acceptable water quality and treatment concentrations potentially affected by the rapid microfaunal growth, the study aquaria were brushed once and siphoned three times each day. Due to this frequent activity, a number of fish probably died due to mechanical injury. Unfortunately, the cause of death could not be confirmed in many cases; the mortality from this activity is believed to have been approximately uniform among treatments and should not have affected the comparison of survival between treatments. Based on mortality in this study and the previous life-span evaluation, the life phase of this study was terminated approximately 13.5 months after hatching. Nominal exposure concentrations of 10, 20, and 40 mg/L resulted in actual exposure concentrations of 9.3, 20.8, and 41.7 mg/L nitromethane, respectively. No differences between control and exposed groups were found in body lengths or weights at the 9-month interim evaluation. Due to mortality, unequal numbers of fish were distributed among the core study and stop-exposure aquaria at 9 months. Differences in lengths and weights were found at 13 months. The biological significance of this finding is unknown. At 9 months, a single cholangiocarcinoma occurred in a 40 mg/L male. Hepatocellular adenomas occurred in two 20 mg/L males and in one 40 mg/L female. In the core study, one cholangioma occurred in a 20 mg/L male, and cholangiocarcinomas were seen in a few exposed males, but none occurred in control males. 1,2,3-TRICHLOROPROPANE - 16-MONTH STUDY IN GUPPIES: The survival of exposed guppies was less than that of the control group at 9 months. Reduced survival was evident at 6 months in the 18.0 mg/L groups and at 7 months in the 4.5 and 9.0 mg/L groups. Survival was significantly reduced in the 18.0 mg/L core study group within 1 month of the 9-month interim evaluation, and mortality in this group was 42.6% between 9 months and study termination. Nominal exposure concentrations of 4.5, 9.0, and 18.0 mg/L resulted in actual exposure concentrations of 4.4, 8.8, and 18.2 mg/L 1, 2,3-trichloropropane, respectively. Guppies in the 18.0 mg/L core study group were significantly longer and weighed more than the controls. Fish in the 18.0 mg/L stop-exposure group also weighed more than the controls. Mortality of fish during the study resulted in unequal numbers of individuals distributed to core study and stop-exposure aquaria at 9 months. This appears to have influenced the length and weight of fish measured at study termination (i.e., the smaller tank population allowed the fish to grow more). Observed differences in weight and length between controls and 18.0 mg/L fish was most likely an artifact of the reduced numbers of fish

in the 18.0 mg/L aquaria. At 9 months, multiple hepatocellular adenomas occurred in one 4.5 mg /L male, and one hepatocellular adenoma occurred in a control male. In the core study, increased incidences of cholangiocellular (bile duct) and hepatocellular neoplasms occurred in exposed groups of males and females. Cholangioma and cholangiocarcinoma were seen in several exposed males and females. In the stop-exposure study, increased incidences of hepatocellular neoplasms occurred in 18.0 mg/L males and increased incidences of cholangiocellular (bile duct) neoplasms occurred in 18.0 mg/L females. (ABSTRACT TRUNCATED)

The utility of the guppy (Poecilia reticulata) and medaka (Oryzias latipes) in evaluation of chemicals for carcinogenicity
Quick ViewOther Sources

- By Kissling Grace E; Bernheim Naomi J; Hawkins William E; Wolfe Marilyn J; Jokinen Micheal P; Smith Cynthia S; Herbert Ronald A; Boorman Gary A
- From Toxicological sciences : an official journal of the Society of Toxicology (2006), 92(1), 143-56. | Language: English, Database: MEDLINE
- There has been considerable interest in the use of small fish models for detecting potential environmental carcinogens. In this study, both guppies (Poecilia reticulata) and medaka (Oryzias latipes) were exposed in the aquaria water to three known rodent carcinogens for up to 16 months. Nitromethane, which caused mammary gland tumors by inhalation exposure in female rats, harderian gland and lung tumors in male and female mice, and liver tumors in female mice by inhalation, failed to increase tumors in either guppies or medaka. Propanediol, which when given in the feed was a multisite carcinogen in both sexes of rats and mice, caused increased liver tumors in male guppies and male medaka. There was reduced survival in female guppies and no increased tumors in female medaka. 1,2,3-Trichloropropane, which when administered by oral gavage was a multisite carcinogen in both sexes of rats and mice, caused an increased incidence of tumors in the liver of both male and female guppies and medaka and in the gallbladder of male and female medaka. The results of this study demonstrate that for these three chemicals, under these specific exposure conditions, the fish appear less sensitive and have a narrower spectrum of tissues affected than rodents. These results suggest that fish models are of limited utility in screening unknown chemicals for potential carcinogenicity.

2,2-bis(Bromomethyl)-1,3-propanediol (technical grade)
Quick ViewOther Sources

- By Anonymous
- From Report on carcinogens : carcinogen profiles / U.S. Dept. of Health and Human Services, Public Health Service, National Toxicology Program (2004), 11, III36-7. | Language: English, Database: MEDLINE

2,2-Bis(bromomethyl)-1,3-propanediol (technical grade)
Quick ViewOther Sources

- No Author and Editor data available
- From Report on carcinogens : carcinogen profiles / U.S. Dept. of Health and Human Services, Public Health Service, National Toxicology Program (2011), 12, 70-1. | Language: English, Database: MEDLINE

Induction of DNA damage in human urothelial cells by the brominated flame retardant 2,2-bis(bromomethyl)-1,3-propanediol: role of oxidative stress
Quick ViewOther Sources

- By Kong Weixi; Kuester Robert K; Gallegos Alfred; Sipes I Glenn
- From Toxicology (2011), 290(2-3), 271-7. | Language: English, Database: MEDLINE
- 2,2-bis(bromomethyl)-1,3-propanediol (BMP) is an extensively used brominated flame retardant found in urethane foams and polyester resins. In a 2-year dietary study conducted by the National Toxicology Program, BMP caused neoplastic lesions at multiple sites including the urinary bladder in both rats and mice. The mechanism of its carcinogenic effect is unknown. In the present study, using SV-40 immortalized human urothelial cells (UROtsa), endpoints associated with BMP induced DNA damage and oxidative stress were investigated. The effects of time (1-24h) and concentration (5-100 μM) on BMP induced DNA strand breaks were assessed via the alkaline comet assay. The results revealed evidence of DNA strand breaks at 1 and 3h

following incubation of cells with non-cytotoxic concentrations of BMP. Strand breaks were not present after 6h of incubation. Evidences for BMP associated oxidative stress include: an elevation of intracellular ROS formation as well as induction of Nrf2 and HSP70 protein levels. In addition, DNA strand breaks were attenuated when cells were pre-treated with N-acetyl-l-cysteine (NAC) and oxidative base modifications were revealed when a lesion specific endonuclease, human 8-hydroxyguanine DNA glycosylase 1 (hOGG1) was introduced into the comet assay. In conclusion, these results demonstrate that BMP induces DNA strand breaks and oxidative base damage in UROtsa cells. Oxidative stress is a significant, determinant factor in mediating these DNA lesions. These early genotoxic events may, in part, contribute to BMP-induced carcinogenesis observed in rodents.

Results of a two-year toxicity and oncogenic study of rats ingesting diets containing dibromoneopentyl glycol (FR-1138)
Quick ViewOther Sources

- By Keyes, D. G.; Kociba, R. J.; Schwetz, R. W.; Wade, C. E.; Dittenber, D. A.; Quinn, T.; Gorzinski, S. J.; Hermann, E. A.; Momany, J. J.; Schwetz, B. A.
- From Journal of Combustion Toxicology (1980), 7(May), 77-98. | Language: English, Database: CAPLUS

- A lifetime toxicity dietary study of FR 1138 [3296-90-0] was conducted in rats to assess the potential for chronic toxicity and possible oncogenesis. Rats ingesting the lower dietary level of 5 mg FR 1138/kg/day had no effects related to the lifetime treatment. Rats ingesting a higher dietary level of 100 mg FR 1138/kg/day had some evidence of toxicity, including degenerative changes in the liver, eye, and possibly thyroid gland; however, there was no oncogenic response, even when FR 1138 was administered at a high dosage to induce some toxicity. Anal. of selected tissues, indicated an increase in bromide content in the tissues of rats ingesting the higher dose, (100 mg FR 1138/kg/day). At the lower dose (5 mg FR 1138/kg/day), there was only a marginal increase in bromide content of some of the tissues, with most values in the same range as the controls.

A collection of guinea pig sensitization test results grouped by chemical class
Quick ViewOther Sources

- By Rao, K. S.; Betso, J. E.; Olson, K. J.
- From Drug and Chemical Toxicology (1977) (1981), 4(4), 331-51. | Language: English, Database: CAPLUS

- Various chem. groups were evaluated for their skin sensitization potential in the guinea pig. In general, amines, acetanilides, pyridines, piperidines, and sulfones were pos. in the guinea pig test. Since these tests were done over a period of years, any further structural-related correlations or predictions should be made with caution due to variability of sample purity or differences in methodol. It is important to realize that every chem. which is pos. in the guinea pig should not be construed as definitive evidence of human skin sensitization.

Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals
Quick ViewOther Sources

- By Mortelmans, Kristien; Haworth, Steve; Lawlor, Timothy; Speck, William; Tainer, Beth; Zeiger, Errol
- From Environmental Mutagenesis (1986), 8(Suppl. 7), 1-119. | Language: English, Database: CAPLUS

- This publication includes data of Salmonella mutagenicity results on 270 coded chems., encompassing 329 tests performed by 3 labs. under contract to the National Toxicol. Program. The preincubation modification of the Salmonella/mammalian microsome assay was used to test chems. in up to 5 Salmonella strains in the presence and absence of rat and hamster liver S-9. With a few exceptions, inter- and intralab. reproducibility was good.

2,2-Bis(bromomethyl)-1,3-propanediol: reproduction and fertility assessment in CD-1 mice when administered in feed (revised January 1986)
Quick ViewOther Sources

- By Gulati, D. K.; Mounce, R. C.; Shaver, S.; Russell, S.; Poonacha, K. B.
- From Report (1986), (NTP-86-063; Order No. PB86-168341/GAR), 522 pp.. | Language: English, Database: CAPLUS

- Rats ingesting 5 mg/kg 2,2-bis(bromomethyl)-1,3-propanediol (BBMP) [3296-90-0] showed no symptoms of toxicity. Treatment at 100 mg/kg resulted in significant toxicity, including degenerative changes in the liver, eyes, and possibly the thyroid gland. BBMP administered in feed at the 0.4% dose level adversely affected reprodn. in mice. The continued BBMP treatment at this dose level also resulted in a significant drop in body wt. When female mice exposed to 0.4% BBMP were mated with control male mice, the fertility index was reduced by almost 60%. The no. of live pups/litter delivered by these breeding pairs was also significantly lower than the 0.4% male X control female pairs.

Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals
Quick ViewOther Sources

- By Galloway, S. M.; Armstrong, M. J.; Reuben, C.; Colman, S.; Brown, B.; Cannon, C.; Bloom, A. D.; Nakamura, F.; Ahmed, M.; et al.
- From Environmental and Molecular Mutagenesis (1987), 10(Suppl. 10), 1-175. | Language: English, Database: CAPLUS

- Results from the testing of 108 coded chems. in CHO cells for the induction of chromosome aberrations and sister chromatid exchanges (SCEs) are presented. All chems. were tested with and without exogenous metabolic activation, using protocols designed to allow testing up to toxic doses. Cell harvest times could also be extended if chem.-induced cell cycle delay was seen. Chromosome aberrations were induced by 43 of the chems., and 66 induced SCEs; 37 of the chems. were pos. for both endpoints.

Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from national toxicology program 13-week studies
Quick ViewOther Sources

- By Morrissey, Richard E.; Schwetz, Bernard A.; Lamb, James C., IV; Ross, Monica D.; Teague, Janet L.; Morris, Richard W.
- From Fundamental and Applied Toxicology (1988), 11(2), 343-58. | Language: English, Database: CAPLUS

- Sperm morphol. and vaginal cytol. examns. (SMVCEs), which include evaluations of motility, concn. and head morphol. of sperm from the cauda epididymis, and male reproductive organ wt. data, were developed by the National Toxicol. Program as a screening system for reproductive toxicants. An anal. was conducted of SMVCE studies carried out at the end of fifty 13-wk studies (25 for rats, 25 for mice) over a 3-yr period. Statistically significant changes in these studies were summarized, as were control data for each male endpoint (mean, SD, 95% confidence limits around the mean, median, and statistical power). Reproductive organ wts. (testis, epididymis, cauda epididymis) and sperm motility were the most statistically powerful endpoints evaluated; sperm head morphol. may also be a sensitive endpoint for detecting reproductive toxicants. For 24 chems. tested in both rats and mice, the concordance of results [i.e., no adverse effect in either species, or at least one SMVCE endpoint (not necessarily the same one) adversely affected in both species] was 58%. These data suggest that detection of potential reproductive toxicants might be best when both species are used. Types of sperm head abnormalities and their relative proportion of the total did not differ among control and treatment groups. Estrous cycle data were obtained in the final week of forty-six 13-wk studies (23 for mice, 23 for rats). Only 3 chems. caused an increase in mean cycle length compared with the control group. More data from breeding studies in which female estrous cycle length is measured are needed to assess fully the assocn. of cycle length with reproductive outcome; stages of the estrous cycle are so variable that they may not be useful in assessing potential toxicity. Interlab. variability in SMVCE values for many endpoints was documented.

Association of sperm, vaginal cytology, and reproductive organ weight data with results of continuous breeding reproduction studies in Swiss (CD-1) mice
Quick ViewOther Sources

- By Morrissey, Richard E.; Lamb, James C., IV; Schwetz, Bernard A.; Teague, Janet L.; Morris, Richard W.
- From Fundamental and Applied Toxicology (1988), 11(2), 359-71. | Language: English, Database: CAPLUS

- In continuous breeding reprodn. studies in which an adverse effect on fertility was detected over an 18-wk treatment period, a crossover mating trial was then conducted to det. the affected sex. Results of 25 crossover breeding studies conducted using Swiss (CD-1) mice were compared with results of sperm morphol. and vaginal cytol. examns. (SMVCEs) conducted at the conclusion of the mating trial. SMVCE endpoints include sperm concn., motility, and morphol., vaginal cytol., and male reproductive organ wts. In most SMVCE studies multiple endpoints were adversely affected. For male reproductive toxicants, sperm motility was decreased in 89% of the studies, and abs. right epididymis and right testis wts. were affected less frequently (80% each). Among studies with no detectable redn. in male breeding performance, 87% exhibited no detectable decrease in epididymis wt. Eighty-two percent had no change in cauda epididymis wt. and 80% had no significant change in sperm concn. An increase in female cycle length was assocd. (100%) with an effect on breeding due to female dysfunction. Overall accuracy, defined as correct identification of toxicants and nontoxicants, was highest for epididymis wt. (84%), followed by cauda epididymis wt. and sperm motility (79% each), and sperm concn. (76%). Female cycle length was so variable that the overall accuracy of the parameter in 13 studies was 69%. With the variety of chems. used in this anal., the assocn. of abnormal sperm morphol. with reproductive outcome was 71%. The statistical sensitivity was relatively high for reproductive organ wts., although it was less for smaller organs such as the prostate. On the basis of both the biol. and statistical analyses, it is recommended that multiple SMVCE endpoints, including sperm measures, be included in screens for reproductive toxicants.

Kidney and urinary bladder lesions in F344/N rats and B6C3F1 mice after 13 weeks of 2,2-bis(bromomethyl)-1,3-propanediol administration
Quick ViewOther Sources

- By Elwell, M. R.; Dunnick, J. K.; Brown, H. R.; Montgomery, C. A.
- From Fundamental and Applied Toxicology (1989), 12(3), 480-90. | Language: English, Database: CAPLUS

- Thirteen-week toxicity studies of the flame retardant 2,2-bis(bromomethyl)-1,3-propanediol (BMP) were conducted in male and female rats and mice. The chem. was administered by oral gavage in corn oil 5 days per wk for 13 wk to rats at doses of 0-800 mg/kg and to mice at doses of 0-400 mg/kg, or in the feed for 13 wk at concns. of 0-20,000 ppm for rats and at 0-10,000 ppm for mice. There was a dose-related decrease in body wt. gain in rats and mice after chem. administration. Mortality attributed to toxicity of BMP was seen in the gavage study in 2/10 high-dose (800 mg/kg) male rats and 3010 high-dose (400 mg/kg) male mice; no dose-related mortality occurred in the feed study. Minimal degeneration in the renal papilla was seen in male rats at 800 mg/kg in the gavage study and at doses of 5000 ppm or more in the feed study. This was also present in one female rat at 20,000 ppm dose. In male mice renal papillary necrosis occurred at 400 mg/kg after dosing by the gavage route and at 2500, 5000, and 10,000 ppm in the dosed-feed study. In female mice papillary necrosis occurred only at the 10,000 ppm dose in the feed study. Tubular cell regeneration of the renal cortex was also present in mice at the same dose levels at which the papillary necrosis was obsd. Transitional cell hyperplasia of the urinary bladder was seen in male rats at 400 and 800 mg/kg and in both sexes of mice at 200 and 400 mg/kg. Hyperplasia of the urinary bladder was also seen when BMP was administered in the feed at doses of 20,000 ppm to male rats; at doses of 2500, 5000, and 10,000 ppm to male mice; and at doses of 5000 and 10,000 ppm to female mice. The kidney and urinary bladder are target organs when BMP is administered by gavage or the dosed-feed route; mice were more sensitive than rats for the development of kidney and

bladder lesions. Male rats and mice were more sensitive than females for the development of renal papillary degeneration or necrosis.

Reproductive toxicity of 2,2-bis(bromomethyl)-1,3-propanediol in a continuous breeding protocol in Swiss (CD-1) mice
Quick ViewOther Sources

- By Treinen, Kimberley A.; Chapin, Robert E.; Gulati, Dushyant K.; Mounce, Robin; Morris, Lisa Z.; Lamb, James C., IV
- From Fundamental and Applied Toxicology (1989), 13(2), 245-55. | Language: English, Database: CAPLUS

- The effect of 2,2-bis(bromomethyl)-1,3-propanediol (BMP) on reprodn. in Swiss CD-1 mice was evaluated by use of a continuous breeding protocol. BMP was administered in the feed at 0.1, 0.2, and 0.4% concns. Both male and female F_0 mice (20 pairs/treatment group, 40 pairs of control animals) were dosed 7 days prior to and during a 98-day cohabitation period. Although the fertility index was unchanged in the high-dose group, BMP exposure significantly decreased the nos. of litters/pair, pups born alive/litter, and pup wt. when adjusted for litter size. Crossover mating between treated and control F_0 animals indicated a specific effect only on female reproductive capacity. At the high dose, BMP caused a body wt. decrease in the F_0 animals of both sexes with no effects on relative organ wts. Sperm concn., motility, morphol., and estrual cyclicity were unaffected by BMP exposure. Histopathol. in the F_0 animals revealed specific kidney lesions in both sexes; males were more sensitive than females. The last litter born in the 98-day breeding phase was reared to age 74 days and then mated to nonsiblings of the same treatment group. The effect of high-dose BMP exposure on F_1 fertility, body and organ wts., sperm parameters, and estrual cyclicity was the same as that for the F_0 animals, with the exception of the lack of renal lesions seen in the F_1 females. Thus, BMP impaired fertility in female mice in both generations in the absence of an effect on reproductive organ wts. and estrual cyclicity.

Prediction of probability of carcinogenicity for a set of ongoing NTP bioassays
Quick ViewOther Sources

- By Enslein, Kurt; Blake, Benjamin W.; Borgstedt, Harold
- From Mutagenesis (1990), 5(4), 305-6. | Language: English, Database: CAPLUS

- Forty-four compds. currently undergoing carcinogenesis bioassay by the National Toxicol. Program were submitted to the Toxicity Prediction by Komputer-Assisted Technol. (TOPKAT) program for prediction of their potential carcinogenicity. Sixteen compds. could not be handled by TOPKAT. Of the 28 for which predictions were made, 26 (93%) had a confidence level in the est. of at least moderate. Seventeen were predicted to be carcinogens and 11 noncarcinogens.

Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals
Quick ViewOther Sources

- By Zeiger, Errol; Anderson, Beth; Haworth, Steve; Lawlor, Timothy; Mortelmans, Kristien
- From Environmental and Molecular Mutagenesis (1992), 19(Suppl. 21), 2-141. | Language: English, Database: CAPLUS

- Three hundred eleven chems. were tested under code, for mutagenicity, in S. typhimurium; 35 of the chems. were tested more than once in the same or different labs. The tests were conducted using a preincubation protocol in the absence of exogenous metabolic activation, and in the presence of liver S-9 from Aroclor-induced male Sprague-Dawley rats and Syrian hamsters. Some of the volatile chems. were also tested in desiccators. A total of 120 chems. were mutagenic or weakly mutagenic, 3 were judged questionable, and 172 were nonmutagenic. The remaining 16 chems. produced different responses in the two or three labs. in which they were tested. The results and data from these tests are presented.

Comparison between rodent carcinogenicity test results of 44 chemicals and a number of predictive systems

Quick ViewOther Sources

- By Lewis, David F. V.
- From Regulatory Toxicology and Pharmacology (1994), 20(3, Pt. 1), 215-22. | Language: English, Database: CAPLUS

- A comparison is made between a no. of predictive systems including bacterial mutagenicity, structure alert and chronic toxicity (R. W. Tennant et al., 1990), COMPACT, Hazardexpert, and DEREK, with the outcome of the two species rodent carcinogenicity bioassay for 44 chems. conducted under the NTP protocol. Following minor updating of the rodent data in the light of pathol. reports, there is a generally good agreement between various predictions and the actual carcinogenicity results. In particular, a combination of two methods of prediction produces an over 80% concordance with the rodent bioassay. Possible reasons for various discrepancies are discussed in light of xenobiotic metab. and activation mechanisms of chem. carcinogenesis.

Toxicology and carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol (FR-1138) (CAS no. 3296-90-0) in F344/N rats and B6C3F1 mice (feed studies)

Quick ViewOther Sources

- By U.S. Department of Health and Human Services
- From National Toxicology Program Technical Report Series (1996), (452), 376 pp.. | Language: English, Database: CAPLUS

- 2,2-Bis(bromomethyl)-1,3-propanediol is used as a fire retardant in unsatd. polyester resins, in molded products, and in rigid polyurethane foam. 2,2-Bis(bromomethyl)-1,3-propanediol was chosen for study because it is a widely used flame retardant and little toxicity and carcinogenicity data were available. Groups of male and female F344/N rats and B6C3F$_1$ mice were exposed to tech. grade 2,2-bis(bromomethyl)-1,3-propanediol (78.6% pure) in feed for 13 wk or 2 yr. Genetic toxicol. studies were conducted in Salmonella typhimurium, cultured Chinese hamster ovary cells, mouse bone marrow, and mouse peripheral blood. Under the conditions of these 2-yr feed studies, there was clear evidence of the carcinogenicity and genotoxicity of 2,2-bis(bromomethyl)-1,3-propanediol.

Prioritization of NTP reproductive toxicants for field studies

Quick ViewOther Sources

- By Moorman, W. J.; Ahlers, H. W.; Chapin, R. E.; Daston, G. P.; Foster, P. M. D.; Kavlock, R. J.; Morawetz, J. S.; Schnorr, T. M.; Schrader, S. M.
- From Reproductive Toxicology (2000), 14(4), 293-301. | Language: English, Database: CAPLUS

- Population studies that evaluate human reproductive impairment are time consuming, expensive, logistically difficult, and with limited resources must be prioritized to effectively prevent the adverse health effects in humans. Interactions among health scientists, unions, and industry can serve to identify populations exposed to potential hazards and develop strategies to evaluate and apply appropriate controls. This report describes a systematic method for prioritizing chems. that may need human reproductive health field studies. Rodent reproductive toxicants identified from the National Toxicol. Program (NTP) Reproductive Assessment by Continuous Breeding (RACB) protocol were prioritized on the basis of potency of toxic effect and population at risk. This model for prioritization links NTP findings with data from the National Occupational Exposure Survey (NOES) and the Hazardous Substance Data Base (HSDB) or the High Prodn. Vol. Chem. Database (HPVC) to prioritize chems. for their potential impact on worker populations. The chems. with the highest priority for field study were: di-Bu phthalate, boric acid, tricresyl phosphate, and N,N-dimethylformamide.

Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program

Quick ViewOther Sources

- By Witt, Kristine L.; Knapton, Alan; Wehr, Carol M.; Hook, Graham J.; Mirsalis, Jon; Shelby, Michael D.; MacGregor, James T.

- From Environmental and Molecular Mutagenesis (2000), 36(3), 163-194. | Language: English, Database: CAPLUS

- The mouse peripheral blood micronucleus (MN) test was performed on samples collected from 20 short-term, 67 subchronic, and 5 chronic toxicity and carcinogenicity studies conducted by the National Toxicol. Program (NTP). Data are presented for studies not previously published. Aspects of protocol that distinguish this test from conventional short-term bone marrow MN tests are duration of exposure and absence of repeat tests and concurrent pos. controls. Furthermore, in contrast to short-term bone marrow MN tests where scoring is limited to polychromatic erythrocytes (PCE), longer-term studies using peripheral blood may evaluate MN in both, or either, the normochromatic (NCE) or PCE populations. The incidence of MN-PCE provides an index of damage induced within 72 h of sampling whereas the incidence of MN in the NCE population at steady state provides an index of av. damage during the 30-day period preceding sampling. The mouse peripheral blood MN test has been proposed as a useful adjunct to rodent toxicity tests and has been effectively incorporated as a routine part of overall toxicity testing by the NTP. Data derived from peripheral blood MN analyses of dosed animals provide a useful indication of the in vivo potential for induced genetic damage and supply an important piece of evidence to be considered in the overall assessment of toxicity and health risk of a particular chem. Although results indicate that the test has low sensitivity for prediction of carcinogenicity, a convincingly pos. result in this assay appears to be highly predictive of rodent carcinogenicity.

Reevaluating cancer risk estimates for short-term exposure scenarios
Quick ViewOther Sources

- By Halmes, N. Christine; Roberts, Stephen M.; Tolson, J. Keith; Portier, Christopher J.
- From Toxicological Sciences (2000), 58(1), 32-42. | Language: English, Database: CAPLUS

- Ests. of cancer risk from short-term exposure to carcinogens generally rely on cancer potency values derived from chronic, lifetime-exposure studies and assume that exposures of limited duration are assocd. with a proportional redn. in cancer risk. The validity of this approach was tested empirically using data from both chronic lifetime and stop-exposure studies of carcinogens conducted by the National Toxicol. Program. Eleven compds. were identified as having data sufficient for comparison of relative cancer potencies from short-term vs. lifetime exposure. The data were modeled using the chronic data alone, and also using the chronic and the stop-exposure data combined, where stop-exposure doses were adjusted to av. lifetime exposure. Maximum likelihood ests. of the dose corresponding to a 1% added cancer risk (ED_{01}) were calcd. along with their assocd. 95% upper and lower confidence bounds. Statistical methods were used to evaluate the degree to which adjusted stop-exposures produced risks equal to those estd. from the chronic exposures. For most chem./cancer endpoint combinations, inclusion of stop-exposure data reduced the ED_{01}, indicating that the chem. had greater apparent potency under stop-exposure conditions. For most chems. and endpoints, consistency in potency between continuous and stop-exposure studies was achieved when the stop-exposure doses were averaged over periods of less than a lifetime-in some cases as short as the exposure duration itself. While the typical linear adjustments for less-than-lifetime exposure in cancer risk assessment can theor. result in under- or overestimation of risks, empirical observations in this anal. suggest that an underestimation of cancer risk from short-term exposures is more likely.

Predominant K-ras Codon 12 G → A Transition in Chemically Induced Lung Neoplasms in B6C3F1 Mice
Quick ViewOther Sources

- By Ton, Thai-Vu T.; Hong, Hue-Hua L.; Anna, Colleen H.; Dunnick, June K.; Devereux, Theodora R.; Sills, Robert C.; Kim, Yongbaek
- From Toxicologic Pathology (2004), 32(1), 16-21. | Language: English, Database: CAPLUS

- Based on long-term toxicity and carcinogenicity studies in B6C3F1 mice conducted by the National Toxicol. Program, 2,2-Bis(bromomethyl)-1,3-propanediol (BMP) and tetranitromethane (TNM) have been identified as carcinogens. Following 2 yr of exposure to 312,

625, or 1,250 ppm BMP in feed, or exposure to 0.5 or 2 ppm TNM by inhalation, increased incidences of lung neoplasms were obsd. in B6C3F1 mice at all exposure concns. compared to unexposed mice. The present study characterizes genetic alterations in the K-ras protooncogene in BMP- and TNM-induced lung neoplasms, resp., and compares the findings to spontaneous lung neoplasms from corresponding control mice. The frequencies of the K-ras mutations were 57% (29/51) in BMP-induced lung neoplasms compared to 15% (3/20) in lung neoplasms from dosed feed control mice, and 54% (14/26) in TNM-induced lung neoplasms compared to 60% (3 /5) in lung neoplasms from inhalation control mice. G → A transitions at the second base of the K-ras codon 12 (GGT GAT) were the most frequent pattern of K-ras mutations identified in BMP-induced (20/29) and TNM-induced lung neoplasms (13/14), which differed from the mutational patterns identified in the lung neoplasms from unexposed control mice. These results indicate that mutations in the K-ras gene are involved in B6C3F1 lung carcinogenesis following BMP- and TNM-exposure, and the high frequency and specificity of the ras mutation profile in lung neoplasms (G → A transition) may be due to in vivo genotoxicity by the parent compds. or their metabolites.

Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity

Quick ViewOther Sources

- By Kirkland, David; Aardema, Marilyn; Henderson, Leigh; Mueller, Lutz
- From Mutation Research, Genetic Toxicology and Environmental Mutagenesis (2005), 584(1-2), 1-256. | Language: English, Database: CAPLUS

- The performance of a battery of three of the most commonly used in vitro genotoxicity tests, i.e., Ames + mouse lymphoma assay (MLA) + in vitro micronucleus (MN) or chromosomal aberrations (CA) test, was evaluated for its ability to discriminate rodent carcinogens and non-carcinogens, from a large database of over 700 chems. compiled from the CPDB ("Gold"), NTP, IARC and other publications. We re-evaluated many (113 MLA and 30 CA) previously published genotoxicity results in order to categories the performance of these assays using the response categories we established. The sensitivity of the three-test battery was high. Of the 553 carcinogens for which there were valid genotoxicity data, 93% of the rodent carcinogens evaluated in at least one assay gave pos. results in at least one of the three tests. Combinations of two and three test systems had greater sensitivity than individual tests resulting in sensitivities of around 90% or more, depending on test combination. Only 19 carcinogens (out of 206 tested in all three tests, considering CA and MN as alternatives) gave consistently neg. results in a full three-test battery. Most were either carcinogenic via a non-genotoxic mechanism (liver enzyme inducers, peroxisome proliferators, hormonal carcinogens) considered not necessarily relevant for humans, or were extremely weak (presumed) genotoxic carcinogens (e.g. N-nitrosodiphenylamine). Two carcinogens (5-chloro-o-toluidine, 1,1,2,2-tetrachloroethane) may have a genotoxic element to their carcinogenicity and may have been expected to produce pos. results somewhere in the battery. We identified 183 chems. that were non-carcinogenic after testing in both male and female rats and mice. There were genotoxicity data on 177 of these. The specificity of the Ames test was reasonable (73.9%), but all mammalian cell tests had very low specificity (i.e. below 45%), and this declined to extremely low levels in combinations of two and three test systems. When all three tests were performed, 75-95% of non-carcinogens gave pos. (i.e. false pos.) results in at least one test in the battery. The extremely low specificity highlights the importance of understanding the mechanism by which genotoxicity may be induced (whether it is relevant for the whole animal or human) and using wt. of evidence approaches to assess the carcinogenic risk from a pos. genotoxicity signal. It also highlights deficiencies in the current prediction from and understanding of such in vitro results for the in vivo situation. It may even signal the need for either a reassessment of the conditions and criteria for pos. results (cytotoxicity, soly., etc.) or the development and use of a completely new set of in vitro tests (e.g. mutation in transgenic cell lines, systems with inherent metabolic activity avoiding the use of S9, measurement of genetic changes in more cancer-relevant genes or hotspots of genes, etc.). It was very difficult to assess the performance of the in vitro MN test, particularly in combination with other assays, because the published database for this assay is

relatively small at this time. The specificity values for the in vitro MN assay may improve if data from a larger proportion of the known non-carcinogens becomes available, and a larger published database of results with the MN assay is urgently needed if this test is to be appreciated for regulatory use. However, specificity levels of <50% will still be unacceptable. Despite these issues, by adopting a relative predictivity (RP) measure (ratio of real:false results), it was possible to establish that pos. results in all three tests indicate the chem. is greater than three times more likely to be a rodent carcinogen than a non-carcinogen. Likewise, neg. results in all three tests indicate the chem. is greater than two times more likely to be a rodent non-carcinogen than a carcinogen. This RP measure is considered a useful tool for industry to assess the likelihood of a chem. possessing carcinogenic potential from batteries of pos. or neg. results.

Human toxicological effect and damage factors of carcinogenic and noncarcinogenic chemicals for life cycle impact assessment

Quick ViewOther Sources

- By Huijbregts, Mark A. J.; Rombouts, Linda J. A.; Ragas, Ad M. J.; van de Meent, Dik
- From Integrated Environmental Assessment and Management (2005), 1(3), 181-244. | Language: English, Database: CAPLUS

- Chem. fate, effect, and damage should be accounted for in the anal. of human health impacts by toxic chems. in life cycle assessment (LCA). The goal of this article is to present a new method to derive human damage and effect factors of toxic pollutants, starting from a lognormal dose-response function. Human damage factors are expressed as disability-adjusted life-years (DALYs). Human effect factors contain a disease-specific and a substance-specific component. The disease-specific component depends on the probability of disease occurrence and the distribution of sensitivities in the human population. The substance-specific component, equal to the inverse of the ED50, represents the toxic potency of a substance. The new method has been applied to calc. combined human damage and effect factors for 1192 substances. The total range of 7-9 orders of magnitude between the substances is dominated by the range in toxic potencies. For the combined factors, the typical uncertainty, represented by the square root of the ratio of the 97.5th and 2.5th percentiles, is a factor of 25 for carcinogenic effects and a factor of 125 for noncarcinogenic effects. The interspecies conversion factor, the (non)cancer effect conversion factor, and the av. noncancer damage factor dominate the overall uncertainty.

Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity. [Erratum to document cited in CA143:243161]

Quick ViewOther Sources

- By Kirkland, David; Aardema, Marilyn; Henderson, Leigh; Mueller, Lutz
- From Mutation Research, Genetic Toxicology and Environmental Mutagenesis (2005), 588(1), 70. | Language: English, Database: CAPLUS

- On the title page, the URL of the website address in the open star footnote should read: www.lhasalimited.org/cgx. This is where the appendixes have been posted.

Carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol, nitromethane, and 1,2,3-trichloropropane (CAS NOS. 3296-90-0, 75-75-5, and 96-18-4) in guppies (Poecilia reticulata) and medaka (Oryzias latipes)

Quick ViewOther Sources

- By Bernheim, N. J.; Boorman, G. A.; Herbert, R. A.; Bristol, D. W.; Bucher, J. R.; Hailey, J. R.; Haseman, J. K.; Kissling, G. E.; Maronpot, R. R.; Nyska, A.; et al
- From National Toxicology Program Technical Report Series (2005), (528), 1-190. | Language: English, Database: CAPLUS

- The National Toxicol. Program has used rats and mice to test if chems. can cause cancer in animals. We wanted to see if fish could be used as a test animal for cancer testing. In this study, we exposed two species of fish, guppies and medaka, to three different chems. that caused cancer in rodents to see if fish had the same response. Methods We held groups of approx. 110 guppies or 170 medaka in aquaria each contg. a specific concn. of a chem. Three

different chems. were tested, and three different concns. of each chem. were used. Similar groups of fish were held in aquaria contg. only clean water and served as the control groups. The three chems. used were 2,2-bis(bromomethyl)-1,3-propanediol, nitromethane, or 1,2,3-trichloropropane. After nine months of exposure, some of the fish were transferred into aquaria with just clean water, and the others remained exposed to chem. for another four to seven months. At the end of the studies, about 20 tissue sites from each fish were examd. microscopically. At higher concns., 2,2-bis(bromomethyl)-1,3-propanediol was toxic to guppies, and many of the females died. Some of the male guppies and male medaka developed liver cancer, but few female medaka did. Nitromethane in the water promoted the growth of algae in the aquaria, and the tanks had to be cleaned often. The cleaning process may have resulted in the death of several of the fish. Not enough male guppies were left to evaluate, and very few tumors were seen in the surviving female guppies or male or female medaka. Male and female guppies exposed to 1,2,3-trichloropropane had higher rates of liver tumors than the control guppies did, and male and female medaka developed tumors of the liver and gallbladder. While all three test chems. caused cancer in several different tissues in lab. rodents, the response in fish was not as consistent, as cancer was seen only mainly in the liver. Fish that died during the study often were eaten by the other fish or dissolved and could not be evaluated. We conclude that several tech. problems remain to be solved before fish can be a reliable replacement for rodents in cancer testing.

Increasing efficiency of QSAR-analysis of carcinogenic activity of halogenated hydrocarbons
Quick ViewOther Sources

- By Tarasov, A. V.; Abilev, S. K.; Velibekov, R. M.; Tarasov, V. A.
- From Ekologicheskaya Genetika (2005), 3(2), 5-14. | Language: Russian, Database: CAPLUS

- The new principle of anal. of structure activity of carcinogenic compds. is offered. It is based on the use of compd. structural descriptors representing a combination unrelated among themselves of structural fragments of a mol. The computer program is developed, allowing to generate every possible structural fragments of mols. of chem. compds. and their combinations and to carry out selection of the compd. descriptors statistically significantly influencing their biol. activity. It was used for 79 halogenated hydrocarbons for which their carcinogenic activity on rodents is investigated.

Application of genomic biomarkers to predict increased lung tumor incidence in 2-year rodent cancer bioassays
Quick ViewOther Sources

- By Thomas, Russell S.; Pluta, Linda; Yang, Longlong; Halsey, Thomas A.
- From Toxicological Sciences (2007), 97(1), 55-64. | Language: English, Database: CAPLUS

- Rodent cancer bioassays are part of a legacy of safety testing that has not changed significantly over the past 30 years. The bioassays are expensive, time consuming, and use hundreds of animals. Fewer than 1500 chems. have been tested in a rodent cancer bioassay compared to the thousands of environmental and industrial chems. that remain untested for carcinogenic activity. In this study, we used existing data generated by the National Toxicol. Program (NTP) to identify gene expression biomarkers that can predict results from a rodent cancer bioassay. A set of 13 diverse chems. was selected from those tested by the NTP. Seven chems. were pos. for increased lung tumor incidence in female B6C3F1 mice and six were neg. Female mice were exposed subchronically to each of the 13 chems., and microarray anal. was performed on the lung. Statistical classification anal. using the gene expression profiles identified a set of eight probe sets corresponding to six genes whose expression correctly predicted the increase in lung tumor incidence with 93.9% accuracy. The sensitivity and specificity were 95.2 and 91.8%, resp. Among the six genes in the predictive signature, most were enzymes involved in endogenous and xenobiotic metab., and one gene was a growth factor receptor involved in lung development. The results demonstrate that increases in chem. induced lung tumor incidence in female mice can be predicted using gene biomarkers from a subchronic exposure

and may form the basis of a more efficient and economical approach for evaluating the carcinogenic activity of chems.

Deriving a data-based interspecies assessment factor using the NOAEL and the benchmark dose approach

Quick ViewOther Sources

- By Bokkers, Bas G. H.; Slob, Wout
- From Critical Reviews in Toxicology (2007), 37(5), 355-373. | Language: English, Database: CAPLUS
- A review. In deriving human health-based exposure limits from animal data, differences in sensitivity to a compd. between animals and humans must be taken into account. These interspecies differences can be caused by differences in toxicokinetics and/or toxicodynamics. Apart from that, species differ in body size, and this is usually accounted for by scaling doses to body wt. (i.e., expressed as mg/kg body $wt.^{1.0}$/day). A default assessment factor (AF) of 10 is commonly applied to this dose metric to account for potential toxicokinetic and toxicodynamic differences. However, both proportional body wt. (BW) scaling and the default AF as often applied are not directly based on empirical findings. Attempts have been made to derive data-based assessment factors and allometric scaling powers using various toxicol. values such as no-obsd.-adverse-effect-levels (NOAELs). In this study both the NOAEL approach and the benchmark dose (BMD) approach are applied to derive NOAEL ratios and BMD ratios from mouse and rat studies and, based on that information, to est. an allometric scaling power and an interspecies AF. To account for interspecies differences in body size, our results confirm earlier findings that allometric body wt. scaling with a power of around 0.7 is appropriate. The factor needed to rescale the dose in terms of mg/kg BW to the allometric dose scale ranges from around 1.7 (for dogs) to 10 (for mice), similar to other findings. The addnl. factor required for taking into account interspecies toxicokinetic and toxicodynamic differences, when based on the 95th percentile of the relevant ratio distribution, would be 3.1 for a lower Confidence limit of the BMD (BMDL), and 8.3 for a NOAEL (to be applied to the allometrically scaled dose). These results indicate that the generally used default AF of 10 may not cover potential interspecies differences, in particular when applied to results from smaller test species. Therefore, using the default AF of 10 could lead to human exposure limits that are insufficiently protective. Further, our results show that a data-based AF that would be needed for interspecies extrapolation is smaller when the point of departure is a BMDL rather than a NOAEL. In the context of a probabilistic hazard characterization, our results indicate that the (geometric) SD of the interspecies AF distribution should be around 2.0 when the BMDL (or BMD uncertainty distribution) is used, and around 3.4 when the NOAEL is used as a point of departure for further risk assessment.

Structure-Activity Relationship Analysis of Rat Mammary Carcinogens

Quick ViewOther Sources

- By Cunningham, Albert R.; Moss, Shanna T.; Iype, Seena A.; Qian, Gefei; Qamar, Shahid; Cunningham, Suzanne L.
- From Chemical Research in Toxicology (2008), 21(10), 1970-1982. | Language: English, Database: CAPLUS
-

Structure-activity relationship (SAR) models are powerful tools to investigate the mechanisms of action of chem. carcinogens and to predict the potential carcinogenicity of untested compds. We describe here the application of the cat-SAR (categorical-SAR) program to two learning sets of rat mammary carcinogens. One set of developed models was based on a comparison of rat mammary carcinogens to rat noncarcinogens (MC-NC), and the second set compared rat mammary carcinogens to rat nonmammary carcinogens (MC-NMC). On the basis of a leave-one-out validation, the best rat MC-NC model achieved a concordance between exptl. and predicted values of 84%, a sensitivity of 79 %, and a specificity of 89%. Likewise, the best rat MC-MNC model achieved a concordance of 78%, a sensitivity of 82%, and a specificity of 74%. The MC-NMC model was based on a learning set that contained carcinogens in both the active (i.e., mammary carcinogens) and the inactive (i.e.,

carcinogens to sites other than the mammary gland) categories and was able to distinguish between these different types of carcinogens (i.e., tissue specific), not simply between carcinogens and noncarcinogens. On the basis of a structural comparison between this model and one for Salmonella mutagens, there was, as expected, a significant relationship between the two phenomena since a high proportion of breast carcinogens are Salmonella mutagens. However, when analyzing the specific structural features derived from the MC-NC learning set, a dichotomy was obsd. between fragments assocd. with mammary carcinogenesis and mutagenicity and others that were assocd. with estrogenic activity. Overall, these findings suggest that the MC-NC and MC-NMC models are able to identify structural attributes that may in part address the question of "why do some carcinogens cause breast cancer", which is a different question than "why do some chems. cause cancer".

Absorption, distribution, metabolism, and excretion of 2,2-bis(bromomethyl)-1,3-propanediol in male Fischer-344 rats

Quick ViewOther Sources

- By Hoehle, Simone I.; Knudsen, Gabriel A.; Sanders, J. Michael; Sipes, I. Glenn
- From Drug Metabolism and Disposition (2009), 37(2), 408-416. | Language: English, Database: CAPLUS

- 2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant, previously shown to be a multisite carcinogen in exptl. animals. Studies were performed to characterize the dispositional and metabolic fate of BMP after oral or i.v. administration to male Fischer-344 rats. After a single oral administration of [^{14}C]BMP (10 or 100 mg/kg) >80% of the low dose and 48% of the high dose were excreted by 12 h in the urine predominantly as a glucuronide metabolite. After repeated daily oral doses for 5 or 10 days, route and rate of elimination were similar to those obtained after single administrations of BMP. In all studies, the radioactivity recovered in feces was low (<15%). The total amt. of radioactivity remaining in tissues at 72 h after a single oral administration of BMP (100 mg/kg) was less than 1% of the dose, and repeated daily dosing did not lead to retention in tissues. After i.v. administration, the radiolabel found in blood decreased rapidly. Excretion profiles were similar to those after oral administration. Parent BMP and BMP glucuronide were present in blood plasma after oral or i.v. dosing. After an i.v. dose of BMP (15 mg/kg) the hepatic BMP glucuronide was primarily exported into the bile (>50% within 6 h), but it underwent enterohepatic recycling with subsequent elimination in the urine. These data indicate that the extensive extn. and rapid glucuronidation by the liver limits exposure of internal tissues to BMP by greatly reducing its systemic bioavailability after oral exposure.

Use of Short-term Transcriptional Profiles to Assess the Long-term Cancer-Related Safety of Environmental and Industrial Chemicals

Quick ViewOther Sources

- By Thomas, Russell S.; Bao, Wenjun; Chu, Tzu-Ming; Bessarabova, Marina; Nikolskaya, Tatiana; Nikolsky, Yuri; Andersen, Melvin E.; Wolfinger, Russell D.
- From Toxicological Sciences (2009), 112(2), 311-321. | Language: English, Database: CAPLUS

- The process for evaluating chem. safety is inefficient, costly, and animal intensive. There is growing consensus that the current process of safety testing needs to be significantly altered to improve efficiency and reduce the no. of untested chems. In this study, the use of short-term gene expression profiles was evaluated for predicting the increased incidence of mouse lung tumors. Animals were exposed to a total of 26 diverse chems. with matched vehicle controls over a period of 3 years. Upon completion, significant batch-related effects were obsd. Adjustment for batch effects significantly improved the ability to predict increased lung tumor incidence. For the best statistical model, the estd. predictive accuracy under honest fivefold cross-validation was 79.3% with a sensitivity and specificity of 71.4 and 86.3%, resp. A learning curve anal. demonstrated that gains in model performance reached a plateau at 25 chems., indicating that the size of current data set was sufficient to provide a robust classifier. The classification results showed that a small subset of chems. contributed disproportionately to the misclassification rate. For these chems., the misclassification was more closely assocd. with genotoxicity status than with efficacy in the original bioassay. Statistical models were also used

to predict dose-response increases in tumor incidence for methylene chloride and naphthalene. The av. posterior probabilities for the top models matched the results from the bioassay for methylene chloride. For naphthalene, the av. posterior probabilities for the top models overpredicted the tumor response, but the variability in predictions was significantly higher. The study provides both a set of gene expression biomarkers for predicting chem. induced mouse lung tumors and a broad assessment of important exptl. and anal. criteria for developing microarray-based predictors of safety-related end points.

Addition to national toxicology program carcinogens: Community right-to-know toxic chemical release reporting

Quick ViewOther Sources

- By Environmental Protection Agency
- From Federal Register (2010), 75(65), 17333-17349. | Language: English, Database: CAPLUS

- SUMMARY: EPA is proposing to add sixteen chems. to the list of toxic chems. subject to reporting under section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA) of 1986 and section 6607 of the Pollution Prevention Act of 1990 (PPA). These sixteen chems. have been classified by the National Toxicol. Program (NTP) in their Report on Carcinogens (RoC) as "reasonably anticipated to be a human carcinogen." EPA believes that these sixteen chems. meet the EPCRA section 313(d)(2)(B) criteria because they can reasonably be anticipated to cause cancer in humans. As in past chem. reviews, EPA adopted a prodn. vol. screen for the development of this proposed rule to screen out those chems. for which no reports are expected to be submitted. Based on a review of the available prodn. and use information, these sixteen chems. are expected to be manufd., processed, or otherwise used in quantities that would exceed the EPCRA section 313 reporting thresholds.

In vitro glucuronidation of 2,2-bis(bromomethyl)-1,3-propanediol by microsomes and hepatocytes from rats and humans

Quick ViewOther Sources

- By Rad, Golriz; Hoehle, Simone I.; Kuester, Robert K.; Sipes, I. Glenn
- From Drug Metabolism and Disposition (2010), 38(6), 957-962. | Language: English, Database: CAPLUS

- 2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant used in unsatd. polyester resins. In a 2-yr bioassay BMP was shown to be a multisite carcinogen in rats and mice. Because glucuronidation is the key metabolic transformation of BMP by rats, in this study the in vitro hepatic glucuronidation of BMP was compared across several species. In addn., the glucuronidation activities of human intestinal microsomes and specific human hepatic UDP-glucuronosyltransferase (UGT) enzymes for BMP were detd. To explore other possible routes of metab. for BMP, studies were conducted with rat and human hepatocytes. Incubation of hepatic microsomes with BMP in the presence of UDP-glucuronic acid resulted in the formation of a BMP monoglucuronide. The order of hepatic microsomal glucuronidation activity of BMP was rats, mice >> hamsters > monkeys >>> humans. The rate of glucuronidation by rat hepatic microsomes was 90-fold greater than that of human hepatic microsomes. Human intestinal microsomes converted BMP to BMP glucuronide at a rate even lower than that of human hepatic microsomes. Among the human UGT enzymes tested, only UGT2B7 had detectable glucuronidation activity for BMP. BMP monoglucuronide was the only metabolite formed when BMP was incubated with suspensions of freshly isolated hepatocytes from male F-344 rats or with cryopreserved human hepatocytes. Glucuronidation of BMP in human hepatocytes was extremely low. Overall, the results support in vivo studies in rats in which BMP glucuronide was the only metabolite found. The poor glucuronidation capacity of humans for BMP suggests that the pharmacokinetic profile of BMP in humans will be dramatically different from that of rodents.

SMILES-based optimal descriptors: QSAR modeling of carcinogenicity by balance of correlations with ideal slopes

Quick ViewOther Sources

- By Toropov, A. A.; Toropova, A. P.; Benfenati, E.
- From European Journal of Medicinal Chemistry (2010), 45(9), 3581-3587. | Language: English, Database: CAPLUS

- Optimal descriptors which are calcd. using the simplified mol. input line entry system (SMILES) were utilized to build quant. structure-activity relationships (QSAR) of carcinogenicity (log TD50). Three schemes of the modeling have been examd.: 1. The most traditional "classic" training-test system, i.e., models are built with training set and validated with external test set; 2. The correlation balance, i.e., models are built with preliminary estn. of the predictability of the model with the calibration set (this set plays a role of preliminary test set); and 3. The extended correlation balance that takes into account the slopes of regression lines in plots exptl. vs. predicted values of carcinogenicity (in ideal, these slopes should be similar). It has been shown that the extended correlation balance with the ideal slopes gives most robust prediction of carcinogenicity for external test set. These models have been built by Monte Carlo method for three splits into subtraining set, calibration set, and test set. The no. of the N-nitroso groups (i.e., R1-N(R2)-N= O) in a mol. system has been examd. as an addnl. descriptor.

Addition of national toxicology program carcinogens; community right-to-know toxic chemical release reporting

Quick ViewOther Sources

- By Bushman, Daniel R.
- From Federal Register (2010), 75(227), 72727-72734. | Language: English, Database: CAPLUS

- USEPA is adding 16 chems. to the list of toxic chems. subject to reporting under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA) of 1986 and Section 6607 of the Pollution Prevention Act of 1990. These 16 chems. were classified by the National Toxicol. Program in their Report on Carcinogens as: reasonably anticipated to be a human carcinogen. EPA detd. these 16 chems. meet EPCRA Section 313(d)(2)(B) criteria because they can reasonably be anticipated to cause cancer in humans.

Induction of DNA damage in human urothelial cells by the brominated flame retardant 2,2-bis(bromomethyl)-1,3-propanediol: Role of oxidative stress

Quick ViewOther Sources

- By Kong, Weixi; Kuester, Robert K.; Gallegos, Alfred; Sipes, I. Glenn
- From Toxicology (2011), 290(2-3), 272-278. | Language: English, Database: CAPLUS

- 2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is an extensively used brominated flame retardant found in urethane foams and polyester resins. In a 2-yr dietary study conducted by the National Toxicol. Program, BMP caused neoplastic lesions at multiple sites including the urinary bladder in both rats and mice. The mechanism of its carcinogenic effect is unknown. In the present study, using SV-40 immortalized human urothelial cells (UROtsa), endpoints assocd. with BMP induced DNA damage and oxidative stress were investigated. The effects of time (1-24 h) and concn. (5-100 μM) on BMP induced DNA strand breaks were assessed via the alk. comet assay. The results revealed evidence of DNA strand breaks at 1 and 3 h following incubation of cells with non-cytotoxic concns. of BMP. Strand breaks were not present after 6 h of incubation. Evidences for BMP assocd. oxidative stress include: an elevation of intracellular ROS formation as well as induction of Nrf2 and HSP70 protein levels. In addn., DNA strand breaks were attenuated when cells were pre-treated with N-acetyl-L-cysteine (NAC) and oxidative base modifications were revealed when a lesion specific endonuclease, human 8-hydroxyguanine DNA glycosylase 1 (hOGG1) was introduced into the comet assay. In conclusion, these results demonstrate that BMP induces DNA strand breaks and oxidative base damage in UROtsa cells. Oxidative stress is a significant, determinant factor in mediating these DNA lesions. These early genotoxic events may, in part, contribute to BMP-induced carcinogenesis obsd. in rodents.

First report on development of quantitative interspecies structure-carcinogenicity relationship models and exploring discriminatory features for rodent carcinogenicity of diverse organic chemicals using OECD guidelines

Quick ViewOther Sources

- By Kar, Supratik; Roy, Kunal
- From Chemosphere (2012), 87(4), 339-355. | Language: English, Database: CAPLUS

- Different regulatory agencies in food and drug administration and environmental protection worldwide are employing quant. structure-activity relationship (QSAR) models to fill the data gaps related with properties of chems. affecting the environment and human health. Carcinogenicity is a toxicity endpoint of major concern in recent times. Interspecies toxicity correlations may provide a tool for estg. sensitivity towards toxic chem. exposure with known levels of uncertainty for a diversity of wildlife species. In this background, we have developed quant. interspecies structure-carcinogenicity correlation models for rat and mouse [rodent species according to the Organization for Economic Cooperation and Development (OECD) guidelines] based on the carcinogenic potential of 166 org. chems. with wide diversity of mol. structures, spanning a large no. of chem. classes and biol. mechanisms. All the developed models have been assessed according to the OECD principles for the validation of QSAR models. Consensus predictions for carcinogenicity of the individual compds. are presented here for any one species when the data for the other species are available. Informative illustrations of the contributing structural fragments of chems. which are responsible for specific carcinogenicity endpoints are identified by the developed models. The models have also been used to predict mouse carcinogenicities of 247 org. chems. (for which rat carcinogenicities are present) and rat carcinogenicities of 150 chems. (for which mouse carcinogenicities are present). Discriminatory features for rat and mouse carcinogenicity values have also been explored.

Scientific opinion on emerging and novel brominated flame retardants (BFRs) in food

Quick ViewOther Sources

- By Benford, Diane; Ceccatelli, Sandra; Cottrill, Bruce; DiNovi, Michael; Dogliotti, Eugenia; Edler, Lutz; Farmer, Peter; Furst, Peter; Hoogenboom, Laurentius; Knutsen, Helle Katrine; et al
- From EFSA Journal (2012), 10(10), 2908, 125 pp.. | Language: English, Database: CAPLUS

- EFSA was asked to deliver a scientific opinion on brominated flame retardants (BFRs) other than PBDEs, PBBs, HBCDDs, TBBPA and brominated phenols and their derivs. The BFRs that are the subject of the current opinion, were classified in groups termed 'emerging' and 'novel' BFRs. Information on 17 emerging and 10 novel BFRs was collected. The information varied widely for these BFRs. There is a lack of exptl. data on physico-chem. characteristics, stability/reactivity and current use and prodn. vol. of all the emerging and novel BFRs. Due to the very limited information on occurrence, exposure and toxicity, the CONTAM Panel could not perform a risk characterization for any of the BFRs considered. Instead, an attempt was made to identify those BFRs that could be a potential health concern and should be considered first for future investigations. For this purpose the Panel first evaluated the available exptl. data on occurrence in food, behavior in the environment and toxicity. Secondly, a modeling exercise was performed focussing on the potential of the emerging and novel BFRs for persistence in the environment and for their possible bioaccumulation potential. There is convincing evidence that tris(2,3-dibromopropyl) phosphate (TDBPP) and dibromoneopentyl glycol (DBNPG) are genotoxic and carcinogenic, warranting further surveillance of their occurrence in the environment and in food. Based on the limited exptl. data on environmental behavior, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and hexabromobenzene (HBB) were identified as compds. that could raise a concern for bioaccumulation. For the modeling exercise, the CONTAM Panel selected two environmental characteristics, overall persistence and potential for bioaccumulation, as being most relevant to provide insight into the possibility that emerging or novel BFRs might accumulate in the food chain, and thus might appear in food intended for human consumption. The modeling exercise identified ten addnl. BFRs that should be subjected to further in-depth studies.

Carcinogenicity Prediction of Noncongeneric Chemicals by a Support Vector Machine

Quick ViewOther Sources

- By Zhong, Min; Nie, Xianglei; Yan, Aixia; Yuan, Qipeng

- From Chemical Research in Toxicology (2013), 26(5), 741-749. | Language: English, Database: CAPLUS

The ability to identify carcinogenic compds. is of fundamental importance to the safe application of chems. In this study, the authors generated an array of in silico models allowing the classification of compds. into carcinogenic and noncarcinogenic agents based on a data set of 852 noncongeneric chems. collected from the Carcinogenic Potency Database (CPDBAS). Twenty-four mol. descriptors were selected by Pearson correlation, F-score, and stepwise regression anal. These descriptors cover a range of physicochem. properties, including electrophilicity, geometry, mol. wt., size, and soly. The descriptor mutagenic showed the highest correlation coeff. with carcinogenicity. On the basis of these descriptors, a support vector machine-based (SVM) classification model was developed and fine-tuned by a 10-fold cross-validation approach. Both the SVM model (Model A1) and the best model from the 10-fold cross-validation (Model B3) runs gave good results on the test set with prediction accuracy over 80%, sensitivity over 76%, and specificity over 82%. In addn., extended connectivity fingerprints (ECFPs) and the Toxtree software were used to analyze the functional groups and substructures linked to carcinogenicity. It was found that the results of both methods are in good agreement.

Oxidative DNA damage and DNA binding induced by 2, 2-bis (bromomethyl)-1, 3-propanediol: Possible mode of action implicated in its carcinogenicity

Quick ViewOther Sources

- By Kong, Weixi
- No Corporate Source data available | (2012), 169 pp.. | Language: English, Database: CAPLUS

Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays

Quick ViewOther Sources

- By Sipes, Nisha S.; Martin, Matthew T.; Kothiya, Parth; Reif, David M.; Judson, Richard S.; Richard, Ann M.; Houck, Keith A.; Dix, David J.; Kavlock, Robert J.; Knudsen, Thomas B.
- From Chemical Research in Toxicology (2013), 26(6), 878-895. | Language: English, Database: CAPLUS

Understanding potential health risks is a significant challenge due to the large nos. of diverse chems. with poorly characterized exposures and mechanisms of toxicities. The present study analyzes 976 chems. (including failed pharmaceuticals, alternative plasticizers, food additives, and pesticides) in Phases I and II of the U.S. EPA's ToxCast project across 331 cell-free enzymic and ligand-binding high-throughput screening (HTS) assays. Half-maximal activity concns. (AC50) were identified for 729 chems. in 256 assays (7135 chem.-assay pairs). Some of the most commonly affected assays were CYPs (CYP2C9 and CYP2C19), transporters (mitochondrial TSPO, norepinephrine, and dopaminergic), and GPCRs (aminergic). Heavy metals, surfactants, and dithiocarbamate fungicides showed promiscuous but distinctly different patterns of activity, whereas many of the pharmaceutical compds. showed promiscuous activity across GPCRs. Literature anal. confirmed >50% of the activities for the most potent chem.-assay pairs (54) but also revealed 10 missed interactions. Twenty-two chems. with known estrogenic activity were correctly identified for the majority (77%), missing only the weaker interactions. In many cases, novel findings for previously unreported chem.-target combinations clustered with known chem.-target interactions. Results from this large inventory of chem.-biol. interactions can inform read-across methods as well as link potential targets to mol. initiating events in adverse outcome pathways for diverse toxicities.

Comparison of 2,2-bis(bromomethyl)-1,3-propanediol induced genotoxicity in UROtsa cells and primary rat hepatocytes: Relevance of metabolism and oxidative stress

Quick ViewOther Sources

- By Kong, Weixi; Gu, Pengfei; Knudsen, Gabriel A.; Sipes, I. Glenn
- From Toxicology Letters (2013), 222(3), 273-279. | Language: English, Database: CAPLUS

- 2,2-Bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant used in urethane foams and polyester resins. In a two year dietary study, BMP caused neoplastic lesions at multiple sites including the urinary bladder of both rats and mice. However, liver was not a target tissue. The authors previously reported that BMP elicited oxidative DNA damage in a human uroepithelial cell line (UROtsa). The present in vitro study investigated the susceptibility of target (UROtsa cells) and non-target cells (primary rat hepatocytes) to BMP-induced genotoxicity. In contrast to hepatocytes, BMP exhibited greater genotoxic potential in UROtsa cells as evidenced by the concn. dependent increase in DNA strand breaks and DNA binding. Total content of intracellular GSH quantified in UROtsa cells (2.7 nmol/mg protein) was 4-fold lower than that in hepatocytes (10.7 nmol/mg protein). HPLC anal. indicated BMP was not metabolized and/or consumed in UROtsa cells at any of the concns. tested (10-250 μM) but was extensively converted to a mono-glucuronide in hepatocytes. These results demonstrate that a target cell line such as UROtsa cells are more susceptible to BMP-induced DNA damage when compared to non-target cells. This increased susceptibility may relate to the deficiency of antioxidant and/or metabolic capabilities in UROtsa cells.

Assessing the persistence, bioaccumulation potential and toxicity of brominated flame retardants: Data availability and quality for 36 alternative brominated flame retardants
Quick ViewOther Sources

- By Stieger, Greta; Scheringer, Martin; Ng, Carla A.; Hungerbuhler, Konrad
- From Chemosphere (2014), 116, 118-123. | Language: English, Database: CAPLUS

- Polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCDD) are major brominated flame retardants (BFRs) that are now banned or under restrictions in many countries because of their persistence, bioaccumulation potential and toxicity (PBT properties). However, there is a wide range of alternative BFRs, such as decabromodiphenyl ethane and tribromophenol, that are increasingly used as replacements, but which may possess similar hazardous properties. This necessitates hazard and risk assessments of these compds. For a set of 36 alternative BFRs, we searched 25 databases for chem. property data that are needed as input for a PBT assessment. These properties are degrdn. half-life, bioconcn. factor (BCF), octanol-water partition coeff. (K_{ow}), and toxic effect concns. in aquatic organisms. For 17 of the 36 substances, no data at all were found for these properties. Too few persistence data were available to even assess the quality of these data in a systematic way. The available data for K_{ow} and toxicity show surprisingly high variability, which makes it difficult to identify the most reliable values. We propose methods for systematic evaluations of PBT-related chem. property data that should be performed before data are included in publicly available databases. Using these methods, we evaluated the data for K_{ow} and toxicity in more detail and identified several inaccurate values. For most of the 36 alternative BFRs, the amt. and the quality of the PBT-related property data need to be improved before reliable hazard and risk assessments of these substances can be performed.

Effects of seven chemicals on DNA damage in the rat urinary bladder: A comet assay study
Quick ViewOther Sources

- By Wada, Kunio; Yoshida, Toshinori; Takahashi, Naofumi; Matsumoto, Kyomu
- From Mutation Research, Genetic Toxicology and Environmental Mutagenesis (2014), 769, 1-6. | Language: English, Database: CAPLUS

- The in vivo comet assay has been used for the evaluation of DNA damage and repair in various tissues of rodents. However, it can give false-pos. results due to non-specific DNA damage assocd. with cell death. In this study, we examd. whether the in vivo comet assay can distinguish between genotoxic and non-genotoxic DNA damage in urinary bladder cells, by using the following seven chems. related to urinary bladder carcinogenesis in rodents: N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), glycidol, 2,2-bis(bromomethyl)-1,3-propanediol (BMP), 2-nitroanisole (2-NA), benzyl isothiocyanate (BITC), uracil, and melamine. BBN, glycidol, BMP, and 2-NA are known to be Ames test-pos. and they are expected to produce DNA damage in the absence of cytotoxicity. BITC, uracil, and melamine are Ames test-neg. with metabolic activation

but have the potential to induce non-specific DNA damage due to cytotoxicity. The test chems. were administered orally to male Sprague-Dawley rats (five per group) for each of two consecutive days. Urinary bladders were sampled 3 h after the second administration and urothelial cells were analyzed by the comet assay and subjected to histopathol. examn. to evaluate cytotoxicity. In the urinary bladders of rats treated with BBN, glycidol, and BMP, DNA damage was detected. In contrast, 2-NA induced neither DNA damage nor cytotoxicity. The non-genotoxic chems. (BITC, uracil, and melamine) did not induce DNA damage in the urinary bladders under conditions where some histopathol. changes were obsd. The results indicate that the comet assay could distinguish between genotoxic and non-genotoxic chems. and that no false-pos. responses were obtained.

Predictive Endocrine Testing in the 21st Century Using in Vitro Assays of Estrogen Receptor Signaling Responses

Quick ViewOther Sources

- By Rotroff, Daniel M.; Martin, Matt T.; Dix, David J.; Filer, Dayne L.; Houck, Keith A.; Knudsen, Thomas B.; Sipes, Nisha S.; Reif, David M.; Xia, Menghang; Huang, Ruili; et al
- From Environmental Science & Technology (2014), 48(15), 8706-8716. | Language: English, Database: CAPLUS

Thousands of environmental chems. are subject to regulatory review for their potential to be endocrine disruptors (ED). In vitro high-throughput screening (HTS) assays have emerged as a potential tool for prioritizing chems. for ED-related whole-animal tests. In this study, 1814 chems. including pesticide active and inert ingredients, industrial chems., food additives, and pharmaceuticals were evaluated in a panel of 13 in vitro HTS assays. The panel of in vitro assays interrogated multiple end points related to estrogen receptor (ER) signaling, namely binding, agonist, antagonist, and cell growth responses. The results from the in vitro assays were used to create an ER Interaction Score. For 36 ref. chems., an ER Interaction Score >0 showed 100% sensitivity and 87.5% specificity for classifying potential ER activity. The magnitude of the ER Interaction Score was significantly related to the potency classification of the ref. chems. ERα/ERβ selectivity was also evaluated, but relatively few chems. showed significant selectivity for a specific isoform. When applied to a broader set of chems. with in vivo uterotrophic data, the ER Interaction Scores showed 91% sensitivity and 65% specificity. Overall, this study provides a novel method for combining in vitro concn. response data from multiple assays and, when applied to a large set of ER data, accurately predicted estrogenic responses and demonstrated its utility for chem. prioritization.

Physical-chemical properties and evaluative fate modelling of 'emerging' and 'novel' brominated and organophosphorus flame retardants in the indoor and outdoor environment

Quick ViewOther Sources

- By Liagkouridis, Ioannis; Cousins, Anna Palm; Cousins, Ian T.
- From Science of the Total Environment (2015), 524-525, 416-426. | Language: English, Database: CAPLUS

- Several groups of flame retardants (FR) entered the market in recent years as replacements for polybrominated di-Ph ethers (PBDE), but little is known about their physicochem. properties or their environmental transport and fate. This work made best ests. of physicochem. properties and evaluative modeling assessments (indoors and outdoors) for 35 novel and emerging brominated flame retardants (BFR) and 22 organophosphorus flame retardants (OPFR). A quant. structure-property relationship-based technique reduced uncertainty in physicochem. properties and aided property selection for modeling, but it was evident that more, high quality property data are required to improve future assessments. Evaluative modeling results showed many alternative FR, mainly alternative BFR and some halogenated OPFR, behave similarly to PBDE under indoor and outdoor conditions. Alternative FR exhibited high overall persistence (P_{ov}), long-range transport potential (LRTP), and persistent org. pollutant-like behavior, and on that basis, cannot be regarded as suitable replacements for PBDE. A group of low mol. wt. alternative BFR and non-halogenated OPFR demonstrated potentially better environmental performance based on P_{ov} and LRTP metrics. Results must be interpreted

with caution because there are significant uncertainties and limited data to allow for thorough model evaluation. Addnl. environmental parameters (toxicity, bioaccumulative potential, functionality issues) should be considered for an industrial substitution strategy.

	Literature Search Results
Search Term	83165-36-0
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	1
Date	July 23 2015
Comments	

Comparison between rodent carcinogenicity test results of 44 chemicals and a number of predictive systems
Quick ViewOther Sources

- By Lewis, David F. V.
- From Regulatory Toxicology and Pharmacology (1994), 20(3, Pt. 1), 215-22. | Language: English, Database: CAPLUS

- A comparison is made between a no. of predictive systems including bacterial mutagenicity, structure alert and chronic toxicity (R. W. Tennant et al., 1990), COMPACT, Hazardexpert, and DEREK, with the outcome of the two species rodent carcinogenicity bioassay for 44 chems. conducted under the NTP protocol. Following minor updating of the rodent data in the light of pathol. reports, there is a generally good agreement between various predictions and the actual carcinogenicity results. In particular, a combination of two methods of prediction produces an over 80% concordance with the rodent bioassay. Possible reasons for various discrepancies are discussed in light of xenobiotic metab. and activation mechanisms of chem. carcinogenesis.

	Literature Search Results
Search Term	96-13-9
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	92
Date	July 23 2015
Comments	

2-Haloacrylic acids as indicators of mutagenic 2-haloacrolein intermediates in mammalian metabolism of selected promutagens and carcinogens
Quick ViewOther Sources

- By Marsden P J; Casida J E
- From Journal of agricultural and food chemistry (1982), 30(4), 627-31. | Language: English, Database: MEDLINE

2,3-Dibromopropan-1-ol
Quick ViewOther Sources

- By Anonymous
- From IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer (2000), 77, 439-53. | Language: English, Database: MEDLINE

2,3-Dibromo-1-propanol
Quick ViewOther Sources

- No Author and Editor data available

- From Report on carcinogens : carcinogen profiles / U.S. Dept. of Health and Human Services, Public Health Service, National Toxicology Program (2002), 10, 82-3. | Language: English, Database: MEDLINE

2,3-Dibromo-1-propanol
Quick ViewOther Sources

- By Anonymous
- From Report on carcinogens : carcinogen profiles / U.S. Dept. of Health and Human Services, Public Health Service, National Toxicology Program (2004), 11, III84. | Language: English, Database: MEDLINE

2,3-Dibromo-1-propanol
Quick ViewOther Sources

- No Author and Editor data available
- From Report on carcinogens : carcinogen profiles / U.S. Dept. of Health and Human Services, Public Health Service, National Toxicology Program (2011), 12, 138-9. | Language: English, Database: MEDLINE

Influence of some aliphatic compounds on rat liver glutathione levels
Quick ViewOther Sources

- By Johnson, M. K.
- From Biochemical Pharmacology (1965), 14(9), 1383-5. | Language: English, Database: CAPLUS

- Oral administration of bromobutane to rats caused moderate depression of liver glutathione (GSH) levels. Iodomethane has a much greater effect, and has been shown to S-methylate GSH under the influence of a liver enzyme, glutathione S-alkyl transferase, in vivo and in vitro. Examn. of the liver enzyme showed that it had a fairly wide specificity for substrates other than GSH, and reports are given on the effect on rat liver GSH of some aliphatic compds. administered orally. Discrepancies between effects seen in vivo and in vitro are discussed.

Studies of possible absorption of a flame retardant from treated fabrics worn by rats and humans
Quick ViewOther Sources

- By St. John, L. E., Jr.; Eldefrawi, M. E.; Lisk, D. J.
- From Bulletin of Environmental Contamination and Toxicology (1976), 15(2), 192-7. | Language: English, Database: CAPLUS

- When tris(2,3-dibromopropyl) phosphate [126-72-7] was applied to the skin of a rat, its hydrolysis product, 2,3-dibromopropanol [**96-13-9**], was detected in the urine. Rat liver supernatant hydrolyzed the phosphate to dibromopropanol. No dibromopropanol was detected in the urine of a rat, a man, and a boy who wore fabrics contg. the flame retardant for up to 9 days.

Tris(2,3-dibromopropyl) phosphate: mutagenicity of a widely used flame retardant
Quick ViewOther Sources

- By Prival, Michael J.; McCoy, Elena C.; Gutter, Bezalel; Rosenkranz, Herbert S.
- From Science (Washington, DC, United States) (1977), 195(4273), 76-8. | Language: English, Database: CAPLUS

- Tris(2,3-dibromopropyl) phosphate [126-72-7], a widely used flame-retardant additive for textiles, was mutagenic to histidine-requiring strains of Salmonella typhimurium. Exts. of fabrics treated with this compd. were also capable of inducing mutations in these bacterial strains.

Mutagenicity of derivatives of the flame retardant tris(2,3-dibromopropyl)phosphate: halogenated propanols
Quick ViewOther Sources

- By Carr, Howard S.; Rosenkranz, Herbert S.
- From Mutation Research, Genetic Toxicology Testing (1978), 57(3), 381-4. | Language: English, Database: CAPLUS

- Genetic tests on 6 halogenated propanols which are potential derivs. of TBPP (tris(2, 3-dibromopropyl)phosphate) [126-72-7] showed that all except 3-chloro-1-propanol [627-30-5] were mutagenic to Salmonella typhimurium through direct base substitution. The mutagenic

activities of 3-bromo-1-propanol [627-18-9], 2,3-dibromo-1-propanol [**96-13-9**], and TBPP were increased in the presence of rat liver microsomes. Caution is indicated in the selection of flame retardants which are capable of being hydrolyzed to mutagenic alcs. The possibility of developing a flame retardant based on nonmutagenic 3-chloro-1-propanol is suggested.

Children absorb tris-BP flame retardant from sleepwear: urine contains the mutagenic metabolite, 2,3-dibromopropanol
Quick ViewOther Sources

- By Blum, Arlene; Gold, Marian Deborah; Ames, Bruce N.; Kenyon, Christine; Jones, Frank R.; Hett, Eva A.; Dougherty, Ralph C.; Horning, Evan C.; Dzidic, Ismet; et al.
- From Science (Washington, DC, United States) (1978), 201(4360), 1020-3. | Language: English, Database: CAPLUS

- The flame retardant, tris(2,3-dibromopropyl)phosphate (tris-BP) [126-72-7], which is a mutagen and causes cancer and sterility in animals, was absorbed from fabric by people. 2,3-Dibromopropanol [**96-13-9**], a metabolite of tris-BP and a mutagen itself, was found in the urine samples of 10 children who were wearing or who had worn tris-BP-treated sleepwear. Eight of these children were wearing well-washed sleepwear and the possibility of absorption of tris-BP from well-washed sleepwear is discussed. 2,3-Dibromopropanol was not found in the urines of 1 child and 1 adult who had never worn tris-BP-treated garments.

The mutagenicity of halogenated alkanols and their phosphoric acid esters for Salmonella typhimurium
Quick ViewOther Sources

- By Nakamura, Akitada; Tateno, Noriyuki; Kojima, Shigeo; Kaniwa, Masaaki; Kawamura, Taro
- From Mutation Research, Genetic Toxicology Testing (1979), 66(4), 373-80. | Language: English, Database: CAPLUS

- Nine halogenated alkanols, 9 corresponding tris(haloalkyl)phosphates, and 2 bis-(2,3-dibromopropyl)phosphate salts were evaluated for mutagenicity against S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538, with and without rat liver in vitro metabolic activation system (S9 mix). Most of the test samples showed mutagenic activity in the strains TA100 and TA1535, but not in the strains TA98, TA1537, and TA1538. In general, the mutagenic activities of the phosphates obtained with S9 mix were greater than the activities obtained without S9 mix. Among the phosphates, several structure-activity relations were found; i.e., the bromoalkyl derivs. were more mutagenic than the corresponding chloroalkyl derivs., the β-haloethyl derivs. were more mutagenic than the γ-halopropyl derivs., the phosphates having adjacent β and γ halogen atoms in the alkyl moiety, e.g., tris-(2,3-dibromopropyl)phosphate (I) [126-72-7], were particularly potent mutagens, the branched C chain reduced the mutagenic activities in spite of the presence of β-halogen atoms, e.g., tris(1-bromomethyl-2-bromoethyl)phosphate [18713-51-4]. However, such relations did not necessarily apply to the halogenated alkanols. Apparently, the metabolic activation pathway via haloalkanols to mutagens must not be in common with all of I-like phosphates.

Mutagenic activation of tris(2,3-dibromopropyl)phosphate: the role of microsomal oxidative metabolism
Quick ViewOther Sources

- By Soederlund, Erik J.; Nelson, Sidney D.; Dybing, Erik
- From Acta Pharmacologica et Toxicologica (1979), 45(2), 112-21. | Language: English, Database: CAPLUS

- The flame retardant tris(2,3-dibromopropyl)phosphate (Tris-BP) [126-72-7] was converted to products which were mutagenic for Salmonella typhimurium TA 100 in the presence of rat liver microsomes, NADPH, and O. Other bromopropyl compds. were also mutagenic; 2,3-dibromopropene [513-31-5] and 2,3-dibromopropionic acid [600-05-5] were directly mutagenic, whereas 2,3-dibromopropanol [**96-13-9**] and tris(2-bromopropyl)phosphate [31858-09-0] were weakly mutagenic after addn. of liver microsomes and cofactors. Typical in vivo and in vitro inhibitors of cytochrome P-450 inhibited Tris-BP mutagenicity. The effects of inducers of

cytochrome P-450 on Tris-BP mutagenicity was dependent on the concn. of mutagen and microsomal protein in the assay, indicating complexity in the kinetics involved when dealing with possible multiple paths that lead to mutagenicity. Addn. of glutathione strongly inhibited Tris-BP mutagenicity. Tris-BP may be oxidized to a reactive electrophile, possibly the 2-keto deriv., which could react with nucleophilic groups in DNA and thus lead to mutagenic events.

The Escherichia coli Pol A1- assay. A quantitative procedure for diffusible and nondiffusible chemicals

- By Hyman, Julie; Leifer, Zev; Rosenkranz, Herbert S.
- From Mutation Research, Environmental Mutagenesis and Related Subjects (1980), 74(2), 107-11. | Language: English, Database: CAPLUS

- A procedure is described for detg. the preferential killing of DNA repair-deficient E. coli. Dil. suspensions of DNA repair-proficient and -deficient bacteria are exposed to a graded series of test chem. concns. Survivors are detd. by enumeration on agar plates. An expression used to express preferential killing (or lack thereof) is shown to be dose-dependent and potentially useful for detg. genotoxic potencies.

Mutagenicity of 2- and 3-carbon halogenated compounds in the Salmonella/mammalian-microsome test

- By Stolzenberg, S. J.; Hine, C. H.
- From Environmental Mutagenesis (1980), 2(1), 59-66. | Language: English, Database: CAPLUS

- Short-chain, 2- and 3-carbon halogenated hydrocarbons were tested for mutagenicity for Salmonella typhimurium strain TA 100 both with and without the presence of S-9. Without exception, all brominated derivs. were more mutagenic than the chlorinated derivs., usually by a substantial order of magnitude. 2-Fluoroethanol [371-62-0] showed little or no mutagenic activity up to 100 μmol/plate. Trihalogenated compds. with a halogen atom on each of the 3 carbon atoms required metabolic activation with S-9 for full expression of mutagenic activity. The presence of a double bond in the case of 1,2,3-trichloropropene [96-19-5] resulted in a higher level of direct mutagenic activity than 1,2,3-trichloropropane [96-18-4], but activation with S-9 resulted in a further increase in mutagenic activity with the former compd. On the other hand, S-9 caused a substantial decrease in mutagenic activity of most compds. contg. a double bond. With the presence of an alc. group in a compd., the addn. of S-9 caused variable responses, increasing the no. of his^+ revertant colonies due to 2,3-dibromopropanol [**96-13-9**] but had little or no effect with other compds. contg. an alc. group. Evidence is also presented that the position of a double bond in relation to the halogen atoms may influence mutagenic activity.

The relation between the structure of vicinal dihalogen compounds and their mutagenic activation via conjugation to glutathione

- By Van Bladeren, P. J.; Breimer, D. D.; Rotteveel-Smijs, G. M. T.; De Knijff, P.; Mohn, G. R.; Van Meeteren-Walchli, B.; Buijs, W.; Van der Gen, A.
- From Carcinogenesis (1981), 2(6), 499-505. | Language: English, Database: CAPLUS

- Vicinal dihalogen compds. may be activated to mutagenic 2-halogenothioethers by conjugation to glutathione [70-18-8]. This type of metabolic activation was studied in vitro in relation to the chem. structure of such compds. The specific activity of rat liver glutathione transferases catalyzing the conjugation of a series of vicinal dihalogen compds. with glutathione was detd. The results were employed to define optimal conditions for the assessment of the relative mutagenic activity of these compds. towards Salmonella typhimurium TA 100 in the presence of rat liver 100,000 g supernatant. A series of model intermediates, N-acetyl-S-2-halogenalkyl-L-cysteine Me esters, was synthesized and their mutagenic activity was detd. in Salmonella strain TA 100. Alkyl substitution at the halogen-bearing carbon atoms reduces both glutathione transferase activity and mutagenicity of vicinal dihalogen compds. considerably. In the case of Ph substitution, glutathione transferase activity is increased without concomitant

enhancement of the mutagenic activity. Introduction of alkyl substituents into the model conjugates also results in a lower mutagenic activity. The specific activity of the glutathione transferases follows the halide order for leaving group ability, i.e., I > Br > Cl. For the mutagenic activity, however, a different result is obtained: the diiodo and dichloro derivs. are less active than the corresponding chloro-bromo and dibromo compds. The 2-chloroethylthioether is most mutagenic, the 2-bromoethylthioether deriv. is somewhat less active, and the 2-iodoethylthioether is the least mutagenic compd. A major factor in detg. the mutagenicity of such intermediates is presumably their stability in aq. solns., the more reactive compds. having a shorter life-time and thus a lower probability to reach genetically relevant macromols.

In vitro and in vivo covalent binding of the kidney toxicant and carcinogen tris(2,3-dibromopropyl)-phosphate

Quick ViewOther Sources

- By Soederlund, Erik J.; Nelson, Sidney D.; Dybing, Erik
- From Toxicology (1981), 21(4), 291-304. | Language: English, Database: CAPLUS

- Tris(2,3-dibromopropyl)phosphate (Tris-BP) [126-72-7] is activated to products which bind covalently to microsomal protein by a cytochrome P 450 [9035-51-2]-dependent oxidn. reaction. Binding to rat liver microsomes proceeds 15 times faster than with kidney microsomes. The binding in liver microsomes is markedly increased by phenobarbital pretreatment, the apparent V_{max} of the reaction is 175 pmol/mg microsomal protein/min with control microsomes and 1053 pmol/mg protein/min with induced microsomes. Binding with kidney microsomes is doubled after pretreatment with polychlorinated biphenyls. 2,3-Dibromopropanol [**96-13-9**], an hydrolysis product of Tris-BP, is also activated to covalently protein-bound products, but at a much slower rate than Tris-BP. Administration of Tris-BP to rats leads to its covalent binding to proteins in liver and kidney, with 5 times higher binding levels in kidney than in liver, correlating with its relative organotoxic potential in single dose expts. Binding to proteins in the kidney was increased by pretreatment of animals with polychlorinated biphenyls. A covalent interaction of Tris-BP could also be demonstrated to DNA, both when DNA was added to liver microsomal incubations in vitro and to DNA extd. from liver and kidney after administration of Tris-BP in vivo. The binding levels were 4 times higher to kidney DNA than to liver DNA.

Nephrotoxicity of the flame retardant, tris(2,3-dibromopropyl) phosphate, and its metabolites

Quick ViewOther Sources

- By Elliott, W. Clayton; Lynn, Robert K.; Houghton, Donald C.; Kennish, John M.; Bennett, William M.
- From Toxicology and Applied Pharmacology (1982), 62(1), 179-82. | Language: English, Database: CAPLUS

- A single i.p. injection of tris(2,3-dibromopropyl) phosphate (TRIS-BP) [126-72-7], when administered to male rats, caused polyuric acute renal failure with tubular necrosis involving the late proximal tubule. Glomerular filtration rate and in vitro transport of the org. acid, p-aminohippurate, and the org. base, N-[^{14}C]methylnicotinamide, were depressed. An approx. equimolar dose of the TRIS-BP metabolite, bis(2,3-dibromopropyl) phosphate (BIS-BP) [5412-25-9], caused significantly more severe renal failure. In contrast, the metabolite, 2,3-dibromopropanol [**96-13-9**], was nonnephrotoxic. Thus, TRIS-BP nephrotoxicity is mediated via its metabolite BIS-BP.

Metabolism, distribution, and excretion of the flame retardant, tris(2,3-dibromopropyl) phosphate (Tris-BP) in the rat: identification of mutagenic and nephrotoxic metabolites

Quick ViewOther Sources

- By Lynn, Robert K.; Garvie-Gould, Clare; Wong, Kenneth; Kennish, John M.
- From Toxicology and Applied Pharmacology (1982), 63(1), 105-19. | Language: English, Database: CAPLUS

- The mutagenic, nephrotoxic, and carcinogenic flame retardant Tris-BP [126-72-7] underwent rapid and extensive metab. in the rat. Five days after administration of [^{14}C]Tris-BP, 86% of the radiolabel was excreted in the form of metabolites in the urine (58%), feces (9%), and expired air (19% as $^{14}CO_2$); 9% was recovered in the body. [^{14}C]Tris-BP and its metabolites

were analyzed by high-pressure liq. chromatog. and liq. scintillation counting. Tris-BP was not detected in the excreta. A metabolite present in urine, feces, bile, and tissues was identified by mass spectrometry as bis(2,3-dibromopropyl) phosphate (Bis-BP) [5412-25-9]. 2,3-dibromopropanol (DBP) [**96-13-9**] was also identified in tissues and in urine. Biliary excretion and enterohepatic recirculation were major routes in the disposition of Tris-BP. All tissues contained Tris-BP-derived radioactivity; however, the concn. of radiolabel in kidney was 11 times the av. body concn. 5 days after dosing. Bis-BP was mutagenic to Salmonella typhimurium (TA 100) in the presence of Aroclor-induced liver homogenates. The mutagenic potency was greater than that of DBP but less than that of Tris-BP. Also, Bis-BP was an acute nephrotoxin and more potent than Tris-BP itself. Thus, the tissues toward which Tris-BP was selectively toxic, kidney and colon, were also the tissues which were exposed to selectively high concns. of Tris-BP-derived radioactivity. Tris-BP, which has a short in vivo half-life, was metabolized to a long-acting mutagenic and nephrotoxic metabolite, Bis-BP.

The mei-9a test for chromosome loss/breakage in Drosophila is positive in assays of acetin and 2, 3-dibromo-1-propanol
Quick ViewOther Sources

- By Zimmering, S.
- From Mutation Research Letters (1982), 105(5), 329-31. | Language: English, Database: CAPLUS

- acetin [26446-35-5] Gave a pos. response with mei-9^a for complete loss (CL) and partial loss (PL), being ~5.8 and 5.1%, resp. Similarly, 2,3-dibromo-1-propanol (I) [**96-13-9**] was pos. measured by induced CL or PL and had a total loss of ~3.4 and 2.2% following exposure for 48 or 24 h, resp. In comparison, there was no evidence that the st mus302 strain had lost its repair-deficient characteristics. No significant increase in chromosome loss for either compd. tested was obsd. from mating with ordinary y_2 v females. Thus, chromosome loss /breakage is demonstrable in F1 progeny from assays of acetin and I in the mei-9^a test for chromosome loss.

Metabolism and disposition of the flame retardant tris(2,3-dibromopropyl)phosphate in the rat
Quick ViewOther Sources

- By Nomeir, Amin A.; Matthews, H. B.
- From Toxicology and Applied Pharmacology (1983), 67(3), 357-69. | Language: English, Database: CAPLUS

- The metab. and disposition of the flame retardant, tris(2,3-dibromopropyl) phosphate (Tris-BP) [126-72-7] were studied after oral and i.v. administration of the ^{14}C-labeled compd. to the male rat. Tris-BP was readily absorbed from the gastrointestinal tract and rapidly distributed throughout the body. The distribution and excretion of Tris-BP derived radioactivity were similar after either oral or i.v. administration. The only effects of route of administration on tissue distribution were slightly higher concns. in liver after oral administration and in lung after i.v. administration. The initial elimination of Tris-BP derived radioactivity in urine, feces, and as CO_2 accounted for approx. 50% of the dose in 24 h. An anal. of Tris-BP derived radioactivity remaining in the tissues 1 day after administration indicated that most of the radioactivity in all tissues was in the form of various metabolites rather than the parent compd. The terminal clearance of Tris-BP derived radioactivity from most of the tissues was best described by a single component exponential decay with a half-life of approx. 2.5 days. Clearance from liver and kidney was somewhat slower having a half-life of approx. 3.8 days. Approx. 33% of the radioactivity excreted in urine and approx. 50% of the radioactivity excreted in bile were identified by cochromatog. with synthesized stds. on high performance liq. chromatog. Six metabolites and a trace of the parent compd. were identified in urine and bile by this method. The 6 metabolites represent products of dealkylation and dehydrobromination of the parent compd. The metabolites of Tris-BP isolated from urine and bile were also formed in vitro by NADPH-dependent microsomal enzymes from rat liver. The sol. enzymes from liver metabolized Tris-BP to least 3 unidentified polar metabolites.

The mei-9a test for chromosome loss in Drosophila: a review of assays of 21 chemicals for chromosome breakage
Quick ViewOther Sources

- By Zimmering, S.
- From Environmental Mutagenesis (1983), 5(6), 907-21. | Language: English, Database: CAPLUS

- Of 21 compds. (17 carcinogens and 4 unknown), all were pos. for complete loss (CL) of the X or Y chromosome + partial loss (PL) of the Y and all for PL in the mei-9^{a} test. Only 10 of 20 compds.. were pos. for CL and PL, and 2 of 21 for PL with repair proficient females; the mei-9^{a} test being clearly more sensitive than the former (conventional test) for chromosome breakage. Therefore, the mei-9^{a} test is a more rapid and sensitive test for chromosome breakage, both published and unpublished data on the 21 compds is presented.

Comparative genotoxicity studies of the flame retardant tris(2,3-dibromopropyl)phosphate and possible metabolites
Quick ViewOther Sources

- By Holme, Joern A.; Soederlund, Erik J.; Hongslo, Jan K.; Nelson, Sidney D.; Dybing, Erik
- From Mutation Research, Genetic Toxicology Testing (1983), 124(3-4), 213-24. | Language: English, Database: CAPLUS

- Tris(2,3-dibromopropyl)phosphate (Tris-BP) [126-72-7] was activated to mutagens in the Salmonella/microsome quant. test system. Liver microsomes from rats pretreated with phenobarbital (PB) increased the mutagenicity of 0.05 mM Tris-BP to 186% of the activity obtained with liver microsomes from untreated rats. The addn. of 0.02 mM Tris-BP to V79 Chinese hamster cells coincubated with liver microsomes from PB-pretreated rats increased the no. of mutants by a factor of 9.7. Tris-BP also caused genotoxic and cytotoxic responses in primary monolayers of rat hepatocytes. The relative increase in unscheduled DNA synthesis after treatment with 0.05 mM Tris-BP was 2.3-fold as measured by scintillation counting of radiolabeled thymidine incorporated into DNA of isolated nuclei. The use of hepatocytes isolated from PB-pretreated rats reduced the increases in DNA repair synthesis relatively to that in control cells. Monolayers of hepatocytes from untreated rats cocultured with S. typhimurium TA100 activated Tris-BP to mutagenic intermediates which were released into the culture medium. Apparently, the reactive intermediates formed from Tris-BP are sufficiently stable and lipophilic to traverse the various membranes from the site of generation to the resp. cellular targets. The relative degree of genotoxic responses of bis(2,3-dibromopropyl)phosphate [5412-25-9], (2,3-dibromopropyl)phosphate [126-72-7], tris(3-bromopropyl)phosphate [70555-33-8], tris(2-bromopropyl)phosphate [31858-09-0], and 2,3-dibromopropanol [**96-13-9**] in the systems studied did not indicate that these compds. were proximate or ultimate reactive metabolites of Tris-BP in liver-derived activation systems.

Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program
Quick ViewOther Sources

- By Yoon, J. S.; Mason, J. M.; Valencia, R.; Woodruff, R. C.; Zimmering, S.
- From Environmental Mutagenesis (1985), 7(3), 349-67. | Language: English, Database: CAPLUS

- Results from Drosophila mutagenicity tests of 45 chem. compds. assayed for the National Toxicol. Program are presented. Nine compds. were judged pos. and 4 equivocal in the sex-linked recessive lethal test. The nine pos. compds. were acetin [26446-35-5], allyl glycidyl ether (I) [106-92-3], cyclophosphamide [50-18-0], 1,2-dibromo-3-chloropropane [96-12-8], 2,3-dibromo-1-propanol [**96-13-9**], dimethylcarbamyl chloride [79-44-7], 1,2-epoxybutane [106-88-7], lasiocarpine [303-34-4], and N-nitrosopiperidine [100-75-4]. The results for chloral hydrate [302-17-0], maleic hydrazide [123-33-1], propantheline bromide [50-34-0], and trifluralin [1582-09-8] were equivocal. Of the 9 compds. pos. in recessive lethal induction, only I and dimethylcarbamyl chloride failed to induce translocations. The remaining 32 were judged to be nonmutagenic under the conditions used.

Structure-activity-relationship of organic substances and bioindication

Quick ViewOther Sources

- By Schmidt, Christian; Schnabl, Heide
- From Vom Wasser (1988), 70, 21-32. | Language: German, Database: CAPLUS

- Anilines, phenolic and aliph. compds. were tested with 4 different bioassays with respect to possible structure-activity-relationship to octanol-water partition coeff., water-soly., and bioassay results. Several structure-activity relationships can be verified, a classification of structure-dependent effects for a possible prediction of toxicity is not possible.

Yeast - an unicellular model system in ecotoxicology and xenobiochemistry

Quick ViewOther Sources

- By Ahlers, Jan; Benzing, Martin; Gies, Andreas; Pauli, Wilfried; Roesick, Erika
- From Chemosphere (1988), Volume Date1987, 17(8), 1603-15. | Language: English, Database: CAPLUS

- The effects of 57 phenols, anilines, and aliph. compds. on yeast (Saccharomyces cerevisiae) growth rate as well as on structural and functional properties of the plasma membrane were examd. The different systems correlated with each other and with data from higher eukaryotic organisms. Close relations between the activity of the chems. in the different biol. test systems and structural parameters, esp. hydrophobicity, were obsd.

Prediction of the outcome of rodent carcinogenicity bioassays currently being conducted on 44 chemicals by the National Toxicology Program

Quick ViewOther Sources

- By Tennant, Raymond W.; Spalding, Judson; Stasiewicz, Stanley; Ashby, John
- From Mutagenesis (1990), 5(1), 3-14. | Language: English, Database: CAPLUS

- This paper was written to enable evaluation of the concept that knowledge about chem. structure combined with limited short-term genotoxicity and toxicity test results can be used to predict potential carcinogens. Previous attempts have been potentially biased by prior knowledge about the tumorigenicity of chems. in animals or humans, but the 44 chems. that are currently being bioassayed for carcinogenicity by the National Toxicol. Program provide an opportunity prospectively to evaluate factors that may be predictive of chem. carcinogencity. Predictions of rodent carcinogenicity for these 44 agents are presented as an example of what is believed to be the best available approach at this time. This publication will also enable others to make their own predictions (using whatever methods they believe to have high predictive value) before the results of the animal assays are known.

A prospective toxicity evaluation (COMPACT) on 40 chemicals currently being tested by the National Toxicology Program

Quick ViewOther Sources

- By Lewis, David F. V.; Ioannides, Costas; Parke, Dennis V.
- From Mutagenesis (1990), 5(5), 433-5. | Language: English, Database: CAPLUS

- The computer-optimized mol. parametric anal. of chem. toxicity (COMPACT) procedure was used to det. the mol. conformation and electronic structure of a series of 40 chems. (out of a total of 44). The procedure can evaluate whether they interact with the active site of cytochrome P 450 I or to the binding site of the Ah receptor, and hence to manifest carcinogenicity/toxicity. This is in response to the recent publication by R. W. Tennant, et al. (1990) and their invitation to participate in a prospective identification of potential mutagenicity /carcinogenicity of these 44 chems. Correlation of COMPACT with potential genotoxicity was 25 /40 (63%): COMPACT also predicted toxicity/carcinogenicity in 10 chems. (25%) considered to be potentially nongenotoxic [naphthalene, promethazine, resorcinol, p-nitrophenol, tricresyl phosphate, bis(bromoethyl)propanediol, 3,4-dihydrocoumarin, theophylline, triamterene and chloramine] and predicted the absence of toxicity in four chems. (10%) considered to be potentially genotoxic (Me bromide, hydrazoic acid, 2,3-dibromo-1-propanol, and 1,2,3-trichloropropane).

Prediction of the carcinogenicity in rodents of chemicals currently being tested by the US National Toxicology Program: structure-activity correlations
Quick ViewOther Sources

- By Rosenkranz, Herbert S.; Klopman, Gilles
- From Mutagenesis (1990), 5(5), 425-32. | Language: English, Database: CAPLUS

- CASE, an artificial intelligence structure-activity relation system, was used to predict the carcinogenicity of a group of chems. currently being tested in rodent bioassays by the US National Toxicol. Program. The learning set for the CASE predictions consisted of the results of previous 252 rodent carcinogenicity bioassays.

Evaluating the ability of CASE, an artificial intelligence structure-activity relational system, to predict structural alerts for genotoxicity
Quick ViewOther Sources

- By Rosenkranz, Herbert S.; Klopman, Gilles
- From Mutagenesis (1990), 5(6), 525-7. | Language: English, Database: CAPLUS

- CASE, a structure-activity relational system, correctly predicts the presence of structural alerts in 36 of 39 mols. (sensitivity, 1.00; specificity, 0.83; concordance, 92%). The misclassification of two of the mols. is due to either ambiguous or previously unenunciated rules for defining structural alerts.

Computer prediction of possible toxic action from chemical structure; the DEREK system
Quick ViewOther Sources

- By Sanderson, D. M.; Earnshaw, C. G.
- From Human & Experimental Toxicology (1991), 10(4), 261-73. | Language: English, Database: CAPLUS

- The development of DEREK, a computer-based expert system (derived from the LHASA chem. synthesis design program) for the qual. prediction of possible toxic action of compds. on the basis of their chem. structure is described. The system is able to perceive chem. sub-structures within mols. and relate these to a rulebase linking the sub-structures with likely types of toxicity. Structures can be drawn in directly at a computer graphics terminal or retrieved automatically from a suitable inhouse database. The system is intended to aid the selection of compds. based on toxicol. considerations, or sep. to indicate specific toxicol. properties to be tested for early in the evaluation of a compd., so saving time, money and some lab. animals and resources.

QSAR prediction of rodent carcinogenicity for a set of chemicals currently bioassayed by the US National Toxicology Program
Quick ViewOther Sources

- By Benigni, R.
- From Mutagenesis (1991), 6(5), 423-5. | Language: English, Database: CAPLUS

- A QSAR model based on the combination of two mol. descriptors-estd. electrophilic reactivity and Ashby's structural alerts-was used to predict the carcinogenicity of 44 chems. currently bioassayed by the US National Toxicol. Program. These predictions will be compared with the rodent carcinogenicity assay results as the assays are completed.

Nonisocyanate exposures in three flexible polyurethane manufacturing facilities
Quick ViewOther Sources

- By Boeniger, Mark F.
- From Applied Occupational and Environmental Hygiene (1991), 6(11), 945-52. | Language: English, Database: CAPLUS

- Although toluene diisocyanate (I) is widely used for about 50 yr, industrial hygiene characterizations of other chem. exposures used in conjunction with TDI have generally received much less attention. As part of a retrospective, cohort mortality study, industrial hygiene evaluations were performed in three facilities manufg. flexible I-based foam. This report is

concerned only with nonisocyanate exposures in that industry. In each facility, the concn. of several air contaminants, other than I, were assessed, including aliph. amines, nitrosamines, NO_2, thermal degrdn. products, org. solvents, polyurethane dust, and retardants. Air samples were also collected for subsequent assay of mutagenic activity using Salmonella bacterial tester strains TA98 and TA100. Workers had respiratory and probably dermal exposure to CH_2Cl_2 in each facility. Tracers of NO_2 and some aliph. amines were evident. However, N-nitrosamines were not found. Exposure to thermal degrdn. products, polyurethane dust, and to flame retardants is not a problem under reasonable hygienic conditions. However, evidence for the existence of acrolein and acrylonitrile was found in one facility due to the thermal degrdn. of finished foam. Mutagenic activity, appearing only in the particulate fraction, appeared highest when the total isocyanate group air concns. were also high. In one facility, the mutagenic activity was up to 75 times higher than the mutagenic activity of the outside air. The methods used to characterize each of the exposures are provided and the results are discussed.

On the rodent bioassays currently being conducted on 44 chemicals: a RASH analysis to predict test results from the National Toxicology Program
Quick ViewOther Sources

- By Jones, Troyce D.; Easterly, Clay E.
- From Mutagenesis (1991), 6(6), 507-14. | Language: English, Database: CAPLUS

- A method of relative potency comparisons was used to rank the potential strength of 44 compds. being tested in rodent carcinogenicity bioassays. All previous hazard evaluations have been for human conditions where great nos. of simultaneous and serial exposures may act in combination to produce a neoplasm comprised of 2^{20} to 2^{30} cells commonly expected to derive from a single precancerous cell. For human exposures, an initiated target tissue contg. at least one transformed but subcarcinogenic cell per organ was assumed. Thus, for man, the focus was on empirical correspondences that may help to index the monoclonal growth of a particular cell lineage during cancer expansion. In contrast to humans, initiation of target tissues in animals subjected to National Toxicity Program (NTP) bioassays may not be a given condition, because of extensive precautions taken to minimize exposures to contaminates in food, water, and cage environments. For this evaluation, categorical assignments of unlikely, possible, and probable carcinogens adapted from NTP tests were used. Rank ordering, of compds. according to max. doses tested in male mice and male rats, is coded according to the 3 outcomes taken from the NTP tests, but the magnitude of potency depends completely upon the particular method of comparing toxicol. data. A relative potency based anal. of a diversity of toxicol. data may be useful for rank ordering potentially hazardous compds. to be tested by the NTP and for range finding of their effective test doses to be administered during chronic test protocols.

Prospective ke screening of potential carcinogens being tested in rodent bioassays by the US National Toxicology Program
Quick ViewOther Sources

- By Bakale, George; McCreary, Richard D.
- From Mutagenesis (1992), 7(2), 91-4. | Language: English, Database: CAPLUS

- Values of k_e, the rate const. of electron attachment, were measured in cyclohexane for 31 of 44 chems. now being screened for carcinogenicity in rodent bioassays conducted by the US National Toxicol. Program. Thes k_es provide a physicochem. measure of electron-solute interaction in a nonpolar medium which had been found to be correlated with solute carcinogenicity in earlier studies of the k_es of chem. carcinogens and putative noncarcinogens. The k_e test yields 15 neg. and 16 pos. responses for the 31 chems. that were screened in this study. These k_e results are compared with the predictions of other carcinogen-screening methods that have been applied to the same chems.

Application of the group contribution method for predicting the toxicity of organic chemicals
Quick ViewOther Sources

- By Gao, Chao; Govind, Rakesh; Tabak, Henry H.

- From Environmental Toxicology and Chemistry (1992), 11(5), 631-6. | Language: English, Database: CAPLUS

- A group contribution method was developed that uses the LC_{50} values (measured for fathead minnow) for 130 diverse org. compds. The 130 selected compds. were constituted from 16 chem. groups with -log(LC_{50}) values ranging from 0.85 to 6.09. The method was then used to predict the LC_{50} values for 10 addnl. compds., not in the training set, with good agreement between the predicted and measured values.

Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination
Quick ViewOther Sources

- By Vogel, Ekkehart W.; Nivard, Madeleine J. M.
- From Mutagenesis (1993), 8(1), 57-81. | Language: English, Database: CAPLUS

- An evaluation is presented on the effects of 181 chems. in the (white/white) (w/w^+) eye mosaic assay, an in vivo short-term test measuring genetic damage in somatic cells of Drosophila after treatment of larvae. The principal outcome of this anal. is the necessity for classification of responses into 3 categories. (1) Pos., ++. The 92 chems. falling into this category were clearly recombinagenic in the assay, meaning that dose-response relations were obtained (or could have been established as was evident from the strong responses obtained at one or two exposure doses). Among the 92 chems. were 49 promutagens including volatile chems. such as vinyl bromide and vinyl chloride. (2) Marginally pos., $+^w$. The 40 chems. belonging to this category mainly represented 4 distinct types, including (a) procarcinogens, (b) electrophilic chems. of high nucleophilic selectivity, (c) spindle poisons, and (d) nongenotoxic carcinogens. (3) Inactive, -. Among the 49 chems. inactive in the assay were the carcinogens acetamide, auramine-O, chloramphenicol, chloroform, di(2-ethylhexyl) phthalate, DMF, ethylene thiourea, and testosterone - chems. for which DNA reactivity is unlikely. Among the most effective recombinagens were three of the four aflatoxins tested, of which aflatoxin B_1 represents the key chem. in this bioassay. Since cytotoxic effects to the developing organism apparently put a limit to the selection of high-dose levels, the consequence is that the use of nonphysiol. high concns. is largely avoided in this in vivo system.

Detecting membrane impairment caused by xenobiotics
Quick ViewOther Sources

- By Pauli, W.; Berger, S.; Koehler, M.; Gies, A.
- From Environmental Toxicology and Water Quality (1993), 8(2), 173-89. | Language: English, Database: CAPLUS

- The toxicity of 14 substituted anilines and 15 aliph. alcs. was assessed by the electrorotation of yeast cells. Depending on the applied frequency, control cells exhibit both anti- and cofield rotation. From this spectrum, a frequency was selected at which untreated cells do not rotate, but impaired cells rotate with the field. The exptl. spectra are fully in accord with theor. models of the rotational response, calcd. on the basis of changes in membrane permeability. High correlations exist between rotational data and physiol. and biochem. end points (growth rate, plasma membrane ATPase, purine transport of yeast cells, and antihemolytic assays with human erythrocytes). A quant. structure-activity relationship anal. was made, whereby rotation data from 23 phenols were included. The effects of all chems. on cell rotation could be predicted by their lipophilicity. Some residuals and deviations could be accounted for by the inclusion of mol. wt. or connectivity in the anal. 4-Nitrophenol was an outlier, having an effect at least one order of magnitude higher than predicted, suggesting a specific toxic mechanism. Based on lipophilicity and rotation data, the antihemolytic effect on erythrocytes could be accurately estd.

The relationship between use of the maximum tolerated dose and study sensitivity for detecting rodent carcinogenicity
Quick ViewOther Sources

- By Haseman, Joseph K.; Lockhart, Ann

- From Fundamental and Applied Toxicology (1994), 22(3), 382-91. | Language: English, Database: CAPLUS

- The relationship between max. tolerated dose (MTD) and study sensitivity for detecting rodent carcinogenicity was evaluated for 216 chems. found to be carcinogens in lab. animal studies conducted by the National Cancer Institute (NCI) and the National Toxicol. Program (NTP). Approx. two-thirds of these rodent carcinogens would have been detected even without the top dose (estd. MTD), but in many of these studies, some site-specific carcinogenic effects would have been missed. Among the remaining one-third of the rodent carcinogens that required the top dose for statistical significance, approx. 80% had numerically elevated rates of the same site-specific tumors at lower doses as well. Only 13 of the NCI/NTP rodent carcinogens had increased tumor rates limited to the top dose for all sites of carcinogenicity. Alternatively, of the 838 site-specific carcinogenic effects obsd. in the NCI/NTP studies, 447 (53%) would have been detected even without the top dose. Of the remaining effects, 75% (294/391) showed numerically elevated site-specific tumor rates at lower doses. The authors' evaluation indicates that most carcinogenic effects obsd. at the top dose in rodent studies are also present (with reduced incidence that might or might not be statistically significant) at the lower doses typically employed (1/2MTD, 1/4MTD).

Predictions of the carcinogenicity in rodents of chemicals currently under test by the U.S. National Toxicology Program: an update and some considerations

Quick ViewOther Sources

- By Rosenkranz, Herbert S.; Klopman, Gilles
- From Life Science Advances: Biochemistry (1991), 10(3-4), 151-8. | Language: English, Database: CAPLUS

- The authors have demonstrated that the predictive performance of CASE, a structure activity relational expert system is a function of the size and quality of the "learning set" (i.e. the database). Recently the authors predicted the carcinogenicity in rodents of chems. undergoing testing by the U.S. National Toxicol. Program (NTP) (1990). These predictions were based on the reported NTP cancer bioassay results of 252 chems. The availability now of the bioassay results for an addnl. 39 chems. (R. W. Tennant and J. Ashby; 1991) enabled the authors to update the "learning set" and make new predictions regarding the carcinogenicity of the same group of chems. currently being tested by the NTP. Although the majority of the predictions were not altered significantly, a no. of them did change.

Mechanism-based comparisons of acute toxicities elicited by industrial organic chemicals in procaryotic and eucaryotic systems

Quick ViewOther Sources

- By Jaworska, Joanna S.; Schultz, T. Wayne
- From Ecotoxicology and Environmental Safety (1994), 29(2), 200-13. | Language: English, Database: CAPLUS

- Comparisons of toxicities elicited by nonpolar and polar narcotics, weak acid uncouplers of oxidative phosphorylation, and bioreactive chems. between the eucaryotic systems Pimephales promelas and Tetrahymena pyriformis and the procaryotic systems Escherichia coli and Photobacterium phosphoreum were performed. Each chem. had been a priori assigned a mechanism/mode of action based n the results from previous studies with eucaryotic systems. Hydrophobicity-dependent QSARs for nonpolar narcosis for both the E. coli and the P. phosphoreum endpoints were developed. However, due to the lack of a significant relationship between P. phosphoreum toxicity and log K_{ow}, such a QSAR for polar narcosis was developed only for the E. coli endpoint. Except for 4-nitroaniline (the only chem. in the examd. group that required activation to become the Michael receptor), all chems. contg. reactive substructures revealed excess toxicity over polar narcosis QSAR for E. coli endpoints. Moreover, chloroacidic acid and Et chloroacetate in this system also appear to be bioreactive. The only mechanism that seemed to not exist in the procaryotic system was uncoupling of oxidative phosphorylation. Chems. from this group, except 2,4-dinitroaniline, did not exhibit excess toxicity over polar narcosis QSAR. This was thought to be explained by the lack of mitochondria in prokaryotes, the

target site of uncoupling agents in eukaryotes. In addn., evaluation of toxicities of halogen-substituted short-chain carboxylic alcs. indicated that their mechanisms vary, depending upon the type of substitution and the system.

Enhancement of Bacterial Mutagenicity of Bifunctional Alkylating Agents by Expression of Mammalian Glutathione S-Transferase

Quick ViewOther Sources

- By Thier, Ricarda; Muller, Michael; Taylor, John B.; Pemble, Sally E.; Ketterer, Brian; Guengerich, F. Peter
- From Chemical Research in Toxicology (1995), 8(3), 465-72. | Language: English, Database: CAPLUS

- Recently, we inserted the plasmid vector pKK233-2 contg. rat GSH S-transferase (GST) 5-5 cDNA into Salmonella typhimurium TA1535 and found that these bacteria [GST 5-5(+)] expressed the protein and produced mutations when ethylene or methylene dihalides were added [Thier, R., Taylor, J. B., Pemble, S. E., Ketterer, B., Persmark, M., Humphreys, W. G., and Guengerich, F. P. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 8576-8580]. After exposure to the known GST 5-5 substrate 1,2-epoxy-3-(4'-nitrophenoxy)propane, the GST 5-5(+) strain showed fewer mutants than the bacteria transfected with the cDNA clone in a reverse orientation [GST 5-5(-)], suggesting a protective role of GST 5-5. However, mutations were considerably enhanced in the GST 5-5(+) strain [as compared to GST 5-5(-)] when 1,2,3,4-diepoxybutane (butadiene diepoxide) or 1,2-epoxy-4-bromobutane was added. The GST 5-5(+) and GST 5-5(-) bacterial stains showed similar responses to 1,2-epoxypropane, 3,4-epoxy-1-butene, and 1,4-dibromobutane. The results suggest that some bifunctional activated butanes are transformed to mutagenic products through GSH conjugation. We also found that the GST 5-5(+) strain showed enhanced mutagenicity with 1,4-dibromo-2,3-epoxybutane, 1,2-epoxy-3-bromopropane (epibromohydrin), and (±)-1,4-dibromo-2,3-dihydroxybutane. The possibility was considered that a 5-membered thialonium ion may be involved in the mutagenicity. Model thialonium compds. were rather stable to hydrolysis in aq. soln. at pH 7.4 and slowly alkylated 4-(4-nitrobenzyl) pyridine. The presence of a hydroxyl group β to the sulfur did not enhance reactivity. Mechanisms involving episulfonium ions are considered more likely. Potential oxidn. products of the toxic pesticide 1,2-dibromo-3-chloropropane (DBCP) were also considered in this system. DBCP itself gave rather similar results in the two strains. Others have reported that oxidn. of DBCP is required for mutagenicity, along with GST-catalyzed GSH conjugation [Simula, T. P., Glancey, M. J., Soederlund, E. J., Dybing, E., and Wolf, C. R. (1993) Carcinogenesis 14, 2303-2307]. The putative oxidn. product 1,2-dibromopropional did not show a difference between the two strains. However, 1,3-dichloroacetone, a model for the putative oxidn. product 1-bromo-3-chloroacetone, was considerably more mutagenic in the GST 5-5(+) strain.

Toxicity and carcinogenicity of 2,3-dibromo-1-propanol in F344/N rats and B6C3F1 mice

Quick ViewOther Sources

- By Eustis, Scot L.; Haseman, Joseph K.; Mackenzie, William F.; Abdo, Kamal M.
- From Fundamental and Applied Toxicology (1995), 26(1), 41-50. | Language: English, Database: CAPLUS

- Toxicol. and carcinogenesis studies of 2,3-dibromo-1-propanol were conducted by applying the chem. in 95% ethanol to the interscapular skin of male and female F344/N rats and B6C3F$_1$ mice 5 days a week for 13 wk in the prechronic study and 48-55 wk (rats) or 36-42 wk (mice) in the carcinogenicity study. In the 13-wk study, 10 rats and 10 mice of each sex received doses of 0, 44, 88, 177, 375, or 750 mg/kg. Deaths assocd. with chem. application occurred only in the high-dose (750 mg/kg) male mice. Chem.-related lesions were seen in the kidney of male rats, liver of female rats, and liver and lung of both sexes of mice. Based on the toxicity obsd. in the 13-wk study, 50 rats of each sex received doses of 0, 188, or 375 mg/kg and 50 mice of each sex received 0, 88, or 177 mg/kg in the carcinogenicity study. The planned 2-yr study was terminated early because of reduced survival of rats related to chem.-induced neoplasia and because of the appearance of antibodies to lymphocytic choriomeningitis virus in sentinel mice. Nearly all dosed rats had malignant neoplasms at one or more sites, while only one control male

and one control female had malignant neoplasms. In rats, neoplasms induced by 2,3-dibromo-1-propanol occurred in the skin, nasal mucosa, Zymbal's gland, oral mucosa, esophagus, forestomach, intestines, liver, kidney, mammary gland (females), clitoral gland (females), spleen (males), and mesothelium (males). In mice, chem.-induced neoplasms occurred in the skin, forestomach, liver (males), and lung (males).

NTP technical report on the toxicology and carcinogenesis studies of 2,3-dibromo-1-propanol (CAS No. 96-13) in F344/N rats and B6C3F1 mice (Dermal studies)

Quick ViewOther Sources

- By National Toxicology Program
- From Report (1993), (NRP-TR-400; Order No. PB94-206687), 206 pp.. | Language: English, Database: CAPLUS

- 2,3-Dibromo-1-propanol, a colorless liq., has been used as a flame retardant and as an intermediate in the manuf. of pesticides and pharmaceutical prepns. Toxicol. and carcinogenicity studies were conducted by applying 2,3-dibromo-1-propanol (~98% pure) in ethanol to the subscapular area of the skin of male and female F344/N rats and B6C3F1 mice 5 days per wk for 16 days, 13 wk, 48-51 wk (male rats), 52-55 wk (female rats), 36-39 wk (male mice), or 39-42 wk (female mice). Under the conditions of these long-term dermal studies, there was clear evidence of the carcinogenic activity of 2,3-dibromo-1-propanol in male and female F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, liver, and kidney. There was clear evidence of the carcinogenic activity of 2,3-dibromo-1-propanol in male B6C3F1 mice based on increased incidences of neoplasms of the skin, forestomach, liver, and lung. There was clear evidence of the carcinogenic activity of 2,3-dibromo-1-propanol in female B6C3F1 mice based on increased incidences of neoplasms of the skin and the forestomach.

Predictions of rodent carcinogenicity testing results: interpretation in light of the Lave-Omenn value-of-information model

Quick ViewOther Sources

- By Omenn, Gilbert S.; Stuebbe, Seth; Lave, Lester B.
- From Molecular Carcinogenesis (1995), 14(1), 37-45. | Language: English, Database: CAPLUS

- The recent National Institute of Environmental Health Sciences/National Toxicol. Program Carcinogen Prediction Challenge elicited a value array of predictions of the carcinogenicity of chems. tested in the lifetime rodent bioassay. The data warrant addnl. analyses of the similarities and differences of the predictive methods. The authors provide here analyses of the sensitivity, specificity, and false-pos./false-neg. tendencies of the different sets of predictions. The value-of-information model provides guidance to testing agencies and regulatory agencies in detg. the social value of addnl. information and setting up the framework for assessing the social consequences of different test strategies and nontest predictive methods. These considerations deserve attention in the second round of the Carcinogen Prediction Challenge.

Activation and Inactivation of Carcinogenic Dihaloalkanes and Other Compounds by Glutathione S-Transferase 5-5 in Salmonella typhimurium Tester Strain NM5004

Quick ViewOther Sources

- By Shimada, Tsutomu; Yamazaki, Hiroshi; Oda, Yoshimitsu; Hiratsuka, Akira; Watabe, Tadashi; Guengerich, F. Peter
- From Chemical Research in Toxicology (1996), 9(1), 333-40. | Language: English, Database: CAPLUS

- A newly developed tester Salmonella typhimurium NM5004 strain was constructed by introducing a plasmid contg. both rat GSH S-transferase (GST) 5-5 cDNA and the umuC''lacZ operon into the host strain Salmonella typhimurium TA1535 and used to examine whether or not GST modified the genotoxic activities of several dihaloalkanes and other compds. Twenty-nine chems. that were suggested to be conjugated by GST were compared with regard to their abilities to induce umu gene expression and cause cytotoxicity responses in both the NM5004

strain and the original tester strain (S. typhimurium TA1535/pSK1002, which is devoid of GST activity toward 1,2-epoxy-3-(4'-nitrophenoxy)propane). Ten chems.-1,2-dibromoethane, N-(2,3-epoxypropyl)phthalimide, 1,3-dichloroacetone, CH_2I_2, 1,2-epoxy-3-phenoxypropane, 2,3-epoxypropyl p-methoxyphenyl ether, 1-bromo-2-chloroethane, 1-bromo-2,3-dichloropropane, CH_2BrCl, and CH_2Br_2-were found to enhance induction of umu gene expression in the NM5004 strain as compared with the TA1535/pSK1002 strain. 1,2-Epoxy-3-(4'-nitrophenoxy)propane and 2,3-dibromo-1-chloropropane were inactivated by GST 5-5 in the NM5004 tester strain, although these chems. were cytotoxic in both tester strains. Roles of GST 5-5 were also examd. for the inactivation of reactive metabolites of several procarcinogens that were formed through oxidn. by liver microsomes of polychlorinated biphenyl-treated rats. The results suggest that reactive metabolites (possibly epoxides) of aflatoxin B_1, sterigmatocystin, 1,2-dihydro-1,2-dihydroxy-6-aminochrysene, and (+)- and (-)-enantiomers of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene could be trapped as inactivated GSH conjugates in the NM5004 strain. High-performance liq. chromatog. anal. suggested that exo-aflatoxin B_1 8,9-oxide-GSH conjugate was formed during the oxidn. of aflatoxin B_1 by rat and human liver microsomes in the presence of GSH and several GST enzymes including purified rat theta class GST Y_{rs}-Y_{rs} and rat liver GST (a mixt. of alpha and mu class enzymes). Thus, the present results support the view that the theta class rat GST 5-5 enzyme participates in the activation and inactivation of potential environmental carcinogenic chems. This newly developed NM5004 tester strain is of use in the elucidation of roles of GST 5-5 in transformations.

The potential of organ specific toxicity for predicting rodent carcinogenicity

- By Lee, Yongwon; Buchanan, Bruce G.; Klopman, Gilles; Dimayuga, Mario; Rosenkranz, Herbert S.
- From Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis (1996), 358(1), 37-62. | Language: English, Database: CAPLUS

- Relationships between organ specific toxicity (specifications of the presence or absence of 43 morphol. effects in 32 organs) obsd. from 13-wk subchronic studies and rodent carcinogenicity were investigated by manually measuring the concordance of each feature and also automatically using the RL (Rule Learner) induction program. Of the 32 organs, the presence or absence of any effect in liver or kidney was found very relevant to rodent carcinogenicity. While the concordance of Salmonella genotoxicity with rodent carcinogenicity was only 60%, the battery of liver and kidney was 74% accurate with 75% sensitivity and 71% specificity. Further, using the RL program, rule sets based on organ specific toxicity together with the default predictions based on Salmonella mutagenicity were on av. 80% accurate with 83% sensitivity and 82% specificity.

Prediction of rodent carcinogenicity bioassays from molecular structure using inductive logic programming

- By King, Ross D.; Srinivasan, Ashwin
- From Environmental Health Perspectives Supplements (1996), 104(5), 1031-1040. | Language: English, Database: CAPLUS

- The machine learning program Progol was applied to the problem of forming the structure-activity relation (SAR) for a set of compds. tested for carcinogenicity in rodent bioassays by the U.S. National Toxicol. Program (NTP). Progol is the first inductive logic programming (ILP) algorithm to use a fully relational method for describing chem. structure in SARs, based on using atoms and their bond connectivities. Progol is well suited to forming SARs for carcinogenicity as it is designed to produce easily understandable rules (structural alerts) for sets of noncongeneric compds. The Progol SAR method was tested by prediction of a set of compds. that have been widely predicted by other SAR methods (the compds. used in the NTP's first round of carcinogenesis predictions). For these compds. no method (human or machine) was significantly more accurate than Progol. Progol was the most accurate method that did not use data from biol. tests on rodents (however, the difference in accuracy is not significant). The Progol predictions were based solely on chem. structure and the results of tests for Salmonella

mutagenicity. Using the full NTP database, the prediction accuracy of Progol was estd. to be 63 % (±3%) using 5-fold cross validation. A set of structural alerts for carcinogenesis was automatically generated and the chem. rationale for them investigated-these structural alerts are statistically independent of the Salmonella mutagenicity. Carcinogenicity is predicted for the compds. used in the NTP's second round of carcinogenesis predictions. The results for prediction of carcinogenesis, taken together with the previous successful applications of predicting mutagenicity in nitroarom. compds., and inhibition of angiogenesis by suramin analogs, show that Progol has a role to play in understanding the SARs of cancer-related compds.

Structure-mutagenic activity relationships in the Salmonella typhimurium TA100 strain in a series of short-chain halogenated hydrocarbons and alcohols.
Quick ViewOther Sources

- By Kharchevnikova, N. V.; Zholdakova, Z. I.; Zhurkov, V. S.; Polyakova, E. E.; Novikov, S. M.
- From Russian Journal of Genetics (Translation of Genetika (Moscow)) (1997), 33(5), 594-597. | Language: English, Database: CAPLUS

- Structure-mutagenic activity relationships in a series of short-chain halogenated hydrocarbons and alcs. were detd. with the use of the energy difference parameter of frontier mol. orbitales of compds. that was calcd. by the quantum-chem. method.

T25: a simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity
Quick ViewOther Sources

- By Dybing, Erik; Sanner, Tore; Roelfzema, Henk; Kroese, Dinant; Tennant, Raymond W.
- From Pharmacology & Toxicology (Copenhagen) (1997), 80(6), 272-279. | Language: English, Database: CAPLUS

- A simplified carcinogenic potency index, the T25, is proposed as a practical method for the inclusion of potency considerations in carcinogen classification systems. The T25 is the chronic daily dose in mg per kg bodyweight which will give 25% of the animals tumors at a specific tissue site, after correction for spontaneous incidence, within the std. life span of that species. Calcd. T25 values of a set of 113 US National Cancer Institute/National Toxicol. Program (NC/NTP) carcinogens showed excellent correlation (correlation coeff. 0.96) with the carcinogenic potency index TD50 of R. Peto et al. (1984). The mean of T25 values for 51 transspecies, multiple common site NCI/NTP carcinogens were 10-fold lower than those for 62 NCI/NTP single species, single site carcinogens. For these 113 carcinogens, the mean T25 values were approx. 3-fold lower for agents that were also mutagenic in Salmonella compared to the nonmutagenic agents.

An update of the National Toxicology Program database on nasal carcinogens
Quick ViewOther Sources

- By Haseman, J. K.; Hailey, J. R.
- From Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis (1997), 380(1,2), 3-11. | Language: English, Database: CAPLUS

- Nearly 500 long-term rodent carcinogenicity studies carried out by the National Cancer Institute and the National Toxicol. Program were examd., and 12 chems. were identified that produced nasal tumors: allyl glycidol ether, p-cresidine, 1,2-dibromo-3-chloropropane, 1,2-dibromoethane, 2,3-dibromo-1-propanol, dimethylvinyl chloride, 1,4-dioxane, 1,2-epoxybutane, iodinated glycerol, procarbazine, propylene oxide, and 2,6-xylidine. All 12 of these chems. produced nasal tumors in rats, and 5 also produced nasal tumors in mice. Most of the nasal carcinogens (1) produced tumor increases in both sexes, (2) produced tumors at other sites as well, (3) had significantly reduced survival at doses that were carcinogenic, and (4) were genotoxic. Only 5 of the 12 nasal carcinogens were administered by inhalation. A variety of different types of nasal cavity tumors were produced, and specific tumor rates are given for those chems. causing multiple tumor types. Increased incidences of nasal neoplasms were often accompanied by suppurative/acute inflammation, epithelial/focal hyperplasia, and squamous metaplasia. However, high incidences of these nonneoplastic nasal lesions were also frequently

seen in inhalation studies showing no evidence of nasal carcinogenicity, suggesting that in general nasal carcinogenesis is not assocd. with the magnitude of chronic toxicity obsd. at this site.

Allergic contact sensitizing chemicals as environmental carcinogens
Quick ViewOther Sources

- By Albert, Roy E.
- From Environmental Health Perspectives (1997), 105(9), 940-948. | Language: English, Database: CAPLUS

- Chems. that were bioassayed by the National Toxicol. Program (NTP) and that also produce allergic dermatitis (ACD) in humans were evaluated for their tumorigenic characteristics. The impetus for the study was that most contact sensitizers, i.e., those that produce ACD, and genotoxic carcinogens are chem. similar in that they are electrophilic, thereby producing adducts on macro-mols. including protein and DNA. This similarity in chem. behavior suggests that many contact sensitizers might be environmental carcinogens. All of the published NTP bioassays by early 1996 that had both genotoxicity and carcinogenicity studies were included in this anal. The NTP chems. had been chosen for bioassay without regard to their ability to produce ACD. Of the 209 chems. that were bioassayed, there were 36 (17%) that were known to be human contact sensitizers; about half of these were pos. on tumor bioassays. The contact sensitizers differed from the NTP sample as a whole by having a proportionately larger no. of nongenotoxic chems. by the Ames Salmonella assay, presumably because more of them were selected on the basis of widespread usage rather than structural resemblance to known carcinogens. Compared to the nongenotoxic chems., the genotoxics were stronger carcinogens in that they had a higher incidence of pos. tumor bioassays, with twice the no. of organs in which tumors were induced. The nongenotoxic chems. had a preference for tumor induction in parenchymal tissues in contrast to epithelial tissues. The contact sensitizers showed essentially the same characteristics as the whole NTP sample when stratified according to genotoxicity. Judging by the chems. that were chosen primarily for their widespread use rather than for their structural resemblance to carcinogens, the addn. of a test for contact sensitization to the Ames test as a screening tool would increase the tumorigenic detection efficiency by about 40% because of the nongenotoxic tumorigens. A ballpark est. suggests that there could be several thousand contact sensitizers for humans in com. use that are rodent tumorigens.

Comparisons on chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: collaborative study II
Quick ViewOther Sources

- By Watanabe, Kazuko; Sakamoto, Kyoko; Sasaki, Toshiaki
- From Mutation Research, Genetic Toxicology and Environmental Mutagenesis (1998), 412(1), 17-31. | Language: English, Database: CAPLUS

- A collaborative study of chem.-induced mutation was performed using the four bacterial strains Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101, used in the previous successive studies in order to compare the specific spectrum of response to chems. among the four strains and to det. the usefulness (sensitivity) of each strain. Twenty-two labs. participated in this study. Following the previous study, 28 compds. were selected consisting of oxidative agents or crosslinking agents. These compds. were tested for mutagenicity using the plate incorporation method with or without metabolic activation. The tests were performed in two labs. per chem. The no. of chems. which showed pos. results were 18, 17, 16 and 14 with WP2 uvrA/pKM101, TA2638, TA102 and WP2/pKM101, resp. In all four strains 19/28 chems. (68%) were either pos. or neg. while the remaining 9 chems. showed a heterogeneous response. With TA102 and TA2638 89% (25/28) of the test chems. showed the same response, while 75-79% (21-22/28) showed the same response in these two S. typhimurium strains and in WP2 uvrA/pKM101. In conclusion, WP2/pKM101 strain showed the least sensitivity to the chems. used. Among the other three strains, WP2 uvrA

/pKM101 was the most sensitive strain, although the difference in the no. of chems. which showed pos. results was minor. Concerning the spectrum of response to chems., it is apparent that TA102 and TA2638 have almost the same sensitivity, but some chems. induce different responses between the two S. typhimurium strains and WP2 uvrA/pKM101.

Carcinogenic activity of the flame retardant, 2,2-bis(bromomethyl)-1,3-propanediol in rodents, and comparison with the carcinogenicity of other NTP brominated chemicals

- By Dunnick, June K.; Heath, James E.; Farnell, Daniel R.; Prejean, J. David; Haseman, Joseph K.; Elwell, Michael R.
- From Toxicologic Pathology (1997), 25(6), 541-548. | Language: English, Database: CAPLUS
- Several brominated chems. have been shown to be multisite-multispecies carcinogens in lab. animals, and in this paper we report that the flame retardant, 2,2-bis(bromomethyl)-1,3-propanediol (BMP) is also a multisite carcinogen in both sexes of Fischer 344 rats and B6C3F$_1$ mice. BMP was administered continuously in the diet for up to 2 yr to rats at doses of 0, 2,500, 5,000 or 10,000 ppm and to mice at doses of 0, 312, 625, or 1,250 ppm.. Interim groups of rats were examd. at 15 mo. An addnl. recovery group off male rats received the chem. for 3 mo at 20,000 ppm in the feed, and then the control diet for the remainder of the study. Chem. exposure caused neoplasms of the skin, s.c. tissue, mammary gland, Zymbal's gland, oral cavity, esophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas in the male rat; mammary gland, oral cavity, esophagus, and thyroid gland in the female rat; lung, kidney, and Harderian gland in male mice; and s.c. tissue, lung, and Harderian gland in the female mouse. The recovery group of male rats presented with the same spectrum of treatment-related neoplasms as in the core study. In this recovery group, BMP (at 20,000 ppm) caused irreversible effects at numerous sites after 90 days of exposure that was not detectable by histol. examn., but without further exposure resulted in carcinogenic responses at 2 yr. BMP is mutagenic in the salmonella test, but is was not detd. if the BMP-induced effects that eventually lead to development of neoplasms at multiple sites are the same in both species and in all organ systems affected.

A New Highly Specific Method for Predicting the Carcinogenic Potential of Pharmaceuticals in Rodents Using Enhanced MCASE QSAR-ES Software

- By Matthews, Edwin J.; Contrera, Joseph F.
- From Regulatory Toxicology and Pharmacology (1998), 28(3), 242-264. | Language: English, Database: CAPLUS
- This report describes in detail a new quant. structure-activity relational expert system (QSAR-ES) method for predicting the carcinogenic potential of pharmaceuticals and other org. chems. in rodents, and a beta-test evaluation of its performance. The method employs an optimized, computer-automated structure evaluation (MCASE) software program and new database modules which were developed under a Cooperative Research and Development Agreement (CRADA) between FDA and Multicase, Inc. The beta-test utilized 126 compds. with carcinogenicity studies not included in control database modules and three sets of modules, including: A07-9 (Multicase, Inc.), AF1-4 (FDA-OTR/Multicase, Inc.), and AF5-8 (FDA-OTR /proprietary). The investigation demonstrated that the std. MCASE(A07-9) system which had a small data-set, detected few structure alerts (SA) for carcinogenicity, and had poor coverage for beta-test compds. (51%). Conversely, the new, optimized FDA-OTR/MCASE(AF5-8) system had a large data-set, detected many SA and had good coverage (94%). In addn., the study showed the std. MCASE(A07-9) software had poor predictive value for carcinogens and specificity for noncarcinogens (50 and 42%), detected many false positives (58%), and exhibited poor concordance (46%). Conversely, the new, FDA-OTR/MCASE(AF5-8) system demonstrated excellent predictive value for carcinogens and specificity for non-carcinogens (97%, 98%), detected only one false pos. (2%), and exhibited good concordance (75%). The dramatic improvements in the performance of the MCASE were due to numerous modifications, including:

(a) enhancement of the size of the control database modules, (b) optimization of MCASE SAR assay evaluation criteria, (c) incorporation of a carcinogenic potency scale for control compd. activity and MCASE biophores, (d) construction of individual rodent gender- and species-specific modules, and (e) defining assay acceptance criteria for query and control database compds. (c) 1998 Academic Press.

Chemical carcinogenicity: can it be predicted from knowledge of mutagenicity and allergic contact dermatitis?

- By Rosenkranz, Herbert S.; Karol, Meryl H.
- From Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis (1999), 431(1), 81-91. | Language: English, Database: CAPLUS

- We investigated the suggestion [R.E. Albert, (1997)] that results of mutagenicity testing in Salmonella combined with allergic contact dermatitis (ACD) testing in humans would be predictive of carcinogenicity in rodents. Using the cancer bioassay results of the US National Toxicol. Program (NTP), Salmonella mutagenicity tests and a highly predictive structure-activity relational model of ACD, we conclude that the combination is not more predictive than the results of the Salmonella mutagenicity assay alone.

Evaluation of the TOPKAT system for predicting the carcinogenicity of chemicals

- By Prival, Michael J.
- From Environmental and Molecular Mutagenesis (2001), 37(1), 55-69. | Language: English, Database: CAPLUS

- The TOPKAT computer-based system for predicting chem. carcinogens was evaluated by detg. its ability to predict the carcinogenicity of chems. tested by the National Toxicol. Program. TOPKAT was not effective in identifying potential rodent carcinogens and noncarcinogens in the data set analyzed. The chems. in the TOPKAT database of known carcinogens and noncarcinogens that the software identifies as most "similar" to unknown chems. are illustrated using six examples. These "similar" chems. generally bear no apparent relationship to the chem. of interest with regard to metab. or potential mechanism of carcinogenicity.

A simple method for quantitative risk assessment of non-threshold carcinogens based on the dose descriptor T25

- By Sanner, Tore; Dybing, Erik; Willems, Marianne I.; Kroese, E. Dinant
- From Pharmacology & Toxicology (Copenhagen, Denmark) (2001), 88(6), 331-341. | Language: English, Database: CAPLUS

- This report provides guidance for using the dose-descriptor T25 from animal studies as a basis for quant. risk characterization of non-threshold carcinogens. T25 is presently used within the European Union for setting specific concn. limits for carcinogens in relation to labeling of prepns. (formulations). The T25 is defined as the chronic dose rate which will give 25% of the animals tumors at a specific tissue site, after correction for spontaneous incidence, within the std. life-time of that species. The T25 is converted to the corresponding human dose descriptor, HT25, by dividing it with the appropriate scaling factor for interspecies dose scaling based on comparative metabolic rates. Subsequently, the human dose (expressed in mg per kg body-wt. per day) is calcd. from the available exposure data. The corresponding human life-time cancer risk is then obtained by using linear extrapolation by dividing the exposure dose with the coeff. (HT25/0.25). The results with this new method, which can easily be calcd. without computer programs, are in excellent agreement with results from computer-based extrapolation methods such as the linearised multistage model and the benchmark method using LED_{10}, even though the present method only takes into consideration one single dose-response point. To overcome possible shortcomings of the present method, the estd. life-time risks are proposed to be accompanied by a commentary statement giving an overall evaluation of data that may have

bearing on the carcinogenic risk and that may indicate whether the real human risk is likely to be higher or lower than the calcd. life-time risk. By using the present guidance and a harmonized set of criteria and default values, the calcn. of life-time cancer risk should be transparent and easy to comprehend.

Artificial neural network for predicting the toxicity of organic molecules
Quick ViewOther Sources

- By Admans, Gary; Takahashi, Yoshimasa; Ban, Satoshi; Kato, Hiroaki; Abe, Hidetsugu; Hanai, Sosuke
- From Bulletin of the Chemical Society of Japan (2001), 74(12), 2451-2461. | Language: English, Database: CAPLUS

- Structure-activity relationships for aquatic toxicity were studied using neural networks and linear regression anal. The structural features contributing to toxicity were identified in mols. exhibiting a level of toxicity greater than that of non-reactive org. mols. A neural network was trained for the toxicity of non-polar narcotics, polar narcotics, or reactive toxicants. Quant. structure-activity relationships (QSARs) were developed, relating a mol. aquatic toxicity to its log P and to a set of 16 structural descriptors based upon the presence of selected structural features. The inclusion of these structural descriptors into a QSAR was found to enhance the correlation of the equation, and thus to improve its ability for predicting aquatic toxicity.

Quantitative structure-activity relationships based on functional and structural characteristics of organic compounds
Quick ViewOther Sources

- By Kulkarni, S. A.; Raje, D. V.; Chakrabarti, T.
- From SAR and QSAR in Environmental Research (2001), 12(6), 565-591. | Language: English, Database: CAPLUS

- In the present quant. structure-activity relationship (QSAR) modeling, org. compds., including priority pollutants, have been considered and classified based on their functional and structural characteristics. Five physico-chem. characteristics have been used to develop a QSAR model for Pimephales promelas, by means of multiple regression anal. Collinearity diagnostics was carried out using two different approaches based on condition index and K correlation index. The outlier anal. was carried out using the variable subsets obtained through both the approaches. An attempt has been made to justify the deletion of outliers in each group referring to their physico-chem. characteristics. The expressions obtained by using both approaches provide almost the same prediction accuracy, however, the latter approach resulted in expressions with reduced no. of mol. descriptors. The QSARs obtained through this exercise would certainly assist in designing environment-friendly mols. with lower toxicity.

Characteristics of the spectrum of proliferative lesions observed in the kidney and urinary bladder of Fischer 344 rats and B6C3F1 mice
Quick ViewOther Sources

- By Wolf, Jeffrey C.
- From Toxicologic Pathology (2002), 30(6), 657-662. | Language: English, Database: CAPLUS

- Many rodent renal and bladder carcinogens rely upon epigenetic mechanisms of carcinogenesis; such mechanisms are likely to influence the spectrum of urinary tract tumors obsd. in control and treated animals. This is reflected in several features of chem. induced rodent urinary tract neoplasms, including a low overall tumor incidence, an increased prevalence of urinary tract tumors in rats compared to mice and males compared to females, the tendency for epithelial tumors to predominate over nonepithelial types, and demonstrated links to chronic progressive nephropathy and urolithiasis. Such tendencies are also characteristic of spontaneous urinary tract tumors in rodents. Data to support these observations can be derived from large historical databases such as the Toxicol. Data Management System, maintained by National Toxicol. Program.

Development of Binary Classification of Structural Chromosome Aberrations for a Diverse Set of Organic Compounds from Molecular Structure
Quick ViewOther Sources

- By Serra, J. R.; Thompson, E. D.; Jurs, P. C.
- From Chemical Research in Toxicology (2003), 16(2), 153-163. | Language: English, Database: CAPLUS

- Classification models are generated to predict in vitro cytogenetic results for a diverse set of 383 org. compds. Both k-nearest neighbor and support vector machine models are developed. They are based on calcd. mol. structure descriptors. Endpoints used are the labels clastogenic or nonclastogenic according to an in vitro chromosomal aberration assay with Chinese hamster lung cells. Compds. that were tested with both a 24 and 48 h exposure are included. Each compd. is represented by calcd. mol. structure descriptors encoding the topol., electronic, geometrical, or polar surface area aspects of the structure. Subsets of informative descriptors are identified with genetic algorithm feature selection coupled to the appropriate classification algorithm. The overall classification success rate for a k-nearest neighbor classifier built with just 6 topol. descriptors is 81.2% for the training set and 86.5% for an external prediction set. The overall classification success rate for a 3-descriptor support vector machine model is 99.7% for the training set, 92.1% for the cross-validation set, and 83.8% for an external prediction set.

An exploratory study of the use of multivariate techniques to determine mechanisms of toxic action
Quick ViewOther Sources

- By Ren, Shijin; Frymier, Paul D.; Schultz, T. Wayne
- From Ecotoxicology and Environmental Safety (2003), 55(1), 86-97. | Language: English, Database: CAPLUS

- The most successful quant. structure-activity relationships have been developed by sepg. compds. by their mechanisms of toxic action (MOAs). However, to correctly det. the MOA of a compd. is often not easy. The authors investigated the usefulness of discriminant anal. and logistic regression in detg. MOAs. The discriminating variables used were the logarithm of octanol-water partition coeffs. (log K_{ow}) and the exptl. toxicity data obtained from Pimephales promelas and Tetrahymena pyriformis assays. Small total error rates were obtained when sepg. nonpolar narcotic compds. from other compds., however, relatively high total error rates were obtained when sepg. less reactive compds. (polar, ester, and amine narcotics) from more reactive compds. (electrophiles, proelectrophiles, and nucleophiles).

Phthalates, Alkylphenols, Pesticides, Polybrominated Diphenyl Ethers, and Other Endocrine-Disrupting Compounds in Indoor Air and Dust
Quick ViewOther Sources

- By Rudel, Ruthann A.; Camann, David E.; Spengler, John D.; Korn, Leo R.; Brody, Julia G.
- From Environmental Science and Technology (2003), 37(20), 4543-4553. | Language: English, Database: CAPLUS

- Endocrine-disrupting compds. (EDC) have widespread consumer uses, yet little is known about indoor exposure. Indoor air and dust was sampled in 120 homes and analyzed for 89 org. EDC: 52 compds. were detected in air and 66 were detected in dust. These are the first reported measurements in residential environments for >30 of these compds., including several detected at highest concns. The no. of compds. detected/home was 13-28 in air and 6-42 in dust. The most abundant compds. in air included phthalates (plasticizers, emulsifiers), o-phenylphenol (disinfectant), 4-nonylphenol (detergent metabolite), and 4-tert-butylphenol (adhesive), with typical concns. of 50-1500 ng/m^3. Penta- and tetrabrominated di-Ph ethers (flame retardants) were frequently detected in dust, and 2,3-dibromo-1-propanol, the carcinogenic intermediate of a flame retardant banned in 1977, was detected in air and dust. A total of 23 pesticides were detected in air and 27 were detected in dust; the most abundant were permethrins and the synergist, piperonyl butoxide; banned pesticides (heptachlor, chlordane, methoxychlor, DDT) were also frequently detected, suggesting limited indoor degrdn. Detected concns. exceeded government health-based guidelines for 15 compds.; however, no

guidelines are available for 28 compds. and existing guidelines do not consider endocrine effects. Results provided a basis to prioritize toxicol. and exposure research for individual EDC and mixts. and provided new tools for exposure assessment in health studies.

Prediction of Rodent Carcinogenesis: An Evaluation of Prechronic Liver Lesions as Forecasters of Liver Tumors in NTP Carcinogenicity Studies

- By Allen, D. G.; Pearse, G.; Haseman, J. K.; Maronpot, R. R.
- From Toxicologic Pathology (2004), 32(4), 393-401. | Language: English, Database: CAPLUS

- The National Toxicol. Program (NTP) developed the chronic 2-yr bioassay as a mechanism for predicting the carcinogenic potential of chems. in humans. The cost and duration of these studies has limited their use to small nos. of selected chems. Many different short-term methods aimed at increasing predictive accuracy and the no. of chems. evaluated have been developed in attempts to successfully correlate their results with evidence of carcinogenicity (or lack of carcinogenicity) are assessed. Using NTP studies, the effectiveness of correlating prechronic liver lesions with liver cancer encompassing multiple studies using mice (83 compds.) and rats (87 compds.). These lesions include hepatocellular necrosis, hepatocellular hypertrophy, hepatocellular cytomegaly, bile duct hyperplasia, and hepatocellular degeneration, along with increased liver wt. These results indicate that pooling 3 of these prechronic data points (hepatocellular necrosis, hepatocellular hypertrophy, and hepatocellular cytomegaly) can be very predictive of carcinogenicity in the 2-yr study ($p < 0.05$). The inclusion of increased liver wt. as an endpoint in the pool of data points increases the no. of rodent liver carcinogens that are successfully predicted ($p < 0.05$), but also results in the prediction of increased nos. of noncarcinogenic chems. as carcinogens. The use of multiple prechronic study endpoints provides supplementary information that enhances the predictivity of identifying chems. with carcinogenic potential.

Description of the Electronic Structure of Organic Chemicals Using Semiempirical and Ab Initio Methods for Development of Toxicological QSARs

- By Netzeva, Tatiana I.; Aptula, Aynur O.; Benfenati, Emilio; Cronin, Mark T. D.; Gini, Giuseppina; Lessigiarska, Iglika; Maran, Uko; Vracko, Marjan; Schueuermann, Gerrit
- From Journal of Chemical Information and Computer Sciences (2005), 45(1), 106-114. | Language: English, Database: CAPLUS

- The quality of quant. structure-activity relationship (QSAR) models depends on the quality of their constitutive elements including the biol. activity, statistical procedure applied, and the physicochem. and structural descriptors. The aim of this study was to assess the comparative use of ab initio and semiempirical quantum chem. calcns. for the development of toxicol. QSARs applied to a large and chem. diverse data set. A heterogeneous collection of 568 org. compds. with 96 h acute toxicity measured to the fish fathead minnow (Pimephales promelas) was utilized. A total of 162 descriptors were calcd. using the semiempirical AM1 Hamiltonian, and 121 descriptors were compiled using an ab initio (B3LYP/6-31G**) method. The QSARs were derived using multiple linear regression (MLR) and partial least squares (PLS) analyses. Statistically similar models were obtained using AM1 and B3LYP calcd. descriptors supported by the use of the logarithm of the octanol-water partition coeff. (log K_{ow}). The main difference between the models derived by both MLR and PLS with the two sets of quantum chem. descriptors was concd. on the type of descriptors selected. It was concluded that for large-scale predictions, irresp. of the mechanism of toxic action, the use of precise but time-consuming ab initio methods does not offer considerable advantage compared to the semiempirical calcns. and could be avoided.

A topological substructural approach applied to the computational prediction of rodent carcinogenicity

- By Helguera, Aliuska Morales; Cabrera Perez, Miguel Angel; Gonzalez, Maykel Perez; Ruiz, Reinaldo Molina; Gonzalez Diaz, Humberto
- From Bioorganic & Medicinal Chemistry (2005), 13(7), 2477-2488. | Language: English, Database: CAPLUS

- The carcinogenic activity has been investigated by using a topol. substructural mol. approach (TOPS-MODE). A discriminant model was developed to predict the carcinogenic and noncarcinogenic activity on a data set of 189 compds. The percentage of correct classification was 76.32%. The predictive power of the model was validated by three tests: an external test set (compds. not used in the develop of the model, with a 72.97% of good classification), a leave-group-out cross-validation procedure (4-fold full cross-validation, removing 20% of compds. in each cycle, with a good prediction of 76.31%) and two external prediction sets (the first and second exercises of the National Toxicol. Program). This methodol. evidenced that the hydrophobicity increase the carcinogenic activity and the dipole moment of the mol. decrease it; suggesting the capacity of the TOPS-MODE descriptors to est. this property for new drug candidates. Finally, the pos. and neg. fragment contributions to the carcinogenic activity were identified (structural alerts) and their potentialities in the lead generation process and in the design of safer' chems. were evaluated.

Electrophilicity as a possible descriptor for toxicity prediction
Quick ViewOther Sources

- By Roy, D. R.; Parthasarathi, R.; Maiti, B.; Subramanian, V.; Chattaraj, P. K.
- From Bioorganic & Medicinal Chemistry (2005), 13(10), 3405-3412. | Language: English, Database: CAPLUS

- Electrophilicity is one of the cardinal chem. reactivity descriptors successfully employed in various mol. reactivity studies within a structure-activity relationship parlance. The applications of this quantity in the modeling of toxicol. properties have inspired the authors to perform a more exhaustive study to test and/or to validate the application of electrophilicity in assessing its chem. and toxicol. potential. For this reason the toxicity of a large data set of mols. comprising 252 aliph. compds. on the Tetrahymena pyriformis is studied. A quant. structure-activity relationship anal. enabled the authors to model toxicity in terms of global and local electrophilicities, which provide a reasonably good prediction of aliph. toxicity. It is heartening to note that the global and local electrophilicity values together can explain the toxicity of a large variety of aliph. compds. nicely without resorting to any other descriptor or other microscopic /macroscopic physicochem. properties as is the situation in all other QSAR studies.

Validation of a QSAR model for acute toxicity
Quick ViewOther Sources

- By Pavan, M.; Netzeva, T. I.; Worth, A. P.
- From SAR and QSAR in Environmental Research (2006), 17(2), 147-171. | Language: English, Database: CAPLUS

- In the present study, a quant. structure - activity relationship (QSAR) model has been developed for predicting acute toxicity to the fathead minnow (Pimephales promelas), the aim being to demonstrate how statistical validation and domain definition are both required to establish model validity and to provide reliable predictions. A dataset of 408 heterogeneous chems. was modeled by a diverse set of theor. mol. descriptors by using multivariate linear regression (MLR) and Genetic Algorithm - Variable Subset Selection (GA-VSS). This QSAR model was developed to generate reliable predictions of toxicity for org. chems. not yet tested, so particular emphasis was given to statistical validity and applicability domain. External validation was performed by using OECD Screening Information Data Set (SIDS) data for 177 High Prodn. Vol. (HPV) chems., and a good predictivity was obtained (Q^2_{ext} = 72.1). The model was evaluated according to the OECD principles for QSAR validation, and compliance with all five principles was established. The model could therefore be useful for the regulatory assessment of chems. For example, it could be used to fill data gaps within its chem. domain and contribute to the prioritization of chems. for aquatic toxicity testing.

Automatic extraction of structural alerts for predicting chromosome aberrations of organic compounds
Quick ViewOther Sources

- By Estrada, Ernesto; Molina, Enrique
- From Journal of Molecular Graphics & Modelling (2006), 25(3), 275-288. | Language: English, Database: CAPLUS

- We use the topol. sub-structural mol. design (TOPS-MODE) approach to formulate structural alert rules for chromosome aberration (CA) of org. compds. First, a classification model was developed to group chems. as active/inactive respect to CA. A procedure for extg. structural information from orthogonalized TOPS-MODE descriptors was then implemented. The contributions of bonds to CA in all the mols. studied were then generated using the orthogonalized classification model. Using this information we propose 22 structural alert rules which are ready to be implemented in expert systems for the automatic prediction of CA. They include, among others, structural alerts for N-nitroso compds. (ureas, urethanes, guanidines, triazines), nitro compds. (arom. and heteroarom.), alkyl esters or phosphoric acids, alkyl methanesulfonates, sulfonic acids and sulfonamides, epoxides, arom. amines, azaphenanthrene hydrocarbons, etc. The chemico-biol. anal. of some of the structural alerts found is also carried out showing the potential of TOPS-MODE as a knowledge generator.

Identification of the Structural Requirements for Mutagenicity, by Incorporating Molecular Flexibility and Metabolic Activation of Chemicals. II. General Ames Mutagenicity Model
Quick ViewOther Sources

- By Serafimova, R.; Todorov, M.; Pavlov, T.; Kotov, S.; Jacob, E.; Aptula, A.; Mekenyan, O.
- From Chemical Research in Toxicology (2007), 20(4), 662-676. | Language: English, Database: CAPLUS

The tissue metabolic simulator (TIMES) modeling approach is a hybrid expert system that couples a metabolic simulator together with structure toxicity rules, underpinned by structural alerts, to predict interaction of chems. or their metabolites with target macromols. Some of the structural alerts representing the reactivity pattern-causing effect could interact directly with the target whereas others necessitated a combination with two- or three-dimensional quant. structure-activity relationship models describing the firing of the alerts from the rest of the mols. Recently, TIMES has been used to model bacterial mutagenicity (O. Mekenyan, O., et al.,2004). The original model was derived for a single tester strain, Salmonella typhimurium (TA100), using the Ames test by the National Toxicol. Program (NTP). The model correctly identified 82% of the primary acting mutagens, 94% of the nonmutagens, and 77% of the metabolically activated chems. in a training set. The identified high correlation between activities across different strains changed the initial strategic direction to look at the other strains in the next modeling developments. In this respect, the focus of the present work was to build a general mutagenicity model predicting mutagenicity with respect to any of the Ames tester strains. The use of all reactivity alerts in the model was justified by their interaction mechanisms with DNA, found in the literature. The alerts identified for the current model were analyzed by comparison with other established alerts derived from human experts. In the new model, the original NTP training set with 1341 structures was expanded by 1626 proprietary chems. provided by BASF AG. Eventually, the training set consisted of 435 chems., which are mutagenic as parents, 397 chems. that are mutagenic after S9 metabolic activation, and 2012 nonmutagenic chems. The general mutagenicity model was found to have 82% sensitivity, 89% specificity, and 88% concordance for training set chems. The model applicability domain was introduced accounting for similarity (structural, mechanistic, etc.) between predicted chems. and training set chems. for which the model performs correctly.

A Novel Logic-Based Approach for Quantitative Toxicology Prediction
Quick ViewOther Sources

- By Amini, Ata; Muggleton, Stephen H.; Lodhi, Huma; Sternberg, Michael J. E.
- From Journal of Chemical Information and Modeling (2007), 47(3), 998-1006. | Language: English, Database: CAPLUS
-

There is a pressing need for accurate in silico methods to predict the toxicity of mols. that are being introduced into the environment or are being developed into new pharmaceuticals. Predictive toxicol. is in the realm of structure activity relationships (SAR), and many approaches have been used to derive such SAR. Previous work has shown that inductive logic programming (ILP) is a powerful approach that circumvents several major difficulties, such as mol. superposition, faced by some other SAR methods. The ILP approach reasons with chem. substructures within a relational framework and yields chem. understandable rules. Here, we report a general new approach, support vector inductive logic programming (SVILP), which extends the essentially qual. ILP-based SAR to quant. modeling. First, ILP is used to learn rules, the predictions of which are then used within a novel kernel to derive a support-vector generalization model. For a highly heterogeneous dataset of 576 mols. with known fathead minnow fish toxicity, the cross-validated correlation coeffs. (R^2_{cv}) from a chem. descriptor method (CHEM) and SVILP are 0.52 and 0.66, resp. The ILP, CHEM, and SVILP approaches correctly predict 55, 58, and 73%, resp., of toxic mols. In a set of 165 unseen mols., the R^2 values from the com. software TOPKAT and SVILP are 0.26 and 0.57, resp. In all calcns., SVILP showed significant improvements in comparison with the other methods. The SVILP approach has a major advantage in that it uses ILP automatically and consistently to derive rules, mostly novel, describing fragments that are toxicity alerts. The SVILP is a general machine-learning approach and has the potential of tackling many problems relevant to chemoinformatics including in silico drug design.

Absorption, distribution, metabolism and excretion of intravenously and orally administered tetrabromobisphenol A [2,3-dibromopropyl ether] in male Fischer-344 rats

Quick ViewOther Sources

- By Knudsen, G. A.; Jacobs, L. M.; Kuester, R. K.; Sipes, I. G.
- From Toxicology (2007), 237(1-3), 158-167. | Language: English, Database: CAPLUS
- Tetrabromobisphenol A bis[2,3-dibromopropyl ether], 2,2-bis[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl]propane is a brominated flame retardant with substantial U.S. prodn. Due to the likelihood of human exposure to TBBPA-DBPE and its probable metabolites, studies regarding the absorption, distribution, metab., and excretion were conducted. Male Fischer-344 rats were dosed with TBBPA-DBPE (20 mg/kg) by oral gavage or i.v. administration. Following a single oral administration of TBBPA-DBPE, elimination of [^{14}C] equiv. in the feces was extensive and rapid (95% of dose by 36 h). Following repeated daily oral doses for 5 or 10 days, route and rate of elimination was similar to single administrations of TBBPA-DBPE. After i.v. administration, fecal excretion of [^{14}C] equiv. was much slower (27% of dose eliminated by 36 h, 71% by 96 h). Urinary elimination was minimal (<0.1%) following oral or i.v. administration. A single peak that co-eluted with the std. of TBBPA-DBPE was detected in exts. of whole blood following oral or IV administration. TBBPA-DBPE elimination from the blood was slow. Kinetic consts. following i.v. dosing were-$t_{1/2\beta}$: 24.8 h; CL_b: 0.1 mL min^{-1}. Kinetic consts. following oral dosing were: $t_{1/2\alpha}$: 2.5 h; $t_{1/2\beta}$: 13.9 h; CL_b: 4.6 mL min^{-1}. Systemic bioavailability was 2.2%. Liver was the major site of disposition following oral or IV administration. After oral administration, 1% of the dose was eliminated in bile in 24 h (as metabolites). In in vitro expts. utilizing hepatocytes or liver microsomal protein, no detectable metab. of TBBPA-DBPE occurred. These data indicate that TBBPA-DBPE is poorly absorbed from the gastrointestinal tract. Compd. which is absorbed is sequestered in the liver, slowly metabolized, and eliminated in the feces.

Identification of the Structural Requirements for Mutagenicity, by Incorporating Molecular Flexibility and Metabolic Activation of Chemicals. II. General Ames Mutagenicity Model. [Erratum to document cited in CA146:516278]

Quick ViewOther Sources

- By Serafimova, R.; Todorov, M.; Pavlov, T.; Kotov, S.; Jacob, E.; Aptula, A.; Mekenyan, O.

- From Chemical Research in Toxicology (2007), 20(8), 1225. | Language: English, Database: CAPLUS
- On page 673, in the conclusion section, the text, "As a comparative exercise, the alerts used in the present work were compared with three alert lists of Ashby, Kazius, and Benigni," should read: "As a comparative exercise, the alerts used in the present work were compared with alert lists of Ashby and Kazius, as well as the lists reported by Benigni in his review.".

Halogenated derivatives QSAR model using spectral moments to predict haloacetic acids (HAA) mutagenicity
Quick ViewOther Sources

- By Perez-Garrido, Alfonso; Gonzalez, Maykel Perez; Escudero, Amalio Garrido
- From Bioorganic & Medicinal Chemistry (2008), 16(10), 5720-5732. | Language: English, Database: CAPLUS
- The risk of the presence of haloacetic acids in drinking water as chlorination byproducts and the shortage of exptl. mutagenicity data for most of them requires a research work. This paper describes a QSAR model to predict direct mutagenicity for these chems. The model, able to describe more than 90% of the variance in the exptl. activity, was developed with the use of the spectral moment descriptors. The model, using these descriptors with multiplicative effects provides better results than other linear descriptors models based on Geometrical, RDF, WHIM, eigenvalue-based indexes, 2D-autocorrelation ones, and information descriptors, taking into account the statistical parameters of the model and the cross-validation results. The structural alerts and the mutagenicity-predicted values from the model output are in agreement with refs. from other authors. The mutagenicity predicted values for the three haloacetic acids, which have available exptl. data (TCAA-Trichloroacetic acid, BDCAA-Bromodichloroacetic acid, and TBAA-Tribromoacetic acid), are reasonably close to their exptl. values, specially for the latest two.

Summary of chemically induced pulmonary lesions in the National toxicology program (NTP) toxicology and carcinogenesis studies
Quick ViewOther Sources

- By Dixon, Darlene; Herbert, Ronald A.; Kissling, Grace E.; Brix, Amy E.; Miller, Rodney A.; Maronpot, Robert R.
- From Toxicologic Pathology (2008), 36(3), 428-439. | Language: English, Database: CAPLUS
- A review. The lung is the second most common target site of neoplasia of chems. tested by the National Toxicol. Program (NTP). Of all peer-reviewed NTP studies to date (N = 545), a total of sixty-four chems. in sixty-six reports produced significant site-specific neoplasia in the lungs of rats and/or mice. Of the studies assocd. with lung tumor induction, approx. 35% were inhalation and 35% were gavage studies, with dosed-feed, dosed-water, topical, i.p., or in utero routes of chem. administration accounting for 18%, 6%, 3%, 1%, and 1% of the studies, resp. The most commonly induced lung tumors were alveolar/bronchiolar (A/B) adenoma and/or carcinoma for both species. The most frequently obsd. nonneoplastic lesions included hyperplasia and inflammation in both species. The liver was the most common primary site of origin of metastatic lesions to the lungs of mice; however, skin was most often the primary site of origin of metastatic lesions to the lungs of rats. In summary, A/B adenoma and carcinoma were the most frequently diagnosed chem. induced tumors in the lungs of both rats and mice in the NTP toxicol. and carcinogenesis bioassays, and hyperplasia and inflammation were the most common nonneoplastic changes obsd.

Quantitative structure toxicity relationship (QSTR) study on a series of aliphatic alcohol derivatives
Quick ViewOther Sources

- By Singh, R. K.; Khan, A. K. R.
- From Organic Chemistry: An Indian Journal (2008), 4(2), 91-98. | Language: English, Database: CAPLUS

- The quant. structure toxicity relationship of 89 derivs. of alc. have been studied with the help of total energy, abs. hardness and electronegativity. The alcs. have been divided into four groups. The first group consists of derivs. of amino alc., second consists of derivs. of diol, the third and fourth resp. consist of derivs. of halogenated and unsatd. alcs. A direct relationship between the toxicity of all groups of alcs. and electronegativity has been obsd. The QSTR model of all the four sets have been developed. The best QSTR model of first and second set of compds. have correlation coeff. value above 0.94 and 0.7 resp., which has been derived by combination of all the three descriptors. The best QSTR model of third set and fourth set of compds. have correlation coeff. value above 0.86 and 0.65 resp., which has been derived by combination of descriptors consisting total energy, abs. hardness and electronegativity.

Quantum mechanical quantitative structure-activity relationships to avoid mutagenicity
Quick ViewOther Sources

- By Holder, Andrew J.; Ye, Lin
- From Dental Materials (2009), 25(1), 20-25. | Language: English, Database: CAPLUS

- Objective: The purpose of this work is to develop a quantum mech. based quant. structure-activity relationship (QMQSAR or QSAR hereafter) adequate to predict and explain Ames TA100-derived mutagenicities for a no. of org. mols. Methods: A set of 35 structurally similar mols. with epoxide (oxirane) functionalities and systematic, reliable exptl. data were selected to construct a QSAR model. The SAM1 quantum mech. method was used to perform conformational anal. and properties calcns. This QM information was used to compute a variety of descriptors. From this a two-descriptor regression model was constructed. Results: The two descriptors are ESP-HACA-1/TMSA and HOMO-LUMO energy gap. Statistical results for the model: $R^2 = 0.857$, R adj 2 = 0.818 , R cv 2 = 0.848 , $s^2 = 0.0618$. The variance inflation factor and significance for both descriptors were 1.082 and <0.001, resp. The descriptors are related to transport across a membrane and to reactivity. Significance: The model we have presented here facilitates design of non-mutagenic monomers that may be useful for dental restorative composites. The model also serves as a screening tool for rating the mutagenicity of new candidate materials.

Comparative QSTR study of a series of alcohol derivatives against Tetrahymena pyriformis
Quick ViewOther Sources

- By Singh, R. K.; Khan, A. K. R.; Sahu, V. K.; Singh, P. P.
- From International Journal of Quantum Chemistry (2008), Volume Date2009, 109(2), 185-195. | Language: English, Database: CAPLUS

- The quant. structure toxicity relationship (QSTR) models of 89 alc. derivs. have been made with the help of quantum chem. and topol. descriptors. The mol. modeling and geometry optimization have been carried out with CAChe pro software. The calcns. of quantum chem. descriptors have been done by MOPAC 2002 of topol. descriptors by Dragon software. The study indicates that quantum chem. descriptors better predict the toxicity of amino, halogenated and unsatd. alc. derivs., while toxicity of acetylenic, diols, and satd. alc. derivs. are better predicted by topol. descriptors as indicated by correlation coeff. and cross validation coeff. (rCV%2) values of the QSTR models. The predicted toxicity (PT) values obtained by these QSTR models are close to obsd. toxicity.

Comparative QSTR study of saturated alcohols based on topological, constitutional, geometrical, and getaway descriptors
Quick ViewOther Sources

- By Khan, A. K. R.; Sahu, V. K.; Singh, R. K.; Khan, S. A.
- From Medicinal Chemistry Research (2009), 18(9), 770-781. | Language: English, Database: CAPLUS

- Comparative quant. structure-toxicity relationship (QSTR) models of a series of satd. alcs. were constructed based on topol., constitutional, geometrical, and getaway descriptors. In the first step, all groups of descriptors were applied to 29 satd. alc. derivs. Mol. modeling and geometry optimization was carried out with CAChe Pro software. Calcn. of descriptors was done

by using Dragon software and multilinear regression anal. by using Project Leader software. Various QSTR models for each set of descriptor in different combinations were then developed, and only the top five models for each set of descriptors were chosen. Of the top five, the best QSTR model of each set of descriptors was used for comparative study. The study shows that the best QSTR model (^{c}RE5 = -0.0795186 M_w + 0.110997 AM_w + 23.0915 S_v - 4.62153 Se - 11.9932 Sp - 0.613194 Ss + 5.68544) is made from constitutional descriptors. The best model was selected on the basis of values of correlation coeff. (r^2 = 0.853) followed by other regression quality parameters such as cross-validation coeff. (r_{CV}^2 = 0.453), std. error (SE = 0.453) and std. error of estn. (SEE = 0.346), F-statistic (F = 25.57), and p values (p = 0.00) that were calcd. by statistical software. Secondly, it has been reported that there is a direct relationship between reported biol. toxicity of the alcs. and the sum of Kier-Hall electrotopol. states (Ss) descriptor of the constitutional group. Sum of Kier-Hall electrotopol. states (Ss) can alone be helpful for searching for alcs. of reliable toxicities before their synthesis.

Quantitative structure-toxicity models for heterogeneous aliphatic compounds
Quick ViewOther Sources

- By Duchowicz, Pablo R.; Ocsachoque, Marco A.
- From QSAR & Combinatorial Science (2009), 28(3), 281-295. | Language: English, Database: CAPLUS

- The presented work deals with the Quant. Structure - Activity Relationships (QSAR) anal. for the growth inhibition of the ciliated protozoan Tetrahymena pyriformis by a mechanistically diverse set of aliph. org. compds. A pool of 1509 theor. descriptors coding for lipophilic, constitutional, steric, and electronic properties of these mols. is calcd. with the Dragon software. The simultaneous linear regression analyses on 370 compds. led to a five-parameter model characterized by R = 0.909 and leave more out $R_{l\text{-}30\%\text{-}o}$ = 0.843. An external test set of 100 structurally related derivs. that is not employed during the model development demonstrates that the relationship found shows good predictive power, with R_{val} = 0.881. Finally, the application of this subset of descriptors on the complete set of 470 aliph. structures outperforms previous reported results. The authors' study corroborates that hydrophobicity, calcd. among more than a thousand of structural variables, represents an important factor for predicting the toxicity of aliph. compds.

Quantitative structure toxicity relationship (QSTR) study on a series of aliphatic alcohol derivatives with the help of topological descriptors
Quick ViewOther Sources

- By Kumar, Dinesh; Singh, R. N.; Khan, A. K. R.; Singh, P. P.
- From Organic Chemistry: An Indian Journal (2008), 4(5), 339-343. | Language: English, Database: CAPLUS

- In this present work, we have taken a series of aliph. alc. derivs. and developed QSTR models with the help of topol. descriptors. The values of topol. descriptors are directly obtained by Dragon software. The first set contains amino alc. derivs. and the correlation coeff. of this set is above 0.80. The second set contains acetylenic and diol derivs. The correlation coeff. of this set is above 0.83. The third and fourth set contains satd. and unsatd. alcs. resp. The correlation coeff. of these sets are above 0.80.

Molecular descriptors based comparative QSTR study of saturated alcohols derivatives
Quick ViewOther Sources

- By Singh, Satyenrdra; Khan, A. K. R.; Singh, R. K.
- From Organic Chemistry: An Indian Journal (2008), 4(6-8), 401-407. | Language: English, Database: CAPLUS

- In this present work, we have taken satd. alc. derivs. and comparative QSTR model have been made with the help of few important groups of descriptors like topol., constitutional descriptors, geometrical and getaway descriptors have been tested and to final QSTR model has been made with the help of the most significant descriptors. The values of these descriptors have been calcd. by Dragon software. Finally, we have made direct relationship between most

significant descriptors (S_e) and obsd. toxicity. The cross validation coeff. and correlation coeff. of best model are 0.453139 and 0.852744 resp.

A segmented principal component analysis-regression approach to quantitative structure-activity relationship modeling

Quick ViewOther Sources

- By Hemmateenejad, Bahram; Elyasi, Maryam
- From Analytica Chimica Acta (2009), 646(1-2), 30-38. | Language: English, Database: CAPLUS

- The major problem assocd. with application of principal component regression (PCR) in QSAR studies is that this model exts. the eigenvectors solely from the matrix of descriptors, which might not have essentially good relationship with the biol. activity. This article describes a novel segmentation approach to PCR (SPCAR), in which the descriptors are firstly segmented to different blocks and then principal component anal. (PCA) is applied on each segment to ext. significant principal components (PCs). In this way, the PCs having useful and redundant information are sepd. A linear regression anal. based on stepwise selection of variables is then employed to connect a relationship between the informative extd. PCs and biol. activity. The proposed method was first applied to model the aq. toxicity of aliph. compds. The effect of the no. of segments on the prediction ability of the method was investigated. Finally, a correlation anal. was achieved to identify those descriptors having significant contribution in the selected PCs and in aq. toxicity. The proposed method was further validated by the anal. of Selwood data set consisting of 31 compds. and 53 descriptors. A comparison between the conventional PCR algorithm and SPCAR reveals the superiority of the latter. For external prediction set, SPCAR represented all requirements to be considered as predicted model, whereas PCR did not. In addn., a comparison was made between the models obtained by SPCAR and those reported previously.

Prediction of chemical carcinogenicity by machine learning approaches

Quick ViewOther Sources

- By Tan, N. X.; Rao, H. B.; Li, Z. R.; Li, X. Y.
- From SAR and QSAR in Environmental Research (2009), 20(1-2), 27-75. | Language: English, Database: CAPLUS

- In this paper, the authors report a successful application of machine learning approaches to the prediction of chem. carcinogenicity. Two different approaches, namely, a support vector machine (SVM) and artificial neural network (ANN), were evaluated for predicting chem. carcinogenicity from mol. structure descriptors. A diverse set of 844 compds., including 600 carcinogenic (CG+) and 244 noncarcinogenic (CG-) mols., was used to est. the accuracies of these approaches. The database was divided into 2 sets: the model construction set and the independent test set. Relevant mol. descriptors were selected by a hybrid feature selection method combining Fischer's score and Monte Carlo simulated annealing from a wide set of mol. descriptors, including physiochem. properties, constitutional, topol., and geometrical descriptors. The first model validation method was based on a 5-fold cross-validation method, in which the model construction set is split into 5 subsets. The 5-fold cross-validation was used to select descriptors and optimize the model parameters by maximizing the averaged overall accuracy. The final SVM model gave an averaged prediction accuracy of 90.7% for CG+ compds., 81.6% for CG- compds., and 88.1% for the overall accuracy, while the corresponding ANN model provided an averaged prediction accuracy of 86.1% for CG+ compds., 79.3% for CG- compds., and 84.2% for the overall accuracy. These results indicate that the hybrid feature selection method is very efficient and the selected descriptors are truly relevant to the carcinogenicity of compds. Another model validation method, i.e., a hold-out method, was used to build the classification model using the selected descriptors and the optimized model parameters, in which the whole model construction set was used to build the classification model and the independent test set was used to test the predictive ability of the model. The SVM model gave a prediction accuracy of 87.6% for CG+ compds., 79.1% for CG- compds., and 85.0% for the overall accuracy. The ANN model gave a prediction accuracy of 85.6% for CG+ compds., 79.1% for CG-

compds., and 83.6% for the overall accuracy. The results indicate that the built models are potentially useful for facilitating the prediction of chem. carcinogenicity of untested compds.

Semivolatile Endocrine-Disrupting Compounds in Paired Indoor and Outdoor Air in Two Northern California Communities

Quick ViewOther Sources

- By Rudel, Ruthann A.; Dodson, Robin E.; Perovich, Laura J.; Morello-Frosch, Rachel; Camann, David E.; Zuniga, Michelle M.; Yau, Alice Y.; Just, Allan C.; Brody, Julia Green
- From Environmental Science & Technology (2010), 44(17), 6583-6590. | Language: English, Database: CAPLUS

- Interest in the health effects of potential endocrine-disrupting compds. which are high prodn. vol. chems. used in consumer products has made exposure assessment and source identification a priority. The authors collected paired indoor and outdoor air samples in 40 non-smoking homes in urban, industrial Richmond, California, and 10 in rural Bolinas, California. Samples were analyzed by gas chromatog.-mass spectrometry for 104 analytes, including phthalates (11), alkylphenols (3), parabens (3), polybrominated di-Ph ether (PBDE) flame retardants (3), polychlorinated biphenyls (PCB, 3), polycyclic arom. hydrocarbons (PAH, 24), pesticides (38), and phenolic compds. (19). In total, 39 analytes were detected in outdoor air and 63 in indoor air. For many phenolic, alkylphenol, phthalate, and PBDE compds., these represent some of the first outdoor measures and first analyses of the relative importance of indoor and outdoor sources in paired samples. Data demonstrated higher indoor concns. for 32 analytes, suggesting primarily indoor sources vs. only 2 which were higher outdoors. Outdoor air concns. were higher in Richmond than Bolinas for 3 phthalates, 10 PAH, and o-phenylphenol; indoor air concns. were more similar between communities, except differences obsd. outdoors were also obsd. indoors. Indoor concns. of the most ubiquitous chems. were generally correlated with each other (4-t-butylphenol, o-phenylphenol, nonylphenol, several phthalates, Me phenanthrenes; Kendall correlation coeffs. 0.2-0.6, $p < 0.05$), indicating possible shared sources and highlighting the importance of considering mixts. in health studies.

A comparative study of two quantum chemical descriptors in predicting toxicity of aliphatic compounds towards Tetrahymena pyriformis

Quick ViewOther Sources

- By Pandith, Altaf Hussain; Giri, S.; Chattaraj, P. K.
- From Organic Chemistry International (2010), 545087, 17 pp.. | Language: English, Database: CAPLUS

- Quantum chem. parameters such as LUMO energy, HOMO energy, ionization energy (I), electron affinity (A), chem. potential (μ), hardness (η) electronegativity (χ), philicity (ω°), and electrophilicity (ω) of a series of aliph. compds. are calcd. at the B3LYP/6-31G(d) level of theory. Quant. structure-activity relationship (QSAR) models are developed for predicting the toxicity ($pIGC_{50}$) of 13 classes of aliph. compds., including 171 electron acceptors and 81 electron donors, towards Tetrahymena pyriformis. The multiple linear regression modeling of toxicity of these compds. is performed by using the mol. descriptor log P (1-octanol/water partition coeff.) in conjunction with two other quantum chem. descriptors, electrophilicity (ω) and energy of the LUMO (E_{LUMO}). A comparison is made towards the toxicity predicting the ability of electrophilicity (ω) vs. E_{LUMO} as a global chem. reactivity descriptor in addn. to log P. The former works marginally better in most cases. There is a slight improvement in the quality of regression by changing the unit of IGC_{50} from mg/L to molarity and by removing the racemates and the diastereoisomers from the data set.

Hardness based quantitative structure toxicity relationship (QSTR) study on a series of aliphatic alcohol derivatives

Quick ViewOther Sources

- By Kumar, Dinesh; Singh, R. N.; Sahu, Sangeeta; Baboo, Vikas
- From Organic Chemistry: An Indian Journal (2011), 7(1), 41-47. | Language: English, Database: CAPLUS

- The quant. structure toxicity relationship of 89 derivs. of alc. have been studied with the help of total energy, abs. hardness and electronegativity. The alcs. have been divided into four groups. The first group consists of derivs. of amino alc., second consists of derivs. of diol, the third and fourth resp. consist of derivs. of halogenated and unsatd. alcs. A direct relationship between the toxicity of all groups of alcs. and electronegativity has been obsd. The QSTR model of all the four sets have been developed. The best QSTR model of first and second set of compds. have correlation coeff. value above 0.94 and 0.7 resp., which has been derived by combination of all the three descriptors. The best QSTR model of third set and fourth set of compds. have correlation coeff. value above 0.86 and 0.65 resp., which has been derived by combination of descriptors consisting total energy, abs. hardness and electronegativity. The abs. hardness is one of the most significant descriptor for searching the low toxicity of alcs.

The use of ex vivo human skin tissue for genotoxicity testing
Quick ViewOther Sources

- By Reus, Astrid A.; Usta, Mustafa; Krul, Cyrille A. M.
- From Toxicology and Applied Pharmacology (2012), 261(2), 154-163. | Language: English, Database: CAPLUS

- As a result of the chem. legislation concerning the registration, evaluation, authorization and restriction of chems. (REACH), and the Seventh Amendment to the Cosmetics Directive, which prohibits animal testing in Europe for cosmetics, alternative methods for safety evaluation of chems. are urgently needed. Current in vitro genotoxicity assays are not sufficiently predictive for the in vivo situation, resulting in an unacceptably high no. of misleading positives. For many chems. and ingredients of personal care products the skin is the first site of contact, but there are no in vitro genotoxicity assays available in the skin for addnl. evaluation of pos. or equivocal responses obsd. in regulatory in vitro genotoxicity assays. In the present study ex vivo human skin tissue obtained from surgery was used for genotoxicity evaluation of chems. by using the comet assay. Fresh ex vivo human skin tissue was cultured in an air-liq. interface and topically exposed to 20 chems., including true pos., misleading pos. and true neg. genotoxins. Based on the results obtained in the present study, the sensitivity, specificity and accuracy of the ex vivo skin comet assay to predict in vivo genotoxicity were 89%, 90% and 89%, resp. Donor and exptl. variability were mainly reflected in the magnitude of the response and not the difference between the presence and absence of a genotoxic response. The present study indicates that human skin obtained from surgery is a promising and robust model for safety evaluation of chems. that are in direct contact with the skin.

Gender differences in the incidence of background and chemically induced primary pulmonary neoplasms in B6C3F1 mice: A retrospective analysis of the National Toxicology Program (NTP) carcinogenicity bioassays
Quick ViewOther Sources

- By Moore, Nigel P.; McFadden, Lisa G.; Landenberger, Bryce D.; Thomas, Johnson
- From Experimental and Toxicologic Pathology (2013), 65(7-8), 1109-1115. | Language: English, Database: CAPLUS

- The National Toxicol. Program (NTP) database of tech. reports on carcinogenicity bioassays has been interrogated for the incidence of primary pulmonary neoplasms in B6C3F$_1$ mice. A total of 170 study reports were selected, from studies that completed the in-life phase during 1983-2007, which included neoplasm incidence data for 180 control groups comprising both male and female mice. The incidence (median and inter-quartile range) of males with alveolar/bronchiolar adenoma was 16% (12-20%), and for females it was 5% (2-8%); the incidence of males with alveolar/bronchiolar carcinoma was 8% (4-12%), and for females it was 2% (0-4%); and the incidence of males with combined alveolar/bronchiolar adenoma or carcinoma was 24% (18-30%), and for females it was 8% (6-12%). Comparing the incidence of animals bearing these lesions on a per study basis showed the median incidence in males to be 3.0-fold, 2.0-fold, and 2.8-fold higher than in females. The incidence of other primary pulmonary neoplasms was <10% of the alveolar/bronchiolar neoplasms. Comparison of gender-specific

response to lung tumorigens showed that the increase in incidence of tumors above control levels was greater in females than in males.

Framework for Identifying Chemicals with Structural Features Associated with the Potential to Act as Developmental or Reproductive Toxicants

Quick ViewOther Sources

- By Wu, Shengde; Fisher, Joan; Naciff, Jorge; Laufersweiler, Michael; Lester, Cathy; Daston, George; Blackburn, Karen
- From Chemical Research in Toxicology (2013), 26(12), 1840-1861. | Language: English, Database: CAPLUS
-

Developmental and reproductive toxicity (DART) end points are important hazard end points that need to be addressed in the risk assessment of chems. to det. whether or not they are the crit. effects in the overall risk assessment. These hazard end points are difficult to predict using current in silico tools because of the diversity of mechanisms of action that elicit DART effects and the potential for narrow windows of vulnerability. DART end points have been projected to consume the majority of animals used for compliance with REACH; thus, addnl. nonanimal predictive tools are urgently needed. This article presents an empirically based decision tree for detg. whether or not a chem. has receptor-binding properties and structural features that are consistent with chem. structures known to have toxicity for DART end points. The decision tree is based on a detailed review of 716 chems. (664 pos., 16 neg., and 36 with insufficient data) that have DART end-point data and are grouped into defined receptor binding and chem. domains. When tested against a group of chems. not included in the training set, the decision tree is shown to identify a high percentage of chems. with known DART effects. It is proposed that this decision tree could be used both as a component of a screening system to identify chems. of potential concern and as a component of wt.-of-evidence decisions based on structure-activity relationships (SAR) to fill data gaps without generating addnl. test data. In addn., the chem. groupings generated could be used as a starting point for the development of hypotheses for in vitro testing to elucidate mode of action and ultimately in the development of refined SAR principles for DART that incorporate mode of action (adverse outcome pathways).

Topological structural alerts modulations of mammalian cell mutagenicity for halogenated derivatives

Quick ViewOther Sources

- By Perez-Garrido, A.; Giron-Rodriguez, F.; Helguera, A. Morales; Borges, F.; Combes, R. D.
- From SAR and QSAR in Environmental Research (2014), 25(1), 17-33. | Language: English, Database: CAPLUS
- Genotoxicity is a key toxicity endpoint for current regulatory requirements regarding new and existing chems. However, genotoxicity testing is time-consuming and costly, and involves the use of lab. animals. This has motivated the development of computational approaches, designed to predict genotoxicity without the need to conduct lab. tests. Currently, many existing computational methods, like quant. structure-activity relationship (QSAR) models, provide limited information about the possible mechanisms involved in mutagenicity or predictions based on structural alerts (SAs) do not take statistical models into account. This paper describes an attempt to address this problem by using the TOPol. Substructural Mol. Design (TOPS-MODE) approach to develop and validate improved QSAR models for predicting the mutagenicity of a range of halogenated derivs. Our most predictive model has an accuracy of 94.12%, exhibits excellent cross-validation and external set statistics. A reasonable interpretation of the model in term of SAs was achieved by means of bond contributions to activity. The results obtained led to the following conclusions: primary halogenated derivs. are more mutagenic than secondary ones; and substitution of chlorine by bromine increases mutagenicity while polyhalogenation decreases activity. The paper demonstrates the potential of the TOPS-MODE approach in developing QSAR models for identifying structural alerts for mutagenicity, combining high predictivity with relevant mechanistic interpretation.

Methods for assigning confidence to toxicity data with multiple values - Identifying experimental outliers
Quick ViewOther Sources

- By Steinmetz, Fabian P.; Enoch, Steven J.; Madden, Judith C.; Nelms, Mark D.; Rodriguez-Sanchez, Neus; Rowe, Phil H.; Wen, Yang; Cronin, Mark T. D.
- From Science of the Total Environment (2014), 482-483, 358-365. | Language: English, Database: CAPLUS
- The assessment of data quality is a crucial element in many disciplines such as predictive toxicol. and risk assessment. Currently, the reliability of toxicity data is assessed on the basis of testing information alone (adherence to Good Lab. Practice (GLP), detailed testing protocols, etc.). Common practice is to take one toxicity data point per compd. - usually the one with the apparently highest reliability. All other toxicity data points (for the same expt. and compd.) from other sources are neglected. To show the benefits of incorporating the "less reliable" data, a simple, independent, statistical approach to assess data quality and reliability on a math. basis was developed. A large data set of toxicity values to Aliivibrio fischeri was assessed. The data set contained 1813 data points for 1227 different compds., including 203 identified as non-polar narcotic. Log K_{ow} values were calcd. and non-polar narcosis quant. structure-activity relationship (QSAR) models were built. A statistical approach to data quality assessment, which is based on data outlier omission and confidence scoring, improved the linear QSARs. The results indicate that a beneficial method for using large data sets contg. multiple data values per compd. and highly variable study data has been developed. Furthermore this statistical approach can help to develop novel QSARs and support risk assessment by obtaining more reliable values for biol. endpoints.

	Literature Search Results
Search Term	1522-92-5
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	5
Date	July 23 2015
Comments	

Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals
Quick ViewOther Sources

- By Mortelmans, Kristien; Haworth, Steve; Lawlor, Timothy; Speck, William; Tainer, Beth; Zeiger, Errol
- From Environmental Mutagenesis (1986), 8(Suppl. 7), 1-119. | Language: English, Database: CAPLUS
- This publication includes data of Salmonella mutagenicity results on 270 coded chems., encompassing 329 tests performed by 3 labs. under contract to the National Toxicol. Program. The preincubation modification of the Salmonella/mammalian microsome assay was used to test chems. in up to 5 Salmonella strains in the presence and absence of rat and hamster liver S-9. With a few exceptions, inter- and intralab. reproducibility was good.

Structure-mutagenic activity relationships in the Salmonella typhimurium TA100 strain in a series of short-chain halogenated hydrocarbons and alcohols.
Quick ViewOther Sources

- By Kharchevnikova, N. V.; Zholdakova, Z. I.; Zhurkov, V. S.; Polyakova, E. E.; Novikov, S. M.
- From Russian Journal of Genetics (Translation of Genetika (Moscow)) (1997), 33(5), 594-597. | Language: English, Database: CAPLUS

- Structure-mutagenic activity relationships in a series of short-chain halogenated hydrocarbons and alcs. were detd. with the use of the energy difference parameter of frontier mol. orbitales of compds. that was calcd. by the quantum-chem. method.

Identification of the Structural Requirements for Mutagenicity, by Incorporating Molecular Flexibility and Metabolic Activation of Chemicals. II. General Ames Mutagenicity Model. [Erratum to document cited in CA146:516278]

- By Serafimova, R.; Todorov, M.; Pavlov, T.; Kotov, S.; Jacob, E.; Aptula, A.; Mekenyan, O.
- From Chemical Research in Toxicology (2007), 20(8), 1225. | Language: English, Database: CAPLUS

- On page 673, in the conclusion section, the text, "As a comparative exercise, the alerts used in the present work were compared with three alert lists of Ashby, Kazius, and Benigni," should read: "As a comparative exercise, the alerts used in the present work were compared with alert lists of Ashby and Kazius, as well as the lists reported by Benigni in his review.".

Identification of the Structural Requirements for Mutagenicity, by Incorporating Molecular Flexibility and Metabolic Activation of Chemicals. II. General Ames Mutagenicity Model

- By Serafimova, R.; Todorov, M.; Pavlov, T.; Kotov, S.; Jacob, E.; Aptula, A.; Mekenyan, O.
- From Chemical Research in Toxicology (2007), 20(4), 662-676. | Language: English, Database: CAPLUS

The tissue metabolic simulator (TIMES) modeling approach is a hybrid expert system that couples a metabolic simulator together with structure toxicity rules, underpinned by structural alerts, to predict interaction of chems. or their metabolites with target macromols. Some of the structural alerts representing the reactivity pattern-causing effect could interact directly with the target whereas others necessitated a combination with two- or three-dimensional quant. structure-activity relationship models describing the firing of the alerts from the rest of the mols. Recently, TIMES has been used to model bacterial mutagenicity (O. Mekenyan, O., et al.,2004). The original model was derived for a single tester strain, Salmonella typhimurium (TA100), using the Ames test by the National Toxicol. Program (NTP). The model correctly identified 82% of the primary acting mutagens, 94% of the nonmutagens, and 77% of the metabolically activated chems. in a training set. The identified high correlation between activities across different strains changed the initial strategic direction to look at the other strains in the next modeling developments. In this respect, the focus of the present work was to build a general mutagenicity model predicting mutagenicity with respect to any of the Ames tester strains. The use of all reactivity alerts in the model was justified by their interaction mechanisms with DNA, found in the literature. The alerts identified for the current model were analyzed by comparison with other established alerts derived from human experts. In the new model, the original NTP training set with 1341 structures was expanded by 1626 proprietary chems. provided by BASF AG. Eventually, the training set consisted of 435 chems., which are mutagenic as parents, 397 chems. that are mutagenic after S9 metabolic activation, and 2012 nonmutagenic chems. The general mutagenicity model was found to have 82% sensitivity, 89% specificity, and 88% concordance for training set chems. The model applicability domain was introduced accounting for similarity (structural, mechanistic, etc.) between predicted chems. and training set chems. for which the model performs correctly.

Scientific opinion on emerging and novel brominated flame retardants (BFRs) in food

- By Benford, Diane; Ceccatelli, Sandra; Cottrill, Bruce; DiNovi, Michael; Dogliotti, Eugenia; Edler, Lutz; Farmer, Peter; Furst, Peter; Hoogenboom, Laurentius; Knutsen, Helle Katrine; et al
- From EFSA Journal (2012), 10(10), 2908, 125 pp.. | Language: English, Database: CAPLUS

- EFSA was asked to deliver a scientific opinion on brominated flame retardants (BFRs) other than PBDEs, PBBs, HBCDDs, TBBPA and brominated phenols and their derivs. The BFRs that are the subject of the current opinion, were classified in groups termed 'emerging' and 'novel' BFRs. Information on 17 emerging and 10 novel BFRs was collected. The information varied widely for these BFRs. There is a lack of exptl. data on physico-chem. characteristics, stability/reactivity and current use and prodn. vol. of all the emerging and novel BFRs. Due to the very limited information on occurrence, exposure and toxicity, the CONTAM Panel could not perform a risk characterization for any of the BFRs considered. Instead, an attempt was made to identify those BFRs that could be a potential health concern and should be considered first for future investigations. For this purpose the Panel first evaluated the available exptl. data on occurrence in food, behavior in the environment and toxicity. Secondly, a modeling exercise was performed focussing on the potential of the emerging and novel BFRs for persistence in the environment and for their possible bioaccumulation potential. There is convincing evidence that tris(2,3-dibromopropyl) phosphate (TDBPP) and dibromoneopentyl glycol (DBNPG) are genotoxic and carcinogenic, warranting further surveillance of their occurrence in the environment and in food. Based on the limited exptl. data on environmental behavior, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and hexabromobenzene (HBB) were identified as compds. that could raise a concern for bioaccumulation. For the modeling exercise, the CONTAM Panel selected two environmental characteristics, overall persistence and potential for bioaccumulation, as being most relevant to provide insight into the possibility that emerging or novel BFRs might accumulate in the food chain, and thus might appear in food intended for human consumption. The modeling exercise identified ten addnl. BFRs that should be subjected to further in-depth studies.

	Literature Search Results
Search Term	100606-66-4
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	
Date	July 23 2015
Comments	

Hydroxylation of pentamidine by rat liver microsomes

Quick ViewOther Sources

- By Berger, B. J.; Reddy, V. V.; Le, S. T.; Lombardy, R. J.; Hall, James Edwin; Tidwell, R. R.
- From Journal of Pharmacology and Experimental Therapeutics (1991), 256(3), 883-9. | Language: English, Database: CAPLUS

- The antiprotozoal/antifungal drug pentamidine is metabolized by rat liver fractions to at least 6 metabolites detectable by HPLC. Two minor metabolites have been identified as N-hydroxypentamidine and N,N'-dihydroxypentamidine. The two major microsomal metabolites have been identified as 1,5-di(4-amidinophenoxy)-2-pentanol and 1,5-bis(4-amidinophenoxy)-3-pentanol. A seventh putative metabolite has been identified as p-hydroxybenzamidine, a fragment of the original drug. Whereas the cytochromes P 450 enzyme system is responsible for pentamidine metab., hydroxylation of the drug was not inducible by phenobarbital, β-naphthoflavone, clofibrate, isosafrole, pregnenolone-16α-carbonitrile, ethanol, or pentamidine pretreatment of rats. The kinetics of the prodn. of the two major microsomal metabolites has K_m = 56 μM and V_{max} = 126 pmol/min/mg microsomal protein for the 2-pentanol analog, and K_m = 28 μM and V_{max} = 195 pmol/min/mg microsomal protein for the 3-pentanol analog. Therefore, the mixed-function oxidases readily convert pentamidine to hydroxylated metabolites, but that which isoenzyme(s) of cytochrome P 450 is responsible is not clear.

	Literature Search Results

Search Term	79033-40-2
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	1
Date	July 22 2015
Comments	

Synthesis and mutagenicity of selectively methylated analogs of tris(2,3-dibromopropyl) phosphate and 1,2-dibromo-3-chloropropane
Quick ViewOther Sources

- By Omichinski, James G.; Soederlund, Erik J.; Bausano, James A.; Dybing, Erik; Nelson, Sidney D.
- From Mutagenesis (1987), 2(4), 287-92. | Language: English, Database: CAPLUS

- Five selectively methylated analogs of the flame retardant tris(2,3-dibromopropyl) phosphate (Tris-BP) and of the nematocide, 1,2-dibromo-3-chloropropane (DBCP), were synthesized and their relative mutagenicities detd. in Salmonella typhimurium TA 100 in the presence of rat liver microsomes. In all cases, ethylation decreased mutagenicity relative to the parent compd., but the relative degree of reduced mutagenicity varied considerably depending on the position of the Me substitution. The mutagenicity studies with the selectively methylated analogs and with suspected mutagenic metabolites (2-bromocrotonaldehyde and Me 1-dibromovinyl ketone) supported earlier work with selectively deuterated analogs of Tris-BP and DBCP. Initial oxidn. at C3, followed by spontaneous dehydrohalogenation and dehydrophosphorylation, was the major route of formation of mutagenic metabolites from Tris BP. In the case of DBCP, formation of mutagenic metabolites can result following initial oxidn. at either C1 or C3.

	Literature Search Results
Search Term	19398-47-1
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	2
Date	July 22 2015
Comments	

Haloalcohols deplete glutathione when incubated with fortified liver fractions
Quick ViewOther Sources

- By Garle, M. J.; Sinclair, C.; Thurley, P.; Fry, J. R.
- From Xenobiotica (1999), 29(5), 533-545. | Language: English, Database: CAPLUS

- This study has examd. the ability of dichloropropanols, haloalcs. and their putative metabolites to deplete glutathione when incubated with liver fractions obtained from untreated and differentially induced rats. 1,3-Dichloropropan-2-ol and 2,3-dichloropropan-1-ol (0-1000 μM) both depleted glutathione in a dose-dependent manner when incubated with cofactors (NADPH generating system) and liver microsomes from the untreated rat. The extent of GSH depletion was significantly enhanced when liver microsomes from the isoniazid- or isosafrole-treated rat were used. Epichlorohydrin produced a moderate, dose-dependent depletion of GSH. By contrast, 1,3-dichloroacetone (identified by TLC as a metabolite of 1,3-dichloropropanol) was a potent depletor of glutathione. N-acetylcysteine was less efficient than glutathione as a nucleophile trap for epichlorohydrin, 1,3-dichloroacetone or reactive metabolites derived from 1, 3-dichloropropan-2-ol. 1,3-Dibromopropan-2-ol and 1,4-dibromobutan-2-ol were potent depletors of GSH but 1-bromopropan-2-ol produced less GSH depletion. Both dibromoalcs. depleted GSH when incubated with dialyzed cytosol derived from the livers of untreated rats. The GSH

depletion mediated by 1,3-dichloropropan-2-ol, 1,3-dibromopropan-2-ol, 1,4-dibromobutan-2-ol and 1-bromopropan-2-ol was inhibited by inclusion of pyridine (1 mM) or cofactor omission. 1,3-Difluoropropanol did not deplete GSH under any of the conditions examd.

The Nature of Halogen Substitution Determines the Mode of Cytotoxicity of Halopropanols
Quick ViewOther Sources

- By Hammond, Alison H.; Garle, Michael J.; Fry, Jeffrey R.
- From Toxicology and Applied Pharmacology (1999), 155(3), 287-291. | Language: English, Database: CAPLUS

- The cytochrome P 450-dependent generation of reactive metabolites from 1,3-dichloropropanol and 1,3-dibromopropanol was assessed in a microsomal thiol depletion assay, while the toxicity of these compds. was assessed in rat hepatocyte cultures and in the 3T3 cell line. Thiol-depleting metabolites of both compds. were generated in the microsomal assay; however, only dibromopropanol extensively depleted glutathione when glutathione S-transferase was used as the enzyme source. The cytotoxicity of dichloropropanol was both cytochrome P 450- and glutathione-dependent, whereas that of dibromopropanol was glutathione-dependent but largely independent of cytochrome P 450. These results indicate that the mechanisms underlying the cytotoxicity of halopropanols are dependent on the nature of the halogen substitution and that microsomal and cellular assays for reactive metabolite generation may yield conflicting results. (c) 1999 Academic Press.

	Literature Search Results
Search Term	15410-44-3
Database	SciFinder
Limitation(s)	
Relevant Papers	1
Date	July 22 2015
Comments	

In Vitro Metabolism of Chloroprene: Species Differences, Epoxide Stereochemistry and a De-chlorination Pathway
Quick ViewOther Sources

- By Cottrell, Lisa; Golding, Bernard T.; Munter, Tony; Watson, William P.
- From Chemical Research in Toxicology (2001), 14(11), 1552-1562. | Language: English, Database: CAPLUS

- Chloroprene was metabolized by liver microsomes from Sprague-Dawley rats, Fischer 344 rats, B6C3F1 mice, and humans to the monoepoxides, (1-chloro-ethenyl)oxirane (I; a/b), and 2-chloro-2-ethenyloxirane (II; a/b). The formation of II a/b was inferred from the identification of their degrdn. products. With male Sprague-Dawley and Fischer 344 rat liver microsomes, there was a ~3:2 preference for the formation of (R)-(1-chloroethenyl)oxirane (Ia) compared to the (S)-enantiomer (Ib). A smaller but distinct enantioselectivity in the formation of (S)-(1-chloro-ethenyl)oxirane occurred with liver microsomes from male mouse (R:S, 0.90:1) or male human (R:S, 0.86:1). 2-Chloro-2-ethenyloxirane was very unstable in the presence of the microsomal mixt. and was rapidly converted to 1-hydroxybut-3-en-2-one and 1-chlorobut-3-en-2-one. An addnl. rearrangement pathway of 2-chloro-2-ethenyloxirane gave rise to 2-chlorobut-3-en-1-al and 2-chlorobut-2-en-1-al. Further reductive metab. of these metabolites occurred to form 1-hydroxybutan-2-one and 1-chlorobutan-2-one. In the absence of an epoxide hydrolase inhibitor, the microsomal incubations converted (1-chloroethenyl)oxirane to 3-chlorobut-3-ene-1, 2-diol. When microsomal incubations were supplemented with glutathione, 1-hydroxybut-3-en-2-one was not detected because of its rapid conjugation with this thiol scavenger.

	Literature Search Results
Search Term	299-70-7

Database	SciFinder
Limitation(s)	NOT 14396-65-7 AND Exclude Patents
Relevant Papers	2
Date	July 22 2015
Comments	

Biological action of D,L-1,4-dibromo-2,3-butanediol
Quick ViewOther Sources

- By Myuller, N. R.; Remizov, A. L.; Belogorodskii, V. V.; Filov, V. A.
- From Farmakologiya i Toksikologiya (Moscow) (1975), 38(5), 590-1. | Language: Russian, Database: CAPLUS
- DL-1,4-dibromo-2,3-butanediol (I) [299-70-7] given i.p. to mice (100-120 mg/kg/day) and to rats (100-140 mg/kg/day) with transplantable tumors showed significant antitumor activity against ascitic Ehrlich, sarcoma 37, and lymphoma NK/Ly tumors, solid sarcoma 180, Walker carcinosarcoma, and sarcoma 45; it was ineffective against solid sarcoma 37. Chronic administration of I to normal or tumor-bearing animals did not produce leukopenia, but instead increased blood leukocyte levels.

Enhancement of Bacterial Mutagenicity of Bifunctional Alkylating Agents by Expression of Mammalian Glutathione S-Transferase
Quick ViewOther Sources

- By Thier, Ricarda; Muller, Michael; Taylor, John B.; Pemble, Sally E.; Ketterer, Brian; Guengerich, F. Peter
- From Chemical Research in Toxicology (1995), 8(3), 465-72. | Language: English, Database: CAPLUS
- Recently, we inserted the plasmid vector pKK233-2 contg. rat GSH S-transferase (GST) 5-5 cDNA into Salmonella typhimurium TA1535 and found that these bacteria [GST 5-5(+)] expressed the protein and produced mutations when ethylene or methylene dihalides were added [Thier, R., Taylor, J. B., Pemble, S. E., Ketterer, B., Persmark, M., Humphreys, W. G., and Guengerich, F. P. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 8576-8580]. After exposure to the known GST 5-5 substrate 1,2-epoxy-3-(4'-nitrophenoxy)propane, the GST 5-5(+) strain showed fewer mutants than the bacteria transfected with the cDNA clone in a reverse orientation [GST 5-5(-)], suggesting a protective role of GST 5-5. However, mutations were considerably enhanced in the GST 5-5(+) strain [as compared to GST 5-5(-)] when 1,2,3,4-diepoxybutane (butadiene diepoxide) or 1,2-epoxy-4-bromobutane was added. The GST 5-5(+) and GST 5-5(-) bacterial stains showed similar responses to 1,2-epoxypropane, 3,4-epoxy-1-butene, and 1,4-dibromobutane. The results suggest that some bifunctional activated butanes are transformed to mutagenic products through GSH conjugation. We also found that the GST 5-5(+) strain showed enhanced mutagenicity with 1,4-dibromo-2,3-epoxybutane, 1,2-epoxy-3-bromopropane (epibromohydrin), and (±)-1,4-dibromo-2,3-dihydroxybutane. The possibility was considered that a 5-membered thialonium ion may be involved in the mutagenicity. Model thialonium compds. were rather stable to hydrolysis in aq. soln. at pH 7.4 and slowly alkylated 4-(4-nitrobenzyl) pyridine. The presence of a hydroxyl group β to the sulfur did not enhance reactivity. Mechanisms involving episulfonium ions are considered more likely. Potential oxidn. products of the toxic pesticide 1,2-dibromo-3-chloropropane (DBCP) were also considered in this system. DBCP itself gave rather similar results in the two strains. Others have reported that oxidn. of DBCP is required for mutagenicity, along with GST-catalyzed GSH conjugation [Simula, T. P., Glancey, M. J., Soederlund, E. J., Dybing, E., and Wolf, C. R. (1993) Carcinogenesis 14, 2303-2307]. The putative oxidn. product 1,2-dibromopropional did not show a difference between the two strains. However, 1,3-dichloroacetone, a model for the putative oxidn. product 1-bromo-3-chloroacetone, was considerably more mutagenic in the GST 5-5(+) strain.

	Literature Search Results

Search Term	14396-65-7
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	1-ish
Date	July 22 2015
Comments	

Activation and Inactivation of Carcinogenic Dihaloalkanes and Other Compounds by Glutathione S-Transferase 5-5 in Salmonella typhimurium Tester Strain NM5004

Quick ViewOther Sources

- By Shimada, Tsutomu; Yamazaki, Hiroshi; Oda, Yoshimitsu; Hiratsuka, Akira; Watabe, Tadashi; Guengerich, F. Peter
- From Chemical Research in Toxicology (1996), 9(1), 333-40. | Language: English, Database: CAPLUS
- A newly developed tester Salmonella typhimurium NM5004 strain was constructed by introducing a plasmid contg. both rat GSH S-transferase (GST) 5-5 cDNA and the umuC''lacZ operon into the host strain Salmonella typhimurium TA1535 and used to examine whether or not GST modified the genotoxic activities of several dihaloalkanes and other compds. Twenty-nine chems. that were suggested to be conjugated by GST were compared with regard to their abilities to induce umu gene expression and cause cytotoxicity responses in both the NM5004 strain and the original tester strain (S. typhimurium TA1535/pSK1002, which is devoid of GST activity toward 1,2-epoxy-3-(4'-nitrophenoxy)propane). Ten chems.-1,2-dibromoethane, N-(2,3-epoxypropyl)phthalimide, 1,3-dichloroacetone, CH_2I_2, 1,2-epoxy-3-phenoxypropane, 2,3-epoxypropyl p-methoxyphenyl ether, 1-bromo-2-chloroethane, 1-bromo-2,3-dichloropropane, CH_2BrCl, and CH_2Br_2-were found to enhance induction of umu gene expression in the NM5004 strain as compared with the TA1535/pSK1002 strain. 1,2-Epoxy-3-(4'-nitrophenoxy)propane and 2,3-dibromo-1-chloropropane were inactivated by GST 5-5 in the NM5004 tester strain, although these chems. were cytotoxic in both tester strains. Roles of GST 5-5 were also examd. for the inactivation of reactive metabolites of several procarcinogens that were formed through oxidn. by liver microsomes of polychlorinated biphenyl-treated rats. The results suggest that reactive metabolites (possibly epoxides)oke of aflatoxin B_1, sterigmatocystin, 1,2-dihydro-1,2-dihydroxy-6-aminochrysene, and (+)- and (-)-enantiomers of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene could be trapped as inactivated GSH conjugates in the NM5004 strain. High-performance liq. chromatog. anal. suggested that exo-aflatoxin B_1 8,9-oxide-GSH conjugate was formed during the oxidn. of aflatoxin B_1 by rat and human liver microsomes in the presence of GSH and several GST enzymes including purified rat theta class GST Y_{rs}-Y_{rs} and rat liver GST (a mixt. of alpha and mu class enzymes). Thus, the present results support the view that the theta class rat GST 5-5 enzyme participates in the activation and inactivation of potential environmental carcinogenic chems. This newly developed NM5004 tester strain is of use in the elucidation of roles of GST 5-5 in transformations.

	Literature Search Results
Search Term	96-21-9
Database	SciFinder
Limitation(s)	Exclude Patents
Relevant Papers	
Date	July 21 2015
Comments	

Influence of the chemical structure on the biological tendency of cytostatic compounds related to dibromomannitol. I. Structure-activity correlations

Quick ViewOther Sources

- By Institoris, Laszlo; Horvath, Irene P.; Csanyi, Endre

- From Arzneimittel-Forschung (1967), 17(2), 145-9. | Language: English, Database: CAPLUS

- The relation between cytostatic activity and chem. structure was investigated in a series of α,ω-substituted alkanes and polyalcs. The tested compds. were injected into rats and mice i.p. The inhibitory effect was expressed as the percentage inhibition of the tumor growth related to untreated groups. Among the polyalcs., bromo derivs. such as 1,6-dibromomannitol (DBM) and 1,6-dibromodulcitol (DBD) had strong cytostatic activity, while dichloromannitol (DCM) and diiodomannitol (DIM) were less effective than DBM and DBD. In the alkanes, dimethyloxyesters such as 1,4-dimethylsulfonyloxybutane were highly inhibitory to tumor growth, whereas dibromoalkanes were inactive. Thus, the structural conditions of cytostatic activity in the Br and methylsulfonyloxy series were not similar.

The mutagenicity of halogenated alkanols and their phosphoric acid esters for Salmonella typhimurium

Quick ViewOther Sources

- By Nakamura, Akitada; Tateno, Noriyuki; Kojima, Shigeo; Kaniwa, Masaaki; Kawamura, Taro
- From Mutation Research, Genetic Toxicology Testing (1979), 66(4), 373-80. | Language: English, Database: CAPLUS

- Nine halogenated alkanols, 9 corresponding tris(haloalkyl)phosphates, and 2 bis-(2,3-dibromopropyl)phosphate salts were evaluated for mutagenicity against S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538, with and without rat liver in vitro metabolic activation system (S9 mix). Most of the test samples showed mutagenic activity in the strains TA100 and TA1535, but not in the strains TA98, TA1537, and TA1538. In general, the mutagenic activities of the phosphates obtained with S9 mix were greater than the activities obtained without S9 mix. Among the phosphates, several structure-activity relations were found; i.e., the bromoalkyl derivs. were more mutagenic than the corresponding chloroalkyl derivs., the β-haloethyl derivs. were more mutagenic than the γ-halopropyl derivs., the phosphates having adjacent β and γ halogen atoms in the alkyl moiety, e.g., tris-(2,3-dibromopropyl)phosphate (I) [126-72-7], were particularly potent mutagens, the branched C chain reduced the mutagenic activities in spite of the presence of β-halogen atoms, e.g., tris(1-bromomethyl-2-bromoethyl)phosphate [18713-51-4]. However, such relations did not necessarily apply to the halogenated alkanols. Apparently, the metabolic activation pathway via haloalkanols to mutagens must not be in common with all of I-like phosphates.

Epoxides as obligatory intermediates in the metabolism of α-halohydrins

Quick ViewOther Sources

- By Jones, A. R.; Fakhouri, G.
- From Xenobiotica (1979), 9(10), 595-9. | Language: English, Database: CAPLUS

- Metab. of 1,3-dibromopropan-2-ol [**96-21-9**], 1,3-dichloropropan-2-ol [96-23-1], 1-bromo-3-chloropropan-2-ol [4540-44-7], 1,2-dibromopropan-3-ol [96-13-9], and 2 halohydrins by rats (50 mg/kg orally, daily for 5 days) resulted in the same 2 mercapturic acid metabolites in urine. These were N,N'-bis(acetyl)-S,S'-[1,3-bis(cysteinyl)]propan-2-ol [71038-56-7] and N-acetyl-S-(2,3-dihydroxypropyl)cysteine [23255-33-6]. Depending on the halogen present, each dihalopropanol produced β-chlorolactate [1713-85-5] or β-bromolactate [32777-03-0] as oxidative metabolites. An epoxide is probably an intermediate of the metabolic path, and this was confirmed by the metab. of 2-chloropropane-1,3-diol [96-24-2] which produced one mercapturic acid, N-acetyl-S-(2,3-dihydroxypropyl)cysteine.

Mutagenicity of 2- and 3-carbon halogenated compounds in the Salmonella/mammalian-microsome test

Quick ViewOther Sources

- By Stolzenberg, S. J.; Hine, C. H.
- From Environmental Mutagenesis (1980), 2(1), 59-66. | Language: English, Database: CAPLUS

- Short-chain, 2- and 3-carbon halogenated hydrocarbons were tested for mutagenicity for Salmonella typhimurium strain TA 100 both with and without the presence of S-9. Without

exception, all brominated derivs. were more mutagenic than the chlorinated derivs., usually by a substantial order of magnitude. 2-Fluoroethanol [371-62-0] showed little or no mutagenic activity up to 100 μmol/plate. Trihalogenated compds. with a halogen atom on each of the 3 carbon atoms required metabolic activation with S-9 for full expression of mutagenic activity. The presence of a double bond in the case of 1,2,3-trichloropropene [96-19-5] resulted in a higher level of direct mutagenic activity than 1,2,3-trichloropropane [96-18-4], but activation with S-9 resulted in a further increase in mutagenic activity with the former compd. On the other hand, S-9 caused a substantial decrease in mutagenic activity of most compds. contg. a double bond. With the presence of an alc. group in a compd., the addn. of S-9 caused variable responses, increasing the no. of his^+ revertant colonies due to 2,3-dibromopropanol [96-13-9] but had little or no effect with other compds. contg. an alc. group. Evidence is also presented that the position of a double bond in relation to the halogen atoms may influence mutagenic activity.

DNA synthesis inhibition in mammalian cells as a test for mutagenic carcinogens
Quick ViewOther Sources

- By Painter, Robert B.
- Edited by Stich, Hans F.; San, R. H. C
- From Short-Term Tests Chem. Carcinog. (1981), 59-64. | Language: English, Database: CAPLUS

- Of 30 chems. including ascorbic acid (I) [50-81-7] tested in the mammalian cell DNA synthesis inhibition test, 23 were pos. The EDs and whether or not they require S9 activation are given. The principle of the method and its usefulness are discussed.

The HeLa DNA-synthesis inhibition test as a rapid screen for mutagenic carcinogens
Quick ViewOther Sources

- By Painter, Robert B.; Howard, Ricci
- From Mutation Research (1982), 92(1-2), 427-37. | Language: English, Database: CAPLUS

- Ninety agents were tested in the HeLa DNA-synthesis inhibition test. This test detected strong mutagens and carcinogens except when difficulties with metabolic activation were encountered. Weaker DNA-damaging agents were generally pos. in the test but required relatively high concns. (≥1 mM). Very weak agents, such as saccharin [81-07-2], were not pos. Only 1 false pos. was encountered and the dose response for it (cytochalasin B [14930-96-2]) was atypical. The test was not suitable for complex mixts., probably because ingredients that did not damage DNA but inhibited DNA synthesis by another mechanism could mask the action of a DNA-damaging agent. Inhibition of DNA synthesis in HeLa and other mammalian cells is best used as a rapid and inexpensive screening procedure to detect relatively strong mutagenic carcinogens.

Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro
Quick ViewOther Sources

- By Perocco, Paolo; Bolognesi, Silvana; Alberghini, William
- From Toxicology Letters (1983), 16(1-2), 69-75. | Language: English, Database: CAPLUS

- Of 17 compds. examd. Me Et ketone, $CHCl_3$, and 1,2-dichloropropane were not toxic in human lymphocyte cultures (as measured by cell viability and [^{3}H]thymidine uptake). chloromethyl Me ether [107-30-2], n-hexane [110-54-3], 1,2-dibromopropane [78-75-1], 1,3-dibromopropane [109-64-8], Chloromethyl Me ether, n-hexane, 1,2-dibromopropane, 1,3-dibromopropane, 1,3-dibromopropanol, 1,2-diiodoethane, tetrachloroethylene, and 1,4-dichlorobenzene (I) were toxic in the absence of an S9 mix. The cytotoxicity of these compds. disappeared in the presence of the metabolizing system. The cytotoxic effects of 1,2-dichlorobenzene and 1,3-dichlorobenzene were not completely inhibited by the S9 mix. EtOAc, benzene, cyclohexane, and cyclohexanone inhibited [^{3}H]thymidine uptake in the absence of the S9 mix, but had no effect on cell viability. Apparently these chems. exercise a cytotoxic action which is not immediately followed by cellular death. Chloromethyl Me ether elevated [^{3}H] thymidine uptake in the presence of the S9 mix.

Effect of bromine and chlorine positioning in the induction of renal and testicular toxicity by halogenated propanes

Quick ViewOther Sources

- By Laag, Marit; Soederlund, Erik J.; Omichinski, James G.; Brunborg, Gunnar; Holme, Joern A.; Dahl, Jon E.; Nelson, Sidney D.; Dybing, Erik
- From Chemical Research in Toxicology (1991), 4(5), 528-34. | Language: English, Database: CAPLUS
- A series of halogenated propanes were studied for renal and testicular necrogenic effects in the rat and correlated to their ability to induce in vivo renal and testicular DNA damage and in vitro testicular DNA damage. 1,2-Dibromo-3-chloropropane (DBCP) and 1,2,3-tribromopropane were most potent in causing organ damage in both kidney and testes. Extensive necrosis was evident at 85 μmol/kg in kidney and at 170 μmol/kg in testis. The dibromomonochlorinated analog 1,3-dibromo-2-chloropropane was less organ toxic than DBCP and 1,2,3-tribromopropane but induced more organ damage than the dichloromonobrominated analogs 1-bromo-2,3-dichloropropane and 1,3-dichloro-2-bromopropane. Dihalogenated propanes were even less necrogenic. These obsd. differences in toxic potency between the halogenated propanes could not be explained by relative differences in tissue concns. The ability of the halogenated propanes to induce DNA damage in vivo correlated well with their ability to induce organ damage. However, DNA damage occurred at lower doses and at a shorter period of exposure than organ necrosis. This indicates that DNA damage might be an initial event in the development of organ necrosis by halogenated propanes in general. Further, testicular DNA damage induced by the halogenated propanes in vivo correlated well with the DNA damage obsd. in isolated testicular cells in vitro, showing that toxicity was due to in situ activation. The nos., positions, and the types of halogen substituents appear to be important determinants in causing DNA damage and necrogenic effects. The toxic potential of the halogenated propanes was in the following order: 1,2,3-tribromopropane ≥ 1,2-dibromo-3-chloropropane > 1,3-dibromo-2-chloropropane > 1,3-dichloro-2-bromopropane ≃ 1-bromo-2,3-dichloropropane > 1,2,3-trichloropropane ≃ 1,2-dibromopropane ≥ 1,3-dibromopropane ≥ 1-bromo-3-chloropropane. The most toxic analogs contain three halogens with at least two vicinal bromines.

Enhancement of Bacterial Mutagenicity of Bifunctional Alkylating Agents by Expression of Mammalian Glutathione S-Transferase

Quick ViewOther Sources

- By Thier, Ricarda; Muller, Michael; Taylor, John B.; Pemble, Sally E.; Ketterer, Brian; Guengerich, F. Peter
- From Chemical Research in Toxicology (1995), 8(3), 465-72. | Language: English, Database: CAPLUS
- Recently, we inserted the plasmid vector pKK233-2 contg. rat GSH S-transferase (GST) 5-5 cDNA into Salmonella typhimurium TA1535 and found that these bacteria [GST 5-5(+)] expressed the protein and produced mutations when ethylene or methylene dihalides were added [Thier, R., Taylor, J. B., Pemble, S. E., Ketterer, B., Persmark, M., Humphreys, W. G., and Guengerich, F. P. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 8576-8580]. After exposure to the known GST 5-5 substrate 1,2-epoxy-3-(4'-nitrophenoxy)propane, the GST 5-5(+) strain showed fewer mutants than the bacteria transfected with the cDNA clone in a reverse orientation [GST 5-5(-)], suggesting a protective role of GST 5-5. However, mutations were considerably enhanced in the GST 5-5(+) strain [as compared to GST 5-5(-)] when 1,2,3,4-diepoxybutane (butadiene diepoxide) or 1,2-epoxy-4-bromobutane was added. The GST 5-5(+) and GST 5-5(-) bacterial stains showed similar responses to 1,2-epoxypropane, 3,4-epoxy-1-butene, and 1,4-dibromobutane. The results suggest that some bifunctional activated butanes are transformed to mutagenic products through GSH conjugation. We also found that the GST 5-5(+) strain showed enhanced mutagenicity with 1,4-dibromo-2,3-epoxybutane, 1,2-epoxy-3-bromopropane (epibromohydrin), and (±)-1,4-dibromo-2,3-dihydroxybutane. The possibility was considered that a 5-membered thialonium ion may be involved in the mutagenicity. Model thialonium compds.

were rather stable to hydrolysis in aq. soln. at pH 7.4 and slowly alkylated 4-(4-nitrobenzyl) pyridine. The presence of a hydroxyl group β to the sulfur did not enhance reactivity. Mechanisms involving episulfonium ions are considered more likely. Potential oxidn. products of the toxic pesticide 1,2-dibromo-3-chloropropane (DBCP) were also considered in this system. DBCP itself gave rather similar results in the two strains. Others have reported that oxidn. of DBCP is required for mutagenicity, along with GST-catalyzed GSH conjugation [Simula, T. P., Glancey, M. J., Soederlund, E. J., Dybing, E., and Wolf, C. R. (1993) Carcinogenesis 14, 2303-2307]. The putative oxidn. product 1,2-dibromopropional did not show a difference between the two strains. However, 1,3-dichloroacetone, a model for the putative oxidn. product 1-bromo-3-chloroacetone, was considerably more mutagenic in the GST 5-5(+) strain.

Thermal Degradation and Decomposition Products of Electronic Boards Containing BFRs
Quick ViewOther Sources

- By Barontini, Federica; Marsanich, Katia; Petarca, Luigi; Cozzani, Valerio
- From Industrial & Engineering Chemistry Research (2005), 44(12), 4186-4199. | Language: English, Database: CAPLUS

- Prodn. of electronic boards contg. brominated flame retardants is constantly increasing, posing important problems with disposal of products contg. these materials. The present study studied the thermal degrdn. behavior of electronic boards manufd. using tetrabromobisphenol A and diglycidyl ether of bisphenol A epoxy resins. Qual. and quant. information was obtained on the products formed in the thermal degrdn. process, and the bromine distribution in the different product fractions was detd. The more important decompn. products included hydrogen bromide, phenol, polybrominated phenols, and polybrominated bisphenol A species. The formation of considerable amts. of hydrogen bromide and high-mol.-wt. organobrominated compds., and the potential formation of limited quantities of polybrominated dibenzo-p-dioxins and dibenzofurans, is an important element of concern in the safety and environmental assessment of the thermal degrdn. processes of electronic boards contg. brominated flame retardants.

Prediction of Aquatic Toxicity: Use of Optimization of Correlation Weights of Local Graph Invariants
Quick ViewOther Sources

- By Toropov, Andrey Andreevich; Schultz, Terry Wayne
- From Journal of Chemical Information and Computer Sciences (2003), 43(2), 560-567. | Language: English, Database: CAPLUS

- Quant. structure-activity relationships (QSARs) were developed for three sets of toxicity data. Chems. in each set represented a no. of narcoses and electrophilic mechanisms of toxic action. A series of quant. structure-toxicity models correlating toxic potency with a no. of optimization of correlation wts. of local graph invariants were developed. In the case of the toxicity of a heterogeneous set of benzene derivs. to Tetrahymena pyriformis, the QSARs were based on the Descriptor of Correlation Wts. (DCW) using atoms and extended connectivity (EC) graph invariants. The model [log (IGC_{50}^{-1}) = 0.0813 DCW(a_k, ^3EC_k) + 2.636; n = 157, r^2 = 0.883, s = 0.27, F = 1170, Pr > F = 0.0001] based on third-order EC of 89 descriptors was obsd. to be best for the benzene data. However, fits for these data of > 0.800 were achieved ECs with as few as 23 variables. The relationship between the toxicity predicted by this model and exptl. toxicity values for the test set [obs. log(IGC_{50}^{-1}) = 0.991 (pred. (log(IGC_{50}^{-1})) - 0.012); n = 60, r^2 = 0.863, s = 0.28, F = 372, Pr > F = 0.0001] is excellent. The utility of the approach was demonstrated by the model [log (IGC_{50}^{-1}) = 0.1744(DCW (a_k, ^2EC) - 3.505); n = 39, r^2 = 0.900, s = 0.35, F = 333, Pr > F = 0.0001] for the toxicity data for T. pyriformis exposed to halo-substituted aliph. compds. and the model [log (IC_{50}^{-1}) = 0.1699(DCW (a_k, ^2EC)) - 2.610; n = 66, r^2 = 0.901, s = 0.31, F = 583, Pr > F = 0.0001] for the Vibrio fischeri toxicity data.

Modulation of hepatocyte thiol content by medium composition: implications for toxicity studies
Quick ViewOther Sources

- By Hammond, A. H.; Garle, M. J.; Sooriakumaran, P.; Fry, J. R.

- From Toxicology in Vitro (2002), 16(3), 259-265. | Language: English, Database: CAPLUS

- Toxicity of compds. requiring glutathione for detoxification, thiol content and synthesis were detd. in 24-h rat hepatocytes cultured in medium contg. different concns. of the sulfur amino acids. Glutathione synthesis was detd. following prior depletion of glutathione with diethylmaleate. L-15 medium, which has high levels of cysteine and methionine (1 mm of each), provided some protection against dichloroacetone, dibromopropanol and dichloropropanol toxicity, and had a small effect on increasing glutathione content and synthesis, relative to Williams' medium E (WE) which has low levels (less than 0.5 mm) of both amino acids. However, WE contg. N-acetylcysteine (NAC) (1 mm final cysteine concn.), with or without methionine (final concn. 1 mm), was a better cytoprotectant medium than L-15, markedly reducing toxicity of all three compds., and rapidly (within 1.5 h) increasing cellular glutathione content. WE supplemented with methionine alone stimulated glutathione synthesis after an initial lag phase, and protected cultures against dichloropropanol, but not dibromopropanol or dichloroacetone, both of which are highly reactive in these cultures. There was a clear assocn. between glutathione content at early time points in culture and toxicity obsd. at later time points, and overall these results indicate that differences in culture medium compn. can alter intracellular glutathione content and xenobiotic toxicity.

Haloalcohols deplete glutathione when incubated with fortified liver fractions
Quick ViewOther Sources

- By Garle, M. J.; Sinclair, C.; Thurley, P.; Fry, J. R.
- From Xenobiotica (1999), 29(5), 533-545. | Language: English, Database: CAPLUS

- This study has examd. the ability of dichloropropanols, haloalcs. and their putative metabolites to deplete glutathione when incubated with liver fractions obtained from untreated and differentially induced rats. 1,3-Dichloropropan-2-ol and 2,3-dichloropropan-1-ol (0-1000 μM) both depleted glutathione in a dose-dependent manner when incubated with cofactors (NADPH generating system) and liver microsomes from the untreated rat. The extent of GSH depletion was significantly enhanced when liver microsomes from the isoniazid- or isosafrole-treated rat were used. Epichlorohydrin produced a moderate, dose-dependent depletion of GSH. By contrast, 1,3-dichloroacetone (identified by TLC as a metabolite of 1,3-dichloropropanol) was a potent depletor of glutathione. N-acetylcysteine was less efficient than glutathione as a nucleophile trap for epichlorohydrin, 1,3-dichloroacetone or reactive metabolites derived from 1, 3-dichloropropan-2-ol. 1,3-Dibromopropan-2-ol and 1,4-dibromobutan-2-ol were potent depletors of GSH but 1-bromopropan-2-ol produced less GSH depletion. Both dibromoalcs. depleted GSH when incubated with dialyzed cytosol derived from the livers of untreated rats. The GSH depletion mediated by 1,3-dichloropropan-2-ol, 1,3-dibromopropan-2-ol, 1,4-dibromobutan-2-ol and 1-bromopropan-2-ol was inhibited by inclusion of pyridine (1 mM) or cofactor omission. 1,3-Difluoropropanol did not deplete GSH under any of the conditions examd.

Structure-toxicity relationships for selected halogenated aliphatic chemicals
Quick ViewOther Sources

- By Akers, Kevin S.; Sinks, Glendon D.; Schultz, T. Wayne
- From Environmental Toxicology and Pharmacology (1999), 7(1), 33-39. | Language: English, Database: CAPLUS

- Toxicity to the ciliate Tetrahymena pyriformis (log (IGC_{50}^{-1})) for 39 halogen-substituted alkanes, alkanols, and alkanitriles were obtained exptl. Log (IGC_{50}^{-1}) along with the hydrophobic term, log K_{ow} (1-octanol/water partition coeff.) and the electrophilic parameter, E_{lumo} (the energy of the LUMO) were used to develop quant. structure-activity relationships (QSARs). Two strong hydrophobic dependent relationships were obtained: one for the haloalkanes and a second for the haloalcs. The relationship for the haloalkanes [log(IGC_{50}^{-1}) = 0.92 (logK_{ow}) -2.58; n = 4, r^2 = 0.993, s = 0.063, f = 276, Pr >f = 0.0036] was not different from baseline toxicity. With the rejection of 1,3-dibromo-2-propanol as a statistical outlier, the relationship [log (IGC_{50}^{-1}) = 0.63(log K_{ow}) - 1.18; n = 19, r^2 = 0.860, s = 0.274, f = 10^4, Pr > f = 0.0001] was obsd. for the haloalcs. No hydrophobicity-dependent model (r^2 = 0.165) was obsd. for the halonitriles.

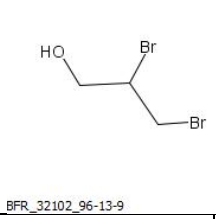
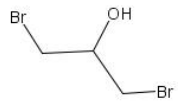
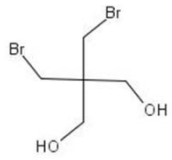
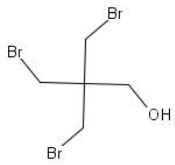
However, an electrophilicity-dependent model [log (IGC_{50}^{-1}) = - 1.245(E_{lumo}) + 0.73; n = 15, r^2 = 0.588, s = 0.764, F = 18.6, Pr > f = 0.0009] was developed for the halonitriles. Addnl. anal. designed to examine surface-response modeling of all three chem. classes met with some success. Following rejection of statistical outliers, the plane [log (IGC_{50}^{-1}) = 0.60(log K_{ow}) - 0.747(E_{lumo}) -0.37; n = 34, r^2 = 0.915, s = 0.297, F = 162, Pr > F= 0.0001] was developed. The halogenated alcs. and nitriles tested all had obsd. toxicity in excess of non-reactive baseline toxicity (non-polar narcosis). This observation along with the complexity of the structure-toxicity relationships developed in this study suggests that the toxicity of haloalcs. and halonitriles is by multiple and/or mixed mechanisms of action which are electro(nucleo)philic in character.

The Nature of Halogen Substitution Determines the Mode of Cytotoxicity of Halopropanols

- By Hammond, Alison H.; Garle, Michael J.; Fry, Jeffrey R.
- From Toxicology and Applied Pharmacology (1999), 155(3), 287-291. | Language: English, Database: CAPLUS

- The cytochrome P 450-dependent generation of reactive metabolites from 1,3-dichloropropanol and 1,3-dibromopropanol was assessed in a microsomal thiol depletion assay, while the toxicity of these compds. was assessed in rat hepatocyte cultures and in the 3T3 cell line. Thiol-depleting metabolites of both compds. were generated in the microsomal assay; however, only dibromopropanol extensively depleted glutathione when glutathione S-transferase was used as the enzyme source. The cytotoxicity of dichloropropanol was both cytochrome P 450- and glutathione-dependent, whereas that of dibromopropanol was glutathione-dependent but largely independent of cytochrome P 450. These results indicate that the mechanisms underlying the cytotoxicity of halopropanols are dependent on the nature of the halogen substitution and that microsomal and cellular assays for reactive metabolite generation may yield conflicting results. (c) 1999 Academic Press.

Appendix 9: Information matrix for category source substances

Substance	2,3-Dibromo-1-propanol (2,3-DBPA), CAS RN 96-13-9	1,3-Dibromo-2-propanol (1,3-DBPA), CAS RN 96-21-9	2,2-Bis(bromomethyl)-1,3-propanediol (DBNPG), CAS RN 3296-90-0	2,2-Bis-(bromomethyl)-3-bromo-1-propanol (TBNPA), CAS RN 36483-57-5
Structure	 BFR_32102_96-13-9	 BFR_32103_96-21-9	 BFR_52229_3296-90-0	 BFR_53142_36483-57-5
Included in the preliminary structural grouping	Yes	No	Yes	Yes
REACH registration / pre-registration	REACH registered	REACH pre-registered	REACH pre-registered	REACH registered
Harmonized cancer/mutagenicity classification	Carc. 1B H350			
Notified cancer/mutagenicity classification		Carc. 2 H351	Muta. 1B H340 / Muta. 2 H341 Carc. 1B H350 / Carc. 2 H351	Muta. 1B H340 / Muta. 2 H341 Carc. 1B H350
ADME experimental information	NA	NA	Available	NA
(Q)SAR predictions of bioavailability	Bioavailable according to Lipinski's rule of 5, and predicted high Human intestinal absorption			
Ames *Salmonella t.* experimental mutagenicity	Positive	NA	Positive	Positive
- Strains tested positive	TA100, TA1535 (base-pair) with and without exogenous metabolic system TA102 (cross-linking) tested only with exogenous metabolic system		TA100 (base-pair) with exogenous metabolic system (Syrian Hamster)	TA100, TA1535 (base-pair) with exogenous metabolic system (hamster)
Other experimental genotoxicity results	Positive ML *in vitro* (without exogenous metabolic system) Positive CHO SCE *in vitro* (with and without exogenous metabolic system) Positive CHO CA *in vitro* (with and	NA	Negative/equivocal CHO SCE *in vitro* Positive CHO CA *in vitro* (with exogenous metabolic system) at doses causing cytotoxicity Positive MN *in vivo* Mouse (peripheral	Positive ML *in vitro* (with exogenous metabolic system) Positive CA *in vitro* (cultured peripheral human lymphocytes , with exogenous metabolic system, however also without at

	without exogenous metabolic system) Positive *Drosophila m.* SLRL and reciprocal translocation Negative MN *in vivo* male Mouse (bone marrow)		blood) Equivocal MN *in vivo* Mouse (bone marrow)	highest tested concentration) Negative UDS *in vivo* Rat (liver) Negative MN *in vivo* Mouse (bone marrow)
Critical effects conclusions from chapter 4.	Multi-site, multispecies carcinogenic effect, most probably caused by a direct genotoxic action of the parent compound	NA	Multi-site, multispecies carcinogenic effect, most probably caused by a genotoxic metabolite of the parent compound	Possible carcinogenic effect, most probably caused by a genotoxic metabolite of the parent compound
Positive predictions in AD in (Q)SAR models for	CU Ashby structural alerts CU SALM, Salmonella mutagenicity (TA97,98,100,1535-1538) (in vitro) DTU Ames Salmonella (TA98, 100, 1535 and either TA1537 or TA97) (in vitro) DTU Ames sub-model Direct (not requiring S9) (in vitro) DTU Ames sub-model Base-pair (in vitro) DTU Ames sub-model Frame shift (in vitro) DTU Chromosomal aberrations CHL (in vitro) DTU UDS rat hepatocytes (in vitro) DTU SHE cell transformation (in vitro) DTU SCE mouse (in vivo)	CU Ashby structural alerts CU SALM, Salmonella mutagenicity (TA97,98,100,1535-1538) (in vitro) DTU Ames sub-model Base-pair (in vitro) DTU SCE mouse (in vivo) DTU Drosophila SLRL (in vivo) CU FDA RCA cancer male rat (in vivo) CU FDA RCA cancer female rat (in vivo) CU FDA RCA cancer male mouse (in vivo) CU FDA RCA cancer female mouse (in vivo) CU FDA RCA cancer rodent (in vivo) CU FDA RCA cancer rat (in vivo) CU FDA RCA cancer mice (in	CU Ashby structural alerts CU SALM, Salmonella mutagenicity (TA97,98,100,1535-1538) (in vitro) DTU Ames Salmonella (TA98, 100, 1535 and either TA1537 or TA97) (in vitro) DTU Ames sub-model Direct (not requiring S9) (in vitro) DTU SCE mouse (in vivo) CU FDA RCA cancer male rat (in vivo) CU FDA RCA cancer female rat (in vivo) CU FDA RCA cancer male mouse (in vivo) CU FDA RCA cancer female mouse (in vivo) CU FDA RCA cancer rodent (in vivo) CU FDA RCA	CU Ashby structural alerts CU SALM, Salmonella mutagenicity (TA97,98,100,1535-1538) (in vitro) DTU Ames Salmonella (TA98, 100, 1535 and either TA1537 or TA97) (in vitro) DTU SCE mouse (in vivo) CU FDA RCA cancer male rat (in vivo) CU FDA RCA cancer female rat (in vivo) CU FDA RCA cancer male mouse (in vivo) CU FDA RCA cancer female mouse (in vivo) CU FDA RCA cancer rodent (in vivo) CU FDA RCA cancer rat (in vivo) CU FDA RCA

	DTU Drosophila SLRL (in vivo) DTU Comet assay (in vivo) CU FDA RCA cancer male rat (in vivo) CU FDA RCA cancer female rat (in vivo) CU FDA RCA cancer male mouse (in vivo) CU FDA RCA cancer female mouse (in vivo) CU FDA RCA cancer rodent (in vivo) CU FDA RCA cancer rat (in vivo) CU FDA RCA cancer mice (in vivo) CU FDA RCA overall cancer call	vivo) CU FDA RCA overall cancer call	cancer rat (in vivo) CU FDA RCA cancer mice (in vivo) CU FDA RCA overall cancer call	cancer mice (in vivo) CU FDA RCA overall cancer call
Negative predictions in AD in (Q)SAR models for	DTU Ames sub-model Potency > 10x ctrl. (in vitro) CU Chromosomal aberrations CHO (in vitro) DTU Mouse micronucleus (bone marrow) (in vivo) DTU Dominant lethal (in vivo)	DTU Ames sub-model Potency > 10x ctrl. (in vitro) DTU Ames sub-model Frame shift (in vitro) CU Chromosomal aberrations CHO (in vitro) DTU Chromosomal aberrations CHL (in vitro) DTU Mouse micronucleus (bone marrow) (in vivo) DTU Dominant lethal (in vivo)	DTU Ames sub-model Frame shift (in vitro) CU Chromosomal aberrations CHO (in vitro) DTU Mouse micronucleus (bone marrow) (in vivo)	DTU Ames sub-model Direct (not requiring S9) (in vitro) DTU Ames sub-model Potency > 10x ctrl. (in vitro) DTU Ames sub-model Frame shift (in vitro) CU Chromosomal aberrations CHO (in vitro) DTU Mouse micronucleus (bone marrow) (in vivo)
OECD (Q)SAR Application Toolbox profiler: DNA binding by OECD identified	Aliphatic halides 1,2-Dihaloalkanes Mono aldehydes	Aliphatic halides Mono aldehydes Epoxides	Aliphatic halides Mono aldehydes	Aliphatic halides Mono aldehydes

alerts (parent or mammalian metabolites)	Epoxides			
OECD (Q)SAR Application Toolbox profiler: DNA binding by OASIS v.1.3 identified alerts (parent or mammalian metabolites)	Haloalkanes Containing Heteroatom Haloalkane Derivatives with Labile Halogen Vicinal Dihaloalkanes Epoxides and Aziridines Haloalcohols	Haloalkanes Containing Heteroatom Haloalkane Derivatives with Labile Halogen Epoxides and Aziridines Haloalcohols	Haloalkane Derivatives with Labile Halogen	Haloalkane Derivatives with Labile Halogen
OECD (Q)SAR Application Toolbox profiler: Protein binding alerts for Chromosomal aberration by OASIS v1.1 identified alerts (parent or mammalian metabolites)	Halogenated Vicinal Hydrocarbons Alpha-Activated Haloalkanes			
OECD (Q)SAR Application Toolbox profiler: in vitro mutagenicity (Ames test) alerts by ISS identified alerts (parent or mammalian metabolites)	Aliphatic halogens Simple aldehyde Epoxides and aziridines	Aliphatic halogens Simple aldehyde Epoxides and aziridines	Aliphatic halogens Simple aldehyde	Aliphatic halogens Simple aldehyde
OECD (Q)SAR Application Toolbox profiler: DNA alerts for AMES, MN and CA by OASIS v.1.3 identified alerts (parent or mammalian metabolites)	Haloalkane Derivatives with Labile Halogen Vicinal Dihaloalkanes Haloalcohols Epoxides and Aziridines	Haloalkane Derivatives with Labile Halogen Haloalcohols Epoxides and Aziridines		
OECD (Q)SAR Application Toolbox profiler: in vivo mutagenicity (Micronucleus) alerts by ISS identified alerts (parent or mammalian metabolites)	Aliphatic halogen Epoxides and aziridines H-acceptor-path3-H-acceptor Simple aldehyde	Aliphatic halogen Epoxides and aziridines H-acceptor-path3-H-acceptor Simple aldehyde	Aliphatic halogen H-acceptor-path3-H-acceptor Simple aldehyde	Aliphatic halogen H-acceptor-path3-H-acceptor Simple aldehyde
OECD (Q)SAR Application Toolbox	Aldehyde Type Compounds	Aldehyde Type Compounds	Aldehyde Type Compounds	Aldehyde Type Compounds

profiler: Oncologic Primary Classification identified alerts (parent or mammalian metabolites)	Alpha, beta-Haloether Reactive Functional Groups Epoxide Reactive Functional Groups	Alpha, beta-Haloether Reactive Functional Groups Reactive Ketone Reactive Functional Groups Epoxide Reactive Functional Groups		
OECD (Q)SAR Application Toolbox profiler: Carcinogenicity (genotox and nongenotox) alerts by ISS identified alerts (parent or mammalian metabolites)	Aliphatic halogens (Genotox) (Poly) Halogenated Cycloalkanes (Nongenotox) Epoxides and aziridines (Genotox) Simple aldehyde (Genotox)	Aliphatic halogens (Genotox) Epoxides and aziridines (Genotox) Simple aldehyde (Genotox)	Aliphatic halogens (Genotox) Simple aldehyde (Genotox)	Aliphatic halogens (Genotox) Simple aldehyde (Genotox)

Category approach for selected brominated flame retardants

The aim of this project was to attempt grouping of a number of identified brominated flame retardants (BFRs). The grouping was performed for 67 brominated flame retardants based on their chemical structures and resulted in 15 preliminary structural groups and 7 substances remaining as "singletons". (Q)SAR predictions for a number of environmental and health effects within these initial groups were generated and investigated.

One of the groups; small linear and branched brominated alkyl alcohols, was chosen for further investigation as a category, in which all compounds having 3-5 carbons, 2-3 bromine atoms and 1-2 alcohol groups were included. The category comprises 61 members, including 3 members with relevant experimental data on human health effects.

Possible read-across for the critical effect from the three category members with experimental data and one further member with a notified classification for the identified critical effect to the remaining 57 structurally similar target analogues in the category is supported by the following observations:

- The toxicity profile of the 3 members with relevant experimental data is comparable and the critical effect is the multiple-organ carcinogenic effect, most probably exerted by a mutagenic/genotoxic mode of action either by the parent compound itself (2,3-DBPA) or by a metabolite of the parent compound (DBNPG and TBNPA).
- The classifications (harmonized or notified) as Muta. 1B H340 / Muta. 2 H341 and/or Carc. 1B H350 / Carc. 2 H351 for these three members and for 1,3-DBPA.
- The (Q)SAR predictions indicate that the 61 category members have a carcinogenic potential with a possible mutagenic/genotoxic mode of action.

Ministry of Environment and Food of Denmark
Environmental Protection Agency
Strandgade 29
1401 Copenhagen K, Denmark
Tel.: (+45) 72 54 40 00

www.mst.dk