Danish Ministry of the Environment
Environmental Protection Agency

Survey and health assesment of thiourea compounds in chloroprene rubber

Survey of chemical substances in consumer products no. 118, 2012

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Survey and health assesment of thiourea compounds in chloroprene rubber

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Preface

This project, "Survey and health assessment of thiourea compounds in chloroprene rubber", was carried out by COWI A/S for the Danish Environmental Protection Agency (DEPA) during the period June 2011 to January 2012.

The background for initiating the project is that contact dermatitis in response to rubber products occurs relatively frequently and the incidence appears to be increasing. The thiourea compounds are among the chemicals used in production of chloroprene rubber and other types of synthetic rubber, and a number of studies have shown that the thiourea compounds may be important contact allergens. With this study, DEPA wishes to establish an overview of the presence of thiourea compounds in chloroprene rubber products and the possible health consequences of exposure to thiourea compounds released from these products.

The project has been advised by a steering committee consisting of Louise Fredsbo Karlsson and Annette Orloff of DEPA, and Carsten Lassen and Sonja Hagen Mikkelsen of COWI.

The project was implemented by a project team consisting of Carsten Lassen (project manager), Sonja Hagen Mikkelsen and Ulla Kristine Brandt of COWI A/S. Analyses of thiourea compounds in chloroprene rubber products and migration of compounds from products were undertaken by the Danish Technological Institute with Eva Jacobsen as contact person.

Summary

COWI has carried out a study of thiourea compounds in chloroprene rubber products for the Danish Environmental Protection Agency. The study has included analyses of thiourea and six thiourea compounds which, according to the literature, are used as accelerators in the production of chloroprene rubber, and which are also known to cause contact dermatitis. The six thiourea compounds are designated by their acronyms ETU, DETU, DBTU, DMTU, DPTU and EBTU. DETU was present in concentrations of 33 to 720 mg/kg in ten of the 14 examined products, whereas DBTU was detected in only one of these ten products in a concentration of 60 mg/kg. The other compounds were not detected. Migration of DETU from five products examined ranged from 1.1 to 2.0 µg/cm^2/h. The concentrations of DETU in ten of the 14 products were of the same magnitude as the concentration in products that are demonstrated to cause the development of contact dermatitis in sensitized individuals and it is expected that all products containing DETU could trigger allergic reactions. It is not possible on the basis of existing knowledge to determine at which exposure levels the sensitization may occur.

Purpose

The study aims to establish an overview of consumer products containing chloroprene rubber marketed in Denmark, in order to measure the content of the substances in the products, to measure the migration of thiourea compounds from selected chloroprene rubber products, and to assess the possible health effects of exposure to thiourea compounds released from the products.

Application of chloroprene rubber products

Chloroprene rubber, which is also known as "neoprene", is a synthetic rubber that is used for both technical and consumer products. Typically, chloroprene rubber with a cell structure that makes the material heat-insulating is used in consumer products, and this study indicates that relatively few different types of chloroprene rubber are used for consumer products. A wide range of consumer products made of chloroprene rubber are marketed in Denmark. The main application areas are water sports products and other leisure activity products such as wetsuits, trunks, hoods, gloves and socks, and waders and boots. Chloroprene rubber is also used for many types of bandages and corsets, and as lining in many types of leisure sandals. The use of these types of products causes the skin to be in prolonged contact with the chloroprene rubber. Chloroprene rubber is also used in a variety of products typically in short-term contact with the skin, such as bags and pouches for various purposes, aprons, balls, tea cups and storage bags. The use of chloroprene rubber for these purposes appears to be increasing. Some jackets and gloves which claim to contain "neoprene" actually contain chloroprene rubber-like textiles.

Thiourea compounds in chloroprene rubber

According to the literature, a number of thiourea compounds are used as accelerators in the manufacturing of chloroprene rubber products. Six of these have been identified as potentially giving rise to allergic reactions: ethyl thiourea (ETU), diethyl thiourea (DETU), dibutyl thiourea (DBTU), dimethyl thiourea (DMTU), diphenyl thiourea (DPTU) and ethylbutyl thiourea (EBTU). The compounds are used either alone or in combination. According to the available information, ETU is the most common thiourea compound used for the production of chloroprene rubber in Europe. The accelerators typically account for 0.2-1% of the total raw materials in a chloroprene rubber compound (corresponding to 2,000-10,000 mg/kg). Through the vulcanization of the chloroprene rubber, the accelerators are oxidized to a certain degree, and consequently they are present in the finished materials in significantly lower concentrations.

The study found DETU in ten of 14 examined products in concentrations of 33 to 720 mg/kg (see Table 1). DBTU was demonstrated to be present in one of the products in a concentration of 60 mg/kg. Surprisingly, ETU was not found in any of the materials. There are two possible explanations: either ETU was not used as an accelerator in any of the studied products, or ETU may degrade through the polymerization of the chloroprene rubber so that it is present in the finished material in concentrations below the detection limit. In four of the products, none of the thiourea compounds were present at concentrations above the detection limit. No information on the accelerators used in these products has been available.

TABLE 1
ANALYSIS RESULT FOR THE TWO THIOUREA COMPOUNDS DETECTED IN THE PRODUCTS AND MIGRATION RATES FOR DETU

Type of product	Concentration mg/kg		Migration rate μg/cm²/h
	DETU	DBTU	DETU
Full-length wetsuit for kids	610	< d.l.	1.8
Short wetsuit for kids	350	< d.l.	0.9
Chloroprene rubber T-shirt for kids	< d.l.	< d.l.	-
Chloroprene rubber socks shirt for kids	33	< d.l.	-
Bathing shoes for kids	720	< d.l.	1.7
Flip-flop sandals with chloroprene rubber strap for adults	< d.l.	< d.l.	-
Sport sandal with chloroprene rubber linings for adults	< d.l.	< d.l.	-
Wrist brace	670	60.	1.1
Elbow support	140	< d.l.	-
Wrist brace	150	< d.l	-
Thigh support	480	< d.l.	0.9
Kayaking gloves	47	< d.l.	-
Chloroprene rubber sleeve for I-PAD	< d.l.	< d.l.	-
Kayaking hood	140	< d.l.	-
Detection limit, mg/kg	6	9	-

d.l. - Detection limit

Releases of thiourea compounds from products of chloroprene rubber

A number of the chloroprene rubber products are in prolonged direct contact with the skin during use, and the user will be exposed to substances released from the products. The exposure time is assumed to be about 6 hours per day in the realistic "worst case" scenario for products used for water sports (such as wetsuits and slippers), whereas it is assumed to be 8 hours per day for corsets and supports. The migration of DETU from five tested products to artificial sweat was determined by migration experiments in which the migration time, based on the realistic "worst case" scenarios, was fixed at 6 and 8 hours. The migration rate for the five tested products varied from 0.9 to 1.8 $\mu g/cm^2/h$. The total migration from the products per day is estimated to be in the range of 5.6 to 10.6 $\mu g/cm^2$. The results show that the content and migration rates were similar among products used for water sports and supports. These results support the assumption that virtually the same materials are used for the various consumer products.

Health effects of thiourea compounds
A number of studies have shown that thiourea compounds in chloroprene rubber can trigger contact dermatitis in users of chloroprene rubber products. Product types that have been shown to cause contact dermatitis include chloroprene rubber, knee braces/supports, waders, wetsuits, keyboard wrist supports and belts. For all of these product types, the skin is in prolonged contact with the chloroprene rubber. No data are available on the total number of individuals with allergic reactions to chloroprene rubber, but investigations in a number of countries of patients with allergic reactions demonstrate that approximately 1% of patients react to the thiourea compounds in patch tests.

One of thiourea compounds, ETU, is classified as teratogenic and harmful if swallowed, and is included in the EU priority list of substances slated for further evaluation of their role in endocrine disruption, but this substance was not detected in any samples. Thiourea is, *inter alia*, suspected to cause cancer and is suspected of teratogenic effects, but this substance was not detected in the samples.

Health risk assessment
The contents of DETU in the investigated products have been measured up to a maximum of 720 mg/kg in bathing shoes for children, which was the product with the highest content of DETU. For comparison purposes, the content of DETU in shoes that have been shown to cause an allergic reaction is 192-251 mg/kg, which is lower than the concentration found in several of the tested products. In another study, an allergic reaction to work gloves with a concentration of DETU of 76-77 mg/kg was demonstrated. Therefore, the concentration of DETU found in ten of 14 products (33-720 mg/kg) is of the same order of magnitude as the concentration of products demonstrated to be able to cause contact eczema in sensitised individuals; it must be assumed that all products containing DETU may potentially cause an allergic reaction. This supposition is supported by laboratory results which demonstrate that the concentration of DETU in the sweat between the skin and the wetsuit in the course of an exposure period of 6 hours can reach a concentration of the same order of magnitude as the levels used in patch tests.

ETU is a teratogen and is classified accordingly in the category 1B. ETU was not detected in the tested samples, but is known to be used to some extent for the production of consumer products of chloroprene rubber.

1 Introduction

1.1 Background

Allergic contact dermatitis caused by rubber products occurs relatively frequently. Thiourea compounds are among the chemicals used in the production of rubber – including chloroprene rubber. Chloroprene rubber is the official terminology for this synthetic rubber based on polychloroprene, but this material is commonly referred to as "neoprene", which is the manufacturer Dupont's trademark for polychloroprene. The substances are not part of the standard allergy test for rubber chemicals in Europe, but the substances are included in the test if deemed necessary.

Contact dermatitis caused by thiourea compounds in chloroprene rubber is described in detail in the medical literature. The causes of the reported allergies include the use of products such as gloves, knee braces, swimming goggles, footwear and diving suits. Among the recent studies is a quantitative assessment of diethyl thiourea (DETU) exposure in two cases of occupational allergic contact dermatitis, conducted by the Danish National Allergy Research Centre (Friis et al., 2011).

Thiourea compounds are used as accelerators in the production of chloroprene rubber and are therefore included in the finished products. A number of thiourea compounds are used as accelerators in the chloroprene rubber manufacturing process, including ethylene thiourea (ETU), diethyl thiourea (DETU), dibutyl thiourea (DBTU), diphenyl thiourea (DPTU), dimethyl thiourea (DMTU) and trimethyl thiourea. The substances used either singly or in combination.

Thiourea compounds that are proven to cause contact dermatitis are the above-mentioned ETU, DETU, DBTU, DPTU and DMTU, as well as ethylbutyl thiourea (EBTU) and thiourea (TU) (Warshaw et al., 2008). In allergy tests, a mixture of DETU and DBTU is referred to as MDTU. MDTU was named "contact allergen of the year" by the American Contact Dermatitis Society (ACDS) in 2009.

A study of chloroprene rubber has previously been undertaken as part of DEPA's programme on surveys of chemical substances in consumer products (Nilsson and Pedersen, 2004). The study estimated the number of consumers who could potentially come into contact with the various chloroprene rubber products. The study focused largely on chloroprene rubber in sports and leisure equipment. The study combined information from importers and suppliers of chloroprene rubber products with information about the number of people engaged in different types of sports and leisure activities. These data were combined to obtain an estimate of the number of people potentially exposed to chloroprene rubber wetsuits (e.g. recreational divers, surfers, water skiers, some dinghy sailors and sea kayak paddlers). The study also included an assessment of the relationship between consumption patterns and exposure.

The study addressed a single thiourea compound, DETU. The substance was detected in two of five examined products (diving suit and waders) at concentrations of 110 and 160 mg/kg, respectively. It is worth noting that this concentration represents approximately 1% of the concentration of the total amount of accelerator used in the production of the chloroprene rubber. Migration could be measured from one product only. The migration from this product to sweat was 0.074 μg/cm^2, whereas no migration to seawater could be demonstrated in another test. In a "real life" scenario, in which DETU was measured in the water inside a diving suit after use, a migration rate of DETU equal to 0.28 ng/cm^2 exposed skin surface was found.

Given the increased attention to thiourea compounds as a possible cause of allergic contact dermatitis, the Environmental Protection Agency has decided to establish an overview of the occurrence of these compounds in consumer products on the Danish market, and to investigate the extent to which the substances are released from products during use, thereby causing allergies.

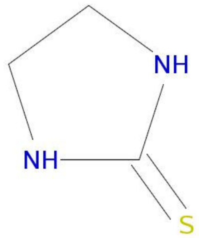
1.2 Purpose

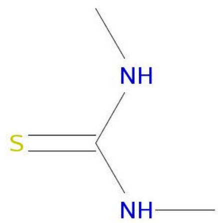
The purpose of this study is:

- To survey consumer chloroprene rubber products marketed in Denmark,
- to measure the content and migration of thiourea compounds from selected chloroprene rubber products, and
- to assess the possible health effects of exposure to thiourea compounds released from the products.

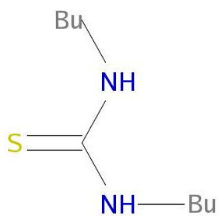
1.3 Substances addressed in the study

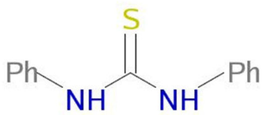
This study includes thiourea and a number of thiourea compounds which are used as accelerators in chloroprene rubber production, which are also shown to be the cause of allergy. The substances listed below are included in the chemical analyses and assessments. One of the substances used as an accelerator, ETU, is classified as teratogenic and is included in the EU list of potential endocrine disruptors. Furthermore, thiourea, which may occur in the products, but which was not used as an accelerator, is classified as category Carc. 2. Consequently, the health assessment will also examine effects aside from the potential induction of allergy.

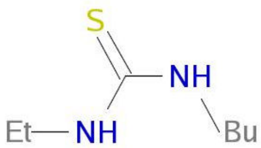
Chemical name:	imidazolidine-2-thione
Synonyms:	ETU; ethylene thiourea, 2-imidazolidinethione
CAS No:	96-45-7
EINECS No:	202-506-9
Structure:	 Source:http://esis.jrc.ec.europa.eu/
Hazard class and category:	Repr. 1B; Acute Tox. 4
Hazard statement codes:	H360-D*** May damage the unborn child. Suspected of damaging fertility. H302: Harmful if swallowed Hazard statements H360 and H361 indicate a general concern for effects on both fertility and development: "May damage/Suspected of damaging fertility or the unborn child". According to the criteria, the general hazard statement can be replaced by the hazard statement indicating only the property of concern, where either fertility or developmental effects are proven to be not relevant. In order not to lose information from the harmonised classifications for fertility and developmental effects under Directive 67/548/EEC, the classifications have been translated only for those effects classified under that Directive. These hazard statements are indicated by the reference ***in Table 3.1 of the CLP Regulation.

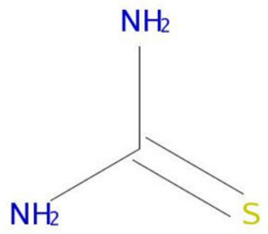
Chemical name:	1,3-dimethyl-2-thiourea
Synonyms:	DMTU; N,N'-dimethylthiourea
CAS No:	534-13-4
EINECS No:	208-588-2
Structure:	 http://esis.jrc.ec.europa.eu/
Hazard class and category:	None

Chemical name:	1,3-diethyl-2-thiourea
Synonyms:	DETU; N,N'-diethylthiourea
CAS No:	105-55-5
EINECS No:	203-308-5
Structure:	 http://esis.jrc.ec.europa.eu/
Hazard class and category:	None

Chemical name:	1,3-dibutyl-2-thiourea
Synonyms:	DBTU
CAS No:	109-46-6
EINECS No:	203-674-6
Structure:	 http://esis.jrc.ec.europa.eu/
Hazard class and category:	None

Chemical name:	1,3-diphenyl-2-thiourea
Synonyms:	DPTU; N,N'-diphenylthiourea
CAS No:	102-08-9
EINECS No:	203-004-2
Structure:	 http://esis.jrc.ec.europa.eu/
Hazard class and category:	None

Chemical name:	1-butyl-3-ethylthiourea
Synonyms:	EBTU, N-butyl-B'-ethyl-thiourea
CAS No:	32900-06-4
EINECS No:	251-285-5
Structure:	 http://esis.jrc.ec.europa.eu/
Hazard class and category:	None

Chemical name:	Thiourea
Synonyms:	Thiocarbamide
CAS No:	62-56-6
EINECS No:	200-543-5
Structure:	 Source: http://esis.jrc.ec.europa.eu/
Hazard class and category:	Carc. 2, Repr. 2, Acute Tox. 4 * , Aquatic Chronic 2
Hazard statement codes:	H351: Suspected of causing cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard) H361d***: Suspected of damaging the unborn child H302: Harmful if swallowed H411: Toxic to aquatic life with long lasting effects *** See explanation under ETU

2 Survey of chloroprene rubber products on the market

As mentioned in the “Background” section, a survey of chloroprene rubber products in Denmark has previously been undertaken. The survey included an inventory of the number of people that may be exposed to the different product types (Nilsson and Pedersen, 2004). It is assumed that no major changes in consumption patterns of these products have occurred since then; consequently, the results of the previous study are used as the basis for this study.

2.1 Use of thiourea compounds in chloroprene rubber

What is chloroprene rubber?
Chloroprene rubber is produced by vulcanization of a polychloroprene which is prepared by polymerization of chloroprene. Chloroprene rubber was the first synthetic elastomer (synthetic rubber) which was introduced commercially and has been in use since 1931. Colloquially the term "Neoprene" is often used as a synonym for chloroprene rubber.

Various grades of chloroprene rubber exist, characterized by differences in flexibility, resistance to wear, surface properties and cell structure, among other properties. Typically, expanded chloroprene grades with closed air-containing cells are used for consumer products as this characteristic gives the material its heat-insulating properties.

Chloroprene rubber has exceptional resistance to weathering, sunlight and ozone, and good wear resistance, as well as resistance to oil, heat and flame. It is used for a wide range of technical products including glue, cable insulation, hoses and belts and a number of consumer products. This study focuses on consumer products, where chloroprene rubber grades with either textile backing or textile reinforcement are most often used.

A number of ingredients are used in the manufacture of chloroprene rubber and are present in the final polymer: acid acceptors (neutralization of the formed hydrochloric acid), vulcanising agents (typically zinc oxide), vulcanization-accelerators, vulcanization-inhibitors, antioxidants, fillers, plasticisers and processing aids.

Thiourea compounds in chloroprene rubber
Thiourea compounds are used as accelerators in the production of chloroprene rubber. The accelerators are added for the polymerization (curing) of the chloroprene compound; the applied accelerators influence the technical characteristics of the finished chloroprene rubber. Through the vulcanization of the chloroprene rubber, cross-links between the chain molecules are formed in a complex chemical process. The cross-links improve elasticity, make the rubber less sticky and improve stability.

As previously mentioned, various thiourea compounds are used as accelerators, including ethylene thiourea (ETU), diethyl thiourea (DETU), dibutyl thiourea (DBTU), diphenyl thiourea (DPTU), di-methyl thiourea

(DMTU) and trimethyl thiourea. According to a guidance document from DuPont Dow, the accelerators are typically used in concentrations of 0.4-1.5 parts per 100 parts chloroprene rubber. The guidance document describes recipes for a wide range of chloroprene rubber grades for different purposes using five of the above-mentioned accelerators (DuPont, 2008). Chloroprene rubber monomer typically accounts for almost half the weight of the raw materials and accelerators consequently typically account for 0.2-1% of the total weight of the ingredients (see example in Table 2.1).

Through the vulcanization of the chloroprene rubber, the accelerators are oxidised to some extent and the accelerators are expected to be present in the finished products in concentrations substantially below 1% (NTP, 2011).

DuPont Dow's guidance indicates that the accelerators are used in conjunction with other substances in accelerator systems with different technical characteristics, but all the systems specified in the guidance incorporate only one of the thiourea compounds. ETU seems to be the thiourea used in the majority of formulations, including wet suits and footwear. By way of example, the formulation from DuPont Dow for the manufacture of the chloroprene rubber material for wetsuits is shown in Table 2.1. In the preparation of the chloroprene rubber, the polychloroprene is vulcanized. In the example shown, the polychloroprene is of the type "Neoprene, GW," which is a sulfur-modified polychloroprene stabilized with the thiuram disulfide (DuPont, 2010).

An examination of the websites of other manufacturers of rubber chemicals reveals that the thiourea compounds are used to some extent in combination; for example, the manufacturer Akrochem indicated that DETU is either used alone or in combination with ETU (Akrochem, 2011).

Polychloroprene is produced by a number of major chemical producers including DuPont, Tosoh and Bayer (SpecialChem, 2008), whereas there are many small producers of thiourea accelerators and other rubber chemicals.

A Swedish study of a chloroprene rubber knee brace identified a combination of EBTU, DETU and DBTU (Bergendorff et al., 2004).

TABLE2.1
EXAMPLE OF THE FORMULATION USED FOR THE MANUFACTURE OF A NEOPRENE MATERIAL FOR WETSUITS WITH A SPECIFIC GRAVITY OF 0.25 G/CM³ (DUPONT, 2008)

Wet Suit Compound

Neoprene GW	100
High-Activity Magnesia	2
Octylated Diphenylamine	2
Stearic Acid	0.5
SRF N772 Carbon Black	15
Calcium Carbonate	25
Brown Factice	25
Naphthenic Process Oil	15
Zinc Oxide	5
ETU Dispersion [75%]	0.5
CBS	0.5
Azodicarbonamide Blowing Agent	10
Cure: 1st Stage: 8 min at 160°C	
2nd Stage: 15 min at 170°C	
Density, g/cm³	0.25

* Neoprene GW is a sulphur-modified polychloroprene stabilized with thiuram disulphide (DuPont. 2010)

In a Swedish study of a chloroprene rubber knee braces a combination of EBTU, DETU and DBTU was found (Bergendorff *et al.*, 2004).

The manufacturers' recommendations on the use of thiourea compounds indicate that the compounds are used for manufacturing of chloroprene rubber and other rubber materials, and examples are provided, but it is not indicated to what extent there is a correlation between the use of each of the accelerators and specific end-uses of the chloroprene rubbers.

An Italian manufacturer of both ETU and DETU indicates the following end uses of elastomers produced with the use of the two accelerators (RDC, 2011):

ETU: Shoe soles, footwear, cables and wires, window frames

DETU: Safety footwear, cables and wires, chloroprene rubber, solid and foamed profiles

The most widely-used thiourea compound in Europe
According to the justification for an ongoing pan-European research project which aims to develop alternatives to thiourea compounds in chloroprene rubber, thiourea-based accelerators are generally used in the vulcanization of chloroprene rubber (Safe Rubber, 2011). It is furthermore stated that ethylene thiourea (ETU) is the accelerator of choice because it produces the highest performance rubber-cure system.

As part of this investigation, the European manufacturers of chloroprene rubber and chloroprene rubber products contacted have confirmed that ETU is widely used as an accelerator in chloroprene rubber production in the EU.

Since many of the finished products are produced outside the EU, websites of Chinese producers of chloroprene rubber accelerators have also been visited. A number of Chinese companies advertise both ETU and DETU, but do not indicate the specific end uses of the chloroprene rubber.

Differences between materials

In order to obtain more information on the prevalence of various thiourea compounds, manufacturers of chloroprene rubber materials were contacted.

The chloroprene rubber materials used in consumer products are manufactured by relatively few companies. These companies sell the fabric to manufacturers of wetsuits, arm supports, bags, etc. which subsequently cut up the materials and glue them into the finished products.

Various grades of chloroprene rubber are produced. The different grades are used for different purposes in consumer products. The grades, hereunder referred to as types, differ in terms of cell structure, hardness and properties in relation to pressure and compacting. Examples from one company of the types used for various consumer products are shown in Table 2.2. The type names are specific to this company and refer to the density of the materials (LS, S, HS, HHS), that the material is flame retardant (NF) or that it is white (W).

Table2.2
Types of chloroprene rubber for consumer products and their application areas (Sedo, 2011)

Type	Properties	Recommended applications
LS	Particularly soft and extendible	Aquatic sports, leisure wear
S	Good extendibility, but strong, resistant and compact	Diving, bandages and protectors, packaging, lining for boots and shoes, diverse application
HS	High cell density, particularly resistant to pressure	Professional diving, equestrian sports
HHS	Very dense cell structure, low flexibility	Technical and other applications
NF	Non-flammable, consistency similar to "S"	Fireproof clothing, special industrial applications
W	Characterized by white colour	Medical sector, especially orthopaedics

For most of the applications described in the following paragraphs, the "LS" and "S" types are used. No major differences between the materials used for wetsuits, supports, packaging and footwear are observed. Differences between the different products are best determined by the origin of the products.

A large English company manufacturing chloroprene rubber products for water sports was contacted. The company stated that it buys fabrics produced both in the EU and imported from countries outside the EU. The company is not aware of the accelerators used in the manufacture of the material, just that the products conform to European legislation on chemicals. The name of the company as well as the names of other companies contacted is not mentioned here, but the names are known to the Danish EPA.

A major supplier of bandages/corsets/protectors for athletic and medical purposes stated that the company uses only high-quality products from major producers in the U.S. and China. The company has been unable to provide information on accelerators used in the products.

Most chloroprene rubber consumer products sold in the EU market appear to be imported from countries outside the EU or produced from fabrics imported from countries outside the EU. It would be difficult to attain reliable information about the content, apart from direct analysis of the products.

Consequently, attempts have not been made to obtain information about accelerators used by importers of finished products.

The presence of the accelerator in the finished products
According to the U.S. National Toxicology Program (NTP, 2011), ETU is converted to other compounds in the cured chloroprene rubber material. Tests have shown that 0.01 mg of non-reacted ETU per square inch (2 $\mu g/cm^2$) could be extracted in water at a temperature of 57 °C over a period of 7 days (NTP, 2011).

The previous Danish survey included one of the thiourea compounds, DETU. The substance was detected in two of five of the examined products in concentrations of 110 and 160 mg/kg, respectively. In this case, the quantities of the accelerator in the finished material represented 1-8% of the quantities typically used in the chloroprene rubber production process.

2.2 Consumer products with chloroprene rubber

In order to get an overview of consumer products with chloroprene rubber marketed in Denmark, an images search on Google with the keyword "Neoprene" was made. The search is limited to websites with URL addresses ending in ".dk".

It is noted by the search each time a product of a particular type was found. The results of the search are shown in Table 2.3. Items marked "*" are considered to be included in the categories of products examined in the previous study of products of chloroprene rubber. Product types for which more than 5 products is found within the used period of 4 hours must be considered to be widespread, while product types for which only one product was found is likely to be less widespread. It should be noted, however, that the method requires that it is specifically indicated on the website that the product is made of neoprene.

The products are divided into products with direct skin contact during use, products with partial skin contact during use and products with only short-term skin contact.

One area where there seems to be a marked increase in the use of chloroprene rubber is for bags; bags made of chloroprene rubber for e.g. laptops and cameras have become common in recent years. These products are included in the group of products with only short-term contact. Some of the products appear to be made of chloroprene rubber-like materials, but are not necessarily made of chloroprene rubber.

The Internet search is supplemented with visits to the baby-equipment shop Babysam, the leisure equipment stores Spejdersport and Friluftsland, an IT shop and Ikea's children's equipment department in Aarhus. These visits resulted in the addition of four products, which are marked with "**" in the table.

By visual inspection, several of the products turned out not to be made of chloroprene rubber, although they are marketed as neoprene. The products were made of chloroprene rubber-like fabrics. All jackets, used as ordinary clothing, were not made of chloroprene rubber, and among the products purchased were a pair of sandals for children and a pair of cycling gloves which did not contain parts made of chloroprene rubber. It is quite likely that other products examined may be made of chloroprene rubber-like fabrics.

According to the website of a leading European producer of fabrics made of chloroprene rubber, the fabric is used for the same type of consumer products as identified in this study (SEDO, 2011). The only use in consumer products listed on the site, but not identified in this study, is the use of chloroprene rubber in furniture.

It should be noted that the chloroprene rubber is used in a wide range of technical products, and parts made of chloroprene rubber will likely be found on certain types of tools and some electrical and electronic products. However, it is difficult to distinguish the chloroprene rubber from other elastomers in these types of products if it is not indicated on the product which type of elastomers is used.

Table 2.3
Consumer products identified via an Internet search and visits to retail stores indicating the number of products found

Products	Number found	Notes
Product types with direct skin contact during use:		
Albow support / brace*	III	
Ankle support/ brace *	I	
Face mask	More than 5	Paintball/hardball
Wrist band	II	For athletes, decoration
Swimming trunks	I	Adult, men
Swimsuit	I	Adult, woman
Swim shoes	More than 5	
Swim shoes for kids	IIII	
Bikini	I	
Diving mask*	I	Strap of chloroprene rubber
Diving socks*	I	
Angling-/water sport gloves*	More than 5	
BBQ glove	III	
Potholders	I	
Half finger gloves	III	Wellness and cycling - bought gloves turned out not contain chloroprene
Wrist support / brace **	III	
Dumbbells	More than 5	Metal coated with chloroprene rubber
Kayaking jacket **	I	
Kayaking socks*	II	
Knee support*	More than 5	
Compression bandage*	I	Can be used for ice bag
Headband	I	
Steering wheel cover	I	For cars
Sandals	I	Turned out not to contain chloroprene rubber –however, later other sandals with

Products	Number found	Notes
		chloroprene rubber were identified
Sailing boots/shoes*	More than 5	
Ski mask	I	
Shoulder bandage*	I	
Socks for kids	I	
Surfing jacket*	I	
Swimsuit for kids	More than 5	Wetsuit with no, short or long sleeves and legs
Svim gloves	I	Edge of chloroprene rubber
Swim vest	I	For small children
Water sport cap*	IIII	Surfing, angling, etc.
Water sport vest *	II	Diwing, kayaking, sailing, surfing
Hot shorts	More than 5	Soccer, scouting, outdoor
Wetsuit*	More than 5	Diwing, kayaking, sailing, surfing
Waders*	More than 5	
Product types with partly skin contact during use		
Fitness belt	I	
BBQ apron	I	
Rubber boots*	More than 5	Of chloroprene rubber
Jacket	More than 5	Ordinary clothing - upon closer examination it appeared that the jackets were not made of chloroprene rubber
Training shoes/sneakers	II	
Kidney belt*	I	
Life jacket	II	
Overshoes	More than 5	For cyclists
Spraydeck	More than 5	Coat that makes the water does not get into a kayak
Product types with short-term skin contact during use		
Spectacle case	I	
Bicycle saddle bag	I	
Bottle holder	I	Insulated holder
Football	I	
First aid kit bag	**	
Head cover for putter (golf)	I	
GPS-bag	I	
Chloroprene rubber sleeve to I-PAD (bag)*	I	
Camera bag	More than 5	
Map holder	I	Chloroprene rubber map holder for exercisers
Calculator bag	I	
Medicine box	I	

Products	Number found	Notes
Mobile phone bag	IIII	
Storage bags of different sizes	**	
Laptop bag	More than 5	
Thermo bag	I	
Tea/coffee cap	I	
Toilet bag	I	
Insulated beer can holder	I	

* Products marked with an asterisk have been included in previous surveys of products of chloroprene rubber.

** Products, which were identified only by the visits in stores.

2.3 Possible exposure to thiourea compounds in chloroprene rubber

In the previous survey of chloroprene rubber products, the relationship between consumption patterns and exposure was assessed, and the contact time per week was estimated for a number of products (Nilsson and Pedersen, 2004).

The results of the survey phase from the previous study as to the number of consumers who come into contact with chloroprene rubber products, exposure conditions and estimated duration of the contact on a weekly basis are summarized in Table 2.4. Despite the extensive information search which was conducted in the study, the figures are largely based on rough estimates, but it is projected that it would require a highly comprehensive data collection to improve these estimates. For details about the calculation method and the interpretation of the results, please consult the previous survey.

The contact conditions in Table 2.4 are divided into direct and non-direct contact. There may be some direct contact between the waders and boots of chloroprene rubber and the legs of the user if the user wears ankle socks and short pants, but it is estimated that there would be only short-term direct skin contact with the products.

The “contact time” column is based on the most enthusiastic persons within the various sports; the values can be considered as realistic "worst case scenarios". On a fishing trip for a week, it is not unusual for anglers to wear waders for more than 10 hours each day according to the study. Windsurfers and kitesurfers also often wear their suits all day, removing only the upper part when on shore. For supports, the lower limit is estimated at 15 hours for normal sportsmen and the upper limit at 60 hours for consumers suffering from joint impairments. These are rough estimates.

No temperature has been indicated for the contact, but since the body temperature is typically 37°C, a temperature of about 30°C does not seem unrealistic. The temperature underneath a support may be higher when running or playing ball due to the higher activity level.

It is also likely that the release of substances from the products increases if the products are used under conditions where the user perspires, as in vigorous sports.

A number of products identified in the current study, considered to have a certain use and having direct skin contact, have been added to the list in Table 2.4. The number of consumers of the products and the contact time is roughly estimated.

One type of product that stands out from the others, sandals with chloroprene rubber, is likely to be used intensively during the summer months by some users. There is, therefore, a particular focus on this application.

Chloroprene rubber is used in cushions and inner lining in certain types of sandals for leisure activities, sports and occupational use. A search on the Internet provides many types of sandals wherein it is stated that the straps are lined with chloroprene rubber. The sandals are used both by adults and children. The material, which is covered by a fabric (such as Lycra®), is typically used on the back of the leather.

A leading manufacturer of shoes reports that the proportion of sandals wherein the material is used, has decreased in recent years. The manufacturers have changed to a two-ply polyester material instead of chloroprene rubber because chloroprene rubber is too hot and retains moisture. The manufacturer estimates that about 25% of leisure sandals available on the Danish market contain chloroprene rubber (not flip flops). The manufacturer, the Danish Sports Trades Association and the Danish Shoe Merchant Association were contacted in an attempt to estimate the volume of leisure sandals with chloroprene rubber in Denmark. Neither of the two trade associations believe that it is possible to calculate that figure and do not want to make an estimate. For the purpose of this study, it is considered that there could be 100,000 people who have sandals with chloroprene rubber, and some users may use the sandals without socks and have direct contact between the sandals and the skin for prolonged periods of the year, e.g. during summer or during holidays and hiking activities.

In two cases from the National Allergy Research Centre from 2011 it was reported that a pair of shoes and a pair of gloves containing chloroprene rubber triggered an allergic reaction (Friis, et al., 2011).

Table 2.4
Consumption pattern for chloroprene rubber products (partly based on Nilsson and Pedersen (2004)

Product type	Number of consumers (minimum)	Estimated contact time per week (hours)	Contact conditions
Products assessed by Nilsson and Pedersen (2004)			
Waders	50,000	50	Usually no direct skin contact
Boots (e.g. rubber boots for hunting)	100,000	50	Usually no direct skin contact
Wetsuits and semi wetsuits	25,000	20-50	Direct skin contact
Dry suits	Approx, 5,000	15	No direct skin contact
Gloves	40,000	20-50	Direct skin contact
Socks	25,000	20-50	Direct skin contact
Boots used together with Wetsuits and semi wetsuits	25,000	20-50	Direct skin contact
Supports	100,000	15-60	Direct skin contact
Added products			
Sandals	100,000	15-60	Direct skin contact
Hot shorts	10,000	16	Direct skin contact
Swimming shoes	100,000	7	Direct skin contact
Dumbbells	75,000	3	Direct skin contact

Chloroprene rubber products have different types of surfaces. In relation to a possible exposure, it is likely that the material surface may be of importance.

A description of chloroprene rubber products for wetsuits indicates three types of surfaces (Terrapin, 2011):

- Skin. The sponge can be cured (a.k.a. melted) again after slicing to get a smooth surface where all the bubbles are sealed.
- Coated. A coating is sprayed on the surface, which causes the material to slide more easily over the skin. The coating material can provide a metal-like appearance. It is also quite common that chloroprene rubber products are coated on the surface facing away from the skin.
- Laminated with fabric. Examples of used textiles are Kevlar ®, kanoko, Cordura ® or Lycra ® nylon. If the chloroprene rubber material is covered with fabric, a glue will also be used.

Painted / printed surfaces will typically turn away from the skin, while the other surfaces may well turn against the skin or away from the skin.

2.4 Market volume

The previous study was unable to estimate the annual turnover of chloroprene rubber as well as which products represented the largest tonnages.

It is possible to find the overall market figures for the turnover of chloroprene rubber in the world, but as chloroprene rubber is largely used for technical purposes, it is not possible using these numbers to get an idea of the market for chloroprene rubber in consumer products in Denmark.

For this study it is estimated by a leading supplier of supports that about 100000-125000 supports of chloroprene rubber are sold in Denmark annually and that the turnover of the retail trade for these supports is of the magnitude of 25 million DKK.

It has been investigated to what extent some of chloroprene rubber products are covered by specific commodity codes in trade statistics, where one can expect that a substantial portion of the product group consists of chloroprene rubber products. The products are registered under more aggregated commodity codes such as "track suits, ski suits and swimwear," "waterproof footwear with outer soles and uppers of rubber or plastic" or "clothing made of plastic." Some commodity codes relate to rubber products, but it is estimated that for none of the codes, products of chloroprene rubber will account for a significant part.

The consumption pattern of chloroprene rubber products (Table 2.4) combined with information on the weight of the products clearly indicates that the largest volumes may be found for products for water sports (different suits), and waders and boots. Supports/bandages of various kinds are in widespread use, but the weight of the each product is relatively small compared with the weight of the suits.

2.5 Alternatives to thiourea compounds in chloroprene rubber

There is currently a project under implementation with funds from the EU’s Seventh Framework Programme, FP7, which aims to develop alternatives to thiourea-based accelerators for chloroprene rubber (Safe Rubber, 2011). The project is entitled: "A Safer alternative replacement for thiourea-based accelerators in the production process of chloroprene rubber" and carried out by several national trade organizations in the rubber trade and a number of European companies.

A major international manufacturer of supports indicates that it is in the process of developing a new type of foamed elastomer that is not harmful. This product has the same properties as chloroprene rubber, except that it is not insulating, but it is expensive.

Various supports based on an anti-bacterial and breathable material called HYDRACINN ® (Mueller Sports Medicine, 2011) are marketed in Denmark and internationally. The material is marketed as neoprene-free and latex-free and it is advertised as a non-allergen. The American manufacturer does not specify on its website what the material is made of and what the mercury Hg80 technology employed giving the material its anti-bacterial properties is.. Since the Hg80 technology is illustrated with drops of mercury, the material does not appear to be the most promising alternative.

2.6 Selection of products

The available information suggests that for most product types similar materials are used and that these materials in principle can be produced using the same accelerators.

When composing the list of products examined in this project, the aim was to get as wide a range of products and brands as possible; however, particular emphasis is put on products where there is direct, prolonged skin contact during use (see Table 2.5). Therefore, waders and boots have not been selected, although the number of users of these products is large. A chloroprene rubber sleeve to an I-PAD was selected because this product could be easily be purchased as a replacement for one of the products which turned out not to be made of chloroprene rubber, and because the market for this product type is increasing markedly.

As to products that are used by both children and adults, i.e. wetsuits, bathing shoes, socks and t-shirts, products used by children were preferably selected. There is a particular interest in relation to children subject to the risk of developing allergies to thiourea compounds.

Since many manufacturers of products in Europe import the fabric from countries outside the EU, there will probably not be much difference between finished products produced in the EU and products produced outside the EU.

Table 2.5
Purchased products

No	Product type	Comment
1	Full-length wetsuit for kids	
2	Short wetsuit for kids	
3	Chloroprene rubber T-shirt for kids	
4	Chloroprene rubber socks shirt for kids	
5	Bathing shoes for kids	
6	Sandal with chloroprene rubber linings for kids	Turned out not to be made of chloroprene rubber
7	Flip-flop sandals with chloroprene rubber strap for adults	
8	Sport Sandal with chloroprene rubber linings for adults	
9	Wrist brace	
10	Elbow support	
11	Wrist brace	
12	Thigh support	
13	Kayaking gloves	
14	Cycling gloves, gel without fingers	Turned out not to be made of chloroprene rubber
15	Chloroprene rubber sleeve for I-PAD	
16	Kayaking hood	

3 Exposure scenarios

Among the applications of chloroprene rubber-containing products that give rise to most contact with the products and longest exposure time is the use of wetsuits and supports. The size of the contact surface is of importance mainly for the extent of the local allergic reaction in sensitized individuals, whereas the duration and frequency of exposure alongside the concentration of the allergen and its potency have implications for the likelihood that the allergy is established (sensitization). Sunglasses with a chloroprene rubber edge can also be expected to be used for extended periods of time, as well as the use of gloves,shoes and socks, which may simultaneously add to the total exposure of athletes, among others.

A realistic worst case scenario, for an adult kayaker for example, could be the simultaneous use of a wet suit, supports, kayaking gloves, sunglasses and shoes, which are the uses that are expected to give the most significant exposure. It can be assumed that the products are used simultaneously for 8 hours per day as realistic worst case situation. In relation to allergies, this simultaneous exposure to a number of sources thus primarily be of importance for where on the body the triggering of the allergic response may occur. However, the simultaneous exposure does not influence the risk of induction, which is determined by the concentration of allergen per area and time and thus the migration rate.

Exposure scenarios are developed in accordance with the REACH guidance: 'Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15 - Consumer exposure estimation ', version 2 from April 2010 (ECHA, 2010).

Algorithms and formulas for dermal exposure are based on the models shown in Section R.15.3.2.2, which deal with dermal exposure and scenarios that relate to non-volatile substances that migrate from articles, and R.15.3.3, which deals with oral exposure.

In the health risk assessment in Chapter 5, the substances will partially be assessed in relation to contact allergy, and partially evaluated in relation to other critical effects.

The exposure scenarios will differ slightly depending on the effects to be assessed. In terms of contact allergy, it is the external dermal exposure of the skin, per cm^2 and duration, which are of importance. For the other systemic effects, the total internal exposure is used in the assessment. It will therefore also be relevant to look at how large an area of skin is exposed, and how much is absorbed through the skin.

Exposure times are included in all scenarios. Table 3.1. shows an overview of anticipated realistic worst case exposure times per day for the selected products. When setting the exposure time, the estimated contact time per week as shown in Table 2.4 is used as starting point and estimated what can be considered as a realistic worst case. Two of the purchased products, which turned out not to contain chloroprene rubber, are not included in the table.

Table 3.1
Overview of realistic worst case exposure times for the selected products

No	Product type	Exposure time per day (realistic worst case)	Comment
1	Full-length wetsuit for kids	6 hours/day	It is assumed that children could keep the wetsuit on for periods of up to 6 hours when they are out of the water.
2	Short wetsuit for kids	6 hours/day	As above.
3	Chloroprene rubber T-shirt for kids	8 hours/day	It is assumed that child is using the T-shirt in the course of most of the day.
4	Chloroprene rubber socks for kids	6 hours/day	It is assumed that children wear the socks for a large part of the time they spend indoors.
5	Bathing shoes for kids	6 hours/day	Same assumptions as for wetsuits to kids.
7	Flip-flop sandals with chloroprene rubber strap for adults	10 hours/day	It is assumed that adults use the sandals most of the day in hot weather.
8	Sport Sandal with chloroprene rubber linings for adults	10 hours/day	As above.
9	Wrist brace	8 hours/day	It is assumed that the support is used in connection with e.g. work or special activities like sports, but not all waking time
10	Elbow support	8 hours/day	As above.
11	Wrist brace	8 hours/day	As above.
12	Thigh support	6 hours/day	It is assumed that the support is not used all day, but use in connection with special activities such as sports, or other physical activities.
13	Kayaking gloves	8 hours/day	It is assumed that the gloves would be used on day trips up to 8 hours.
15	Chloroprene rubber sleeve for I-PAD	2 hours/day	I- PADs is often used throughout the day and the handling of the bag is expected to result in up to 2 hours of exposure
16	Kayaking hood	8 hours/day	Same as for gloves.

3.1 Contact allergy

Contact allergy is generally considered to be a threshold effect, but for many substances, a sufficient data base or possibility to determine a threshold value and derive a DNEL or DMEL[1] does not exist. Consequently, the risk assessment is mostly qualitative.

[1] DNEL, Derived No Effect Level, expresses the level of exposure below which no health effects would be expected. DMEL, Derived Minimal Effect Level, is used for effects for which there is not a threshold below which one can say that there are no health effects.

The threshold value for skin sensitization is defined as a concentration (%) of the allergen in contact with the skin, or more precisely, as a dose per unit area skin (µg/cm^2) (ECHA, 2010).

The external dermal exposure of consumers (i.e. the amount of substance applied to the skin per cm^2) corresponds to the measured migration to sweat per unit surface area and unit time during the exposure period.

The external dermal dose per skin area (cm^2) within the defined time period for realistic worst case scenarios is consequently based on the measurements of migration to a sweat simulant. The dermal load is calculated as follows:

$$L_{der} = Migr \cdot T_{contact}$$

3.2 Other effects

Because some thiourea compounds also are associated with carcinogenic and reprotoxic properties, it is relevant to assess what effect is of most significance for the risk.

Tier 1 exposure scenario
For a Tier 1 assessment (first rough assessment of possible risks) of the exposure from chloroprene rubber products, the migration of thiourea compound from purchased products to a sweat simulant has been measured and the results are taken as a measure of the dermal load:

$$L_{der} = Migr \cdot T_{contact}$$

The external dermal dose = $D_{der, external}$ (the dermal exposure of the skin) in mg per kg body weight (bw) can, based on measurements of migration to sweat simulant, be calculated as follows:

$$D_{der,external} = \frac{L_{der} \cdot A_{skin}}{\mathrm{BW}}$$

If the absorption through the skin is inserted in the formula, the internal dermal dose (the amount which is absorbed and may get into the bloodstream) in mg thiourea compound per kg body weight is calculated as:

$$D_{der,internal} = \frac{L_{der} \cdot A_{skin} \cdot F_{abs}}{BW}$$

Parameters and symbols used in the model formulas from the guidance and in the formulas which are used here for the dermal exposure scenarios are explained in Table 3.2.

Table 3.2
Explanation of symbols used in the exposure scenarios (based on ECHA, 2010)

Parameters	Description	Unit
Migr.	Quantity of the substance that migrates to the skin surface per surface unit of the skin and unit of time	$mg/cm^2/hour$
$T_{contact}$	Duration of contact between the article and skin	hour
A_{skin}	Surface area of the contact surface between the article and the skin	cm^2
BW	Body weight	kg
F_{abs} *	Dermal absorption	%
Result parameters	Description	Unit
L_{der}	Dermal load on the basis of migration	mg/cm^2
$D_{der, external}$	External dermal load per day and unit body weight	mg/kg bw/d
$D_{der, internal}$	Internal dermal load per day and unit body weight	mg/kg bw/d

* For a Tier 1 estimate a dermal absorption of 100% is usually used.

4 Analyses

The survey analyzes the content of thiourea compounds in a number of chloroprene rubber products. From a selection of those products where the highest levels of thiourea compounds were demonstrated, analyses of the migration of compounds into artificial sweat have also been carried out

There is no standardized method for the analysis of thiourea compounds in chloroprene rubber or other rubber materials. Consequently, as part of this study, a new method has been developed. The method has been validated in accordance with DEPA's requirements for documentation of the analytical method.

4.1 Description of the methods for chemical analyses

4.1.1 Analysed thiourea compounds

Using chemical analyses, a quantitative determination of thiourea compounds were carried out as shown in Table 4.1.

Table 4.1
Thiourea compounds included in the analyses programme

Name	Synonym	CAS no.
2-imidazolidinethione	ETU	96-45-7
1,3-dimethyl-2-thiourea	DMTU	534-13-4
1,3-diethyl-2-thiourea	DETU	105-55-5
1,3-dibutyl-2-thiourea	DBTU	109-46-6
1,3-diphenyl-2-thiourea	DPTU	102-08-9
1-butyl-3-ethylthiourea	EBTU	32900-06-4
Thiourea	TU	62-56-6

4.1.2 Analysis method for quantitative analyses

A subsample of the products (about 1 g accurately weighed) was extracted with 10 ml of methanol added internal analysis standards of PBC and pyrene-d_{10} at ultrasonic and mechanical shaking, respectively. The extraction was conducted on the total material, even in the cases where the material was coated or laminated with a textile. It was noted whether the material was coated or laminated with a textile.

In connection with the development of the method, it was initially attempted to extract thiourea compounds with a mixture of dichloromethane and acetone. Compared to extraction with methanol, with this mixture lower values were generally determined, and therefore it was decided to use methanol for extraction.

The extract was directly analysed by capillary gas chromatography with mass spectrometric detection (GC-MS-SCAN). The GC-MS analysis was used to ensure the identity of the substances. The identity of thiourea and EBTU is difficult to determine with other methods.

GC-MS conditions:
GC-column: Varian, Cat. No. CP5871:
CP SIL 8 CB low bleed/MS – 30 m x 0.25 mm ID, df = 0.50 µm
Carrier gas: helium 15 psi
Injector-temp.: 260°C
Column-temp.: 55°C (0.5 min.) - 320°C (20°C/min.) in 12 min. (run time: 26.5 min.)
MS Source: 250°C
MS Quad: 150°C
MS tune: atune
2 µl extract injected

Calibration standards and control standards were prepared, as far as was possible, from certified standards. It was not possible to obtain an EBTU standard, so this substance is only identified by molecular weight ("target ion") and an expected fragment ("qualifier ion") by GC-MS (Table 4.2).

Each sample was analysed in duplicate.

Table 4.2
Applied "target" and "qualifier" ions by the GC-MS analysis

Component	Abbreviation	Target ion	Qualifier ion
2-imidazolidinethione	ETU	102	73, 72
1,3-dimethyl-2-thiourea	DMTU	104	74, 71
1,3-diethyl-2-thiourea	DETU	132	60, 71, 131
1,3-dibutyl-2-thiourea	DBTU	188	72, 57, 155
1,3-diphenyl-2-thiourea	DPTU	*1	*1
Aniline		93	66
Isothiocyanatobenzen		135	77
1-butyl-3-ethylthiourea*2	EBTU	160	72
Thiourea	TU	76	60, 59
Internal standard: PBC		112	56
Internal standard: Pyren-d_{10}		212	

*1 DPTU is decomposed to analine and isothiocyanatobenzene and is determined as these compounds.
*2 It was not possible to obtain a standard.

4.1.3 Validation of the method for quantitative analyses

In accordance with the Danish EPA's document "Chemical analyses. Requirements for documentation of the analytical method" a verification of the method as to detection limit, detection range, blank value and recovery/correctness was performed.

Linearity in the range used (5 µg / ml - 30 µg / ml) was demonstrated for the calibration standards listed in Table 4.2. The measured blank values were below the detection limit and without significance to the analysis results.

The detection limits for the chemical analysis were determined to be 2-30 mg/kg at 10 ml of extraction liquid and 1 g of sample, and the quantification limit was determined as 5 times the detection limit.

Table 4.3
Detection limits and quantification limits, quantitative analyses

Component	Detection limit		Quantification limit	
	µg/mL	µg/g	µg/mL	µg/g
TU	3	30	15	150
DMTU	0.5	5	2.5	25
DETU	0.6	6	3	30
ETU	0.2	2	1	10
DBTU	0.9	9	5	50
DPTU	0.9	9	5	50

An investigation of the recovery was carried out by adding thiourea compounds to sample No. 3. The recovery rates are reported in Table 4.4. There is no immediate explanation of why the recovery of some of the substances was significantly lower when 25 µg/ml was added.

Table 4.4
Recovery, quantitative analyses

Component	Spike with 5 µg/mL		Spike with 25 µg/mL	
	% REC *1	% RSD *1	% REC	% RSD
TU	103	7.6	36	17
DMTU	99	4.0	84	2.2
DETU	94	5.1	82	1.8
ETU	94	8.5	78	2.8
DBTU	83	2.9	75	2.1
DPTU	68	8.7	94	6.2

*1 % REC ("recovery") designates the recovery percentage whereas RSD designates the relative standard deviation.

Based on duplicate determinations of all samples, the uncertainty of the analytical method was estimated at approximately 5%.

4.2 Description of the method for migrations analyses

The five products with the largest concentration of DETU were selected for migration analysis. The intention of the migration analysis was to measure the migration from the surface of the materials to a contact medium.

Since the materials typically have a smooth surface, whereas the interior has a cellular structure, there will be a tendency for the cut surfaces, where the internal cell structure of the material is exposed, to have a relatively large surface area, and there may be an exchange of liquid between the inner structures of the material and the contact medium. By a piece of 1.5 x 10 cm and a thickness of 0.3 cm, the cut surface accounts for 23% of the total surface (macroscopically), but due to the internal cellular structure, the effective surface of the cut surface may well account for a considerably greater portion. Since it was not possible to determine the actual surface of the cut surface, the macroscopic surface area of material samples was used through the calculation of the total surface area.

It was investigated whether it was possible to create a test setup where the cut surface was in contact with the artificial sweat, but a workable method was not found, and it was therefore chosen to use a method where a full piece of the material was immersed in the contact medium

4.2.1 Extraction time

It is estimated that in the actual usage situation there will be a constant movement at the interface between the material and the skin and stirring of the contact medium was consequently used for the migration experiments. In many cases, a film of sweat between the material and the skin may also be formed.

Extraction time was set at 6 and 8 hours, reflecting the estimated realistic worst case exposure time. The exposure calculation assumes that the release of substances will remain constant throughout the exposure period and correspond to the measured average release per hour.

4.2.2 Contact medium for migration analyses

The contact medium is artificial sweat according to ISO 105-E04: 2008 "Textiles - Tests for colour fastness - Part E04: Colour fastness to perspiration." The sweat simulant consists of 1-histidine monohydrochloride, 1-hydrate, sodium chloride, sodium dihydrogenphosphate and sodium hydroxide to adjust the pH to 5.5.

The contact medium is heated to 37 °C before addition to the products occurs.

4.2.3 Method for migration analyses

A subsample of the material (1.5 x 10 cm, a thickness of 0.19 to 0.3 cm) was weighed out. The sub-sample was placed in a glass container, so that the entire sample was in contact with synthetic perspiration, which was heated to 37 °C before pouring. The migration test was carried out at a temperature of 37 °C, as the samples were placed in an oven for 6 or 8 hours.

The extract was analyzed directly by liquid chromatography with mass spectrometric detection (LC-MS-SIM). The content of DETU was quantified by use of external DETU calibration standards, prepared in artificial sweat.

Each sample was analysed in duplicate.

LC-MS conditions:
LC-column: Phenomenex Kinetex C18, Cat. No. OOD-4462-YO
100 mm x 3.0 mm x 2.6 µm
Column temperature: room temperature
Flow: 0.3 ml/min.
Mobile phase:
A: Milli-Q water with 0.1% formic acid
B: Methanol with 0.1% formic acid
Gradient fra A: 80%, B: 20% til A: 80%, B: 20%
Injection: 10 µl
Detection: Tg-ion: 133, Q-ions: 131, 132, 134

4.2.4 Validation of method for migration analyses

In accordance with the Danish EPA's document "Chemical analyses. Requirements for documentation of the analytical method" a verification of the method as to detection limit, detection range, blank value and recovery/correctness was performed.

Linearity was demonstrated for the applied detection range (0.0015 to 2.1 µg/mL) for DETU corresponding to 0.0025 to 3.5 µg/cm^2 in 50 ml of acidified sweat and 30 cm 2 sample (either side of the material: 2 x 1.5 cm x 10 cm). No blank values of significance for the analysis results were demonstrated.

The detection limit was determined to be 0.3 ng mL of artificial sweat equal to 0.5 at 50 ng/cm^2 ml acidic sweat and 30 cm^2 sample. The limit of quantification was determined to be five times the detection limit, corresponding to 1.5 ng/ml and 2.5 ng/cm^2 at 50 ml acidic sweat and 30 cm^2 sample.

A recovery of 98-102% for DETU by the analysis of sample 3 spiked with DETU was demonstrated.

The uncertainty of the analytical method is determined at 3% from 6 spikes of artificial sweat with 0.2 and 0.8 µg/ ml, respectively. Duplicate determinations of the products gave a % RSD (relative standard deviation) up to 13%.

4.3 Results of the chemical analyses

The results of the chemical analyses are shown in Table 4.5. Through a visual assessment it was found in the laboratory that two of the products (6 and 14) did not contain parts of chloroprene rubber, but instead were made of a textile material: Consequently, two more products were purchased.

The table shows the average of duplicate determinations. Basic data for each product are listed in Annex 1

In 10 out of 14 tested products, DETU could be measured above the detection limit of 10 mg/kg, while in one of the samples DBTU was measured above the detection limit of 2 mg/kg. In the sample with DBTU, DETU was also measured. Allergy tests often apply a mixture of DETU and DBTU known as MDTU.

The concentration of DETU in the10 materials having a concentration above the detection limit ranged from 33 to 720 mg/kg. By polymerization of chloroprene rubber the accelerators are typically added in concentrations of 0.4-1.5 parts per 100 parts chloroprene rubber, which corresponds to approximately 2,000-10,000 mg per kg of total raw material weight. The highest measured concentration (720 mg/kg) consequently corresponds to 7 to 36% of the originally added quantity (assuming that this corresponds to the typical level), while the concentration of the other products is lower than this.

From the information obtained by the survey, it was expected that several of the products would contain ETU, which is reported to be the most common accelerator used in Europe. The detection limit for ETU is 30 mg/kg and it cannot be denied that ETU has been used as accelerator in some of the products, but that the residual content in the products is below the detection limit. ETU is to a certain extent used together with DETU; for example, in accelerators for chloroprene rubber marketed by Akrochem Corp.

In four of the products, none of the tested thiourea compounds were identified. This reason may be that other accelerators were used in the manufacture, that the material is a different synthetic rubber than chloroprene rubber, or that the residual content of the accelerators is below the detection limit. It should be noted that all products were marketed as chloroprene rubber products ("neoprene" products) and were estimated to be made of a foamed elastomer by the visual assessment.

In the previous Danish study of chloroprene rubber products, DETU was detected in two of five tested products in concentrations of 110 and 160 mg/kg, respectively (Nilsson and Pedersen, 2004).

In a new study from the National Allergy Research Centre, concentrations of DETU in running shoes made of chloroprene rubber at 192-251 mg/kg and 76-77 mg/kg in work gloves were found (Friis *et al.*, 2011). DETU was extracted with acetone in that study. As discussed later in Chapter 5, these products have given rise to occupational contact dermatitis.

The figures found in other studies are therefore within the concentration range found in this study.

Table 4.5
Results of analysis of the extractable content of thiourea compounds in chloroprene rubber products (average of two independent determinations).

Sample no.	Type af product	Concentration, mg/kg						
		TU	ETU	DMTU	DETU	DBTU	DPTU	EBTU
1	Full-length wetsuit for kids	< d.l.	< d.l.	< d.l.	**610**	< d.l.	< d.l.	< d.g.
2	Short wetsuit for kids	< d.l.	< d.l.	< d.l.	**350**	< d.l.	< d.l.	< d.g.
3	Chloroprene rubber T-shirt for kids	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
4	Chloroprene rubber socks for kids	< d.l.	< d.l.	< d.l.	**33**	< d.l.	< d.l.	< d.g.
5	Bathing shoes for kids	< d.l.	< d.l.	< d.l.	**720**	< d.l.	< d.l.	< d.g.
7	Flip-flop sandals with chloroprene rubber strap for adults	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
8	Sport Sandal with chloroprene rubber linings for adults	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
9	Wrist brace	< d.l.	< d.l.	< d.l.	**670**	**60**	< d.l.	< d.g.
10	Elbow support	< d.l.	< d.l.	< d.l.	**140**	< d.l.	< d.l.	< d.g.
11	Wrist brace	< d.l.	< d.l.	< d.l.	**150**	< d.l.	< d.l.	< d.g.
12	Thigh support	< d.l.	< d.l.	< d.l.	**480**	< d.l.	< d.l.	< d.g.
13	Kayaking gloves	< d.l.	< d.l.	< d.l.	**47**	< d.l.	< d.l.	< d.g.
15	Chloroprene rubber sleeve for I-PAD	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
16	Kayaking hood	< d.l.	< d.l.	< d.l.	**140**	< d.l.	< d.l.	< d.g.
Detection limit, mg/kg		30	2	5	6	9	9	30

< d.l. Below the detection level

4.4 Results of migration analyses

On the basis of the chemical analyses, five products were selected for migration analysis. Since in most of the products only DETU was found by the chemical analysis, the migration analysis examined this substance only.

The applied extraction time is identical to the estimated real "worst case" exposure time, which is reported in Table 3.1. For the wet suit and the slippers for children, it was therefore 6 hours, whereas for the two supports, is was 8 hours.

The results are shown in Table 4.6. The total migration of DETU during the extraction period ranged from 5.6 to10.6 $\mu g/cm^2$. The total migration corresponds to the quantity which the user may be exposed to as a maximum during a day. The migration rate, expressed as the migration per hour, ranged from 0.9 to 1.8 $\mu g/cm^2$/hour.

In order to compare the migration rate with the total content of the materials, it is necessary to estimate content of DETU per cm^2 of material. The thicknesses of the investigated chloroprene rubber materials were between 1.9 and 3 mm. The density of chloroprene rubber materials varies with the cell structure, but for materials for wetsuits it is reported to be 0.25 g/cm^3 (DuPont, 2008.) When assuming a density of 0.25 g/cm^3, the total content of the material which had the highest content (slippers for children), can be calculated at 50 µg/cm^2 (720 µg / g * 0.25 g/cm^3 * 0.28 cm). The lowest value of the five materials can be calculated at 24 µg/cm^2 (short wet suit for kids). Compared to these values, the quantities measured by the migration analysis are relatively large. For all products, the migration during the test period corresponds to 24% to 34% of the total extractable content.

In the previous study of chloroprene rubber, the release of DETU from waders over an extraction period of 6 hours was determined to be 0.074 µg/cm^2 in the laboratory (Nilsson and Pedersen, 2004). This release corresponds to an average rate of 0.012 µg/cm^2/hour. In a "real life" experiment, where DETU was measured in the water from a diving suit after use for 155 minutes, a total release to water of 0.00028 µg/cm^2 was found (indicated in the report per cm^2 skin area, but it is assumed here that the ratio of skin area to the area of the surface of the diving suit, which faces the skin, is approx. 1:1) (Nilsson and Pedersen, 2004). Therefore, in the previous study migration rates were measured that were two to three orders of magnitude smaller than the measured rates in this study. There has been no explanation for this difference.

In the laboratory experiment the migration was, similar to the situation in the present study, measured from one full piece of chloroprene rubber material immersed in the contact medium. There was also contact between the cut surface and the contact medium in the previous study. The difference between the two studies is therefore not explained by differences in the experimental conditions as to the contact between the contact medium and the internal cell structure of the cut surfaces.

As stated in section 2.1, U.S. studies of ETU has demonstrated release rates of 2 µg ETU per cm^2 from a chloroprene rubber material by extraction in water at 57 °C over 7 days (NTP, 2011) (stated as 0.02 mg/sqr inch). The total migration of DETU over a 6-8 hour test period is 4-6 times higher than the overall migration of ETU over a 7 day period in the U.S. investigation. Calculated as migration per hour the difference is much greater.

Compared to these two studies relatively high migration rates have been found in this study.

Table 4.6
Results of the investigation of the migration of DETU from chloroprene rubber products

No.	Type of product	Content mg/kg	Extraction time, hours	Sample size (width x length) cm	Thickness mm	Total surface area , cm² *1	Total migration during the extraction µg/cm²	Migration rate µg/cm²/hour
1	Full-length wetsuit for kids	610	6	1.5 x 10	2.4	35.5	10.6	1.8
2	Short wetsuit for kids	350	6	1.5 x 10	2.7	36.2	5.6	0.9
5	Chloroprene rubber socks for kids	720	6	1.5 x 10	2.8	36.4	10.0	1.7
9	Wrist brace	670	8	1.5 x 10	1.9	34.4	8.6	1.1
12	Thigh support	480	8	1.5 x 10	3.0	36.9	7.1	0.9

*1 Indicates the total surface of upper side + bottom + cut surface (it is not taken into account that the cut surface may have a larger surface area microscopically)

5 Health assessment

5.1 Contact allergy – mechanisms and terminology

A variety of low molecular weight substances, such as thiourea compounds, are able to induce contact allergy in humans. Allergic contact dermatitis is the clinical manifestation of allergy, which is triggered locally in sensitized individuals exposed to the allergen over a certain level. Contact allergy is also called Type IV allergy or delayed hypersensitivity. The latter indicates that the clinical response in the form of eczema usually does not occur until 18-48 hours following the exposure, as the immune response involves a number of cell-mediated processes, which takes time.

Figure 5.1
Examples of contact allergy to products of chloroprene rubber (Photos courtesy of National Allergy Research Centre).

In Denmark it is assumed that at least 10% of the population suffers from contact allergy to one or more substances (National Allergy Research Centre)[2]. It is not yet known why some people more readily develop contact allergy than others, but factors such as hereditary predisposition, skin protein composition and various environmental factorsplay a role. Among the environmental factors are the potency of the contact allergen, the concentration of the allergen, duration and frequency of exposure and possible concurrent exposure to skin-irritating substances (National Allergy Research Centre)[3].

Contact allergens are predominantly haptens, i.e. low molecular weight substances that can permeate into the skin and react with proteins in the top layer, thus forming immunogenic hapten-protein complexes that are regarded as foreign by the body. But even substances which do not readily react with proteins can trigger contact allergies by bioactivation to reactive compounds in the skin. These so-called prohaptens contains structural elements which are sensitive to metabolic transformation and the formation of protein-reactive compounds, which can trigger a contact allergy. This transformation can also occur in the skin by the cytochrome P450 enzymes.

Basically, the allergy develops in two phases, an initial sensitization phase or induction and a subsequent provocation or elicitation phase.

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[2] http://www.videncenterforallergi.dk/
[3] http:// http://www.videncenterforallergi.dk/allergi-eksem-sygdomsmekanisme.html

In the sensitization phase, the immune system reacts to the exposure, where the formed hapten-protein complexes are captured by the so-called Langerhans cells, which transport substances to the draining lymph nodes. Here they come into contact with T lymphocytes, which thereby are activated and are capable of recognizing the allergen by the next exposure.

The cells divide and form a clone of T cells, which is spread in the blood stream and the lymphatic vessels and will be capable of recognizing the hapten-protein complex by subsequent exposure to the allergen. Hereby, the allergy is formed. There are no symptoms during this phase.

By the next exposure, the allergen is recognized by the T-cells that will stimulate the formation of a series of signal substances, which in turn activate other cells of the immune system, including macrophages. Macrophages will then attack the cells with the allergen on the surface and release destructive enzymes, resulting in tissue destruction and eczema in the areas of skin where exposure takes place. This phase is called the elicitation phase and the symptoms which usually occur 24-48 hours after exposure.

Very potent allergens may cause sensitization by the first contact, while less potent allergens only work by sensitizing after several years of exposure.

Contact allergy is usually considered to be a threshold effect, but individual differences in sensitivity are determinants for the induction and elicitation levels. Therefore, significant variations from person to person are seen, and setting a DNEL for the risk assessment can be difficult. The potency of the allergen will particularly influence the threshold value for the induction, while the elicitation is less dependent on the potency. In connection with the exposure and risk assessment the dose per skin area is essential (WHO, 2008). The induction and elicitation thresholds can be used as a measure of the potency of the substance and is calculated based on the concentration tested, the size of the application site (exposed area) and the application volume.

Commonly accepted methods for testing of the allergenic properties of chemicals are all based on experimental animal models. The tests are designed primarily to indicate the risk of induction, i.e. the risk of healthy people to develop allergies.

The most widely used model has previously been Guinea Pig Maximization Test (GPMT), wherein the test substance is injected into guinea pigs in conjunction with Freund's complete adjuvant (a solution of antigen is added to boost the immunization). An allergic reaction is triggered subsequently by a closed patch test in which the substance is applied to the skin surface at a concentration slightly below the concentration that may cause irritation. The test has been used for the potency assessment based on the number of animals reacting in the test, but gives no information about the threshold dose, as usually only a set of concentrations is tested.

Today tests in local lymph nodes in mice are preferred, and these tests are also more suitable for evaluating the potency. The principle of the Local Lymph Node Assay (LLNA) test is that allergens cause a greater division of white blood cells in the lymph node which drain the area where the allergen is applied. The more the substance applied, and the more potent it is, the stronger the division that occurs in the white blood cells in the lymph node. The degree of cell division is compared with the untreated control animals. The relationship between treated and untreated mice can be expressed in an index number, and this index must be at least 3 in order to consider the substance as a possible allergen.

In a more sensitive version of the lymph node-test, Sensitive Lymph Node Assay (SLNA) sensitization is determined by evaluating the total activity in the lymph node.

Knowledge of induction levels can also be obtained from healthy humans' common use of specific products with a known content of allergenic substances. However, it is often not known what the content has been in products that are used and the induction phase does not result in symptoms. Therefore, the problem is not recognised before the eczema is induced, i.e. when the elicitation occurs. In general, the amount required to induce allergy will be higher than the dose that can induce eczema in the sensitized (elicitation). If the elicitation level is applied as the threshold value in the risk assessment, healthy people will be protected from developing allergies and those already sensitized protected from developing allergic dermatitis.

The risk of both induction and elicitation depend in practice on the specific exposure conditions e.g. the frequency of applications, the skin areas, vehicles, etc.

Knowledge of elicitation in practice is obtained only from studies of people who have developed allergies to specific substances.

Diagnosis of contact allergy in humans is carried out using a patch test, also called the epicutaneous test, whereby the patient is exposed to small amounts of the allergen in a small chamber that is attached on the back with a patch. Here it is allowed to sit for 48 hours; hereafter, the skin reaction is read in accordance with an international standard. The reaction may take up to 8-14 days to manifest itself.

A glossary of relevant terms used in connection with contact allergy is shown in the box below.

Allergens are substances that cause allergies.

Contact allergy is a specific cell-mediated allergic reaction of the skin to low molecular weight substances (haptens), which are absorbed into the skin and activate the immune system in the form of T-cells capable of recognizing the substance on subsequent exposure. Contact allergy develops in two stages called *induction* or *sensitization* and *elicitation*. Contact allergy is also called Type IV allergy or delayed hypersensitivity. Both phases are dependent on several factors, including the potency of the substance and the extent of the exposure, i.e. concentration, duration and frequency.

The symptoms of contact allergy are eczema, which causes redness and swelling of the skin. It may occur as bumps and blisters that turn into sores. If the eczema is not treated, there will be cracks in the skin. The skin becomes thick and scaly, and eczema can become chronic. Eczema occurs first on the skin where the affected individual has been in contact with the allergen.

Induction or **sensitization** is the stage where the person is first exposed to the allergen, which is captured by the Langerhans cells of the immune system. Those cells transport the substance to the deep lymph vessels, and on to the lymph nodes, where there is an activation of specific T-cells. The T-cells divide and spread in the blood stream where they will subsequently be able to recognize the allergen. The induction phase is asymptomatic.

Elicitation or **provocation** is the phase where the sensitized person is exposed to the allergen that is recognized by the activated T-cells. T-cells bind to the allergen, and elicit the reaction of the immune system, leading to local tissue destruction, and symptoms of eczema.

The elicitation threshold is the highest concentration (in %) or dose per area of skin (μg/cm2) of the allergen, which does not trigger any allergic reaction in a sensitized subject. In principle, this concentration will be lower than the concentration that results in the induction.

MET10 (MET = minimum threshold elicitation) is the minimum elicitation level that induces a response in 10% of the individuals tested.

Potency can be defined as the relative ability of a chemical to induce sensitization, which is determined by the amount of chemical per unit area required for the acquisition of skin sensitization in an individual not previously exposed (WHO, 2008). Data for assessment of potency can come from both human experience / studies, tests in local lymph nodes in mice (LLNA / SLNA) and possibly other animal (Guinea Pig Maximization Test and the Buehler test). Results from the latter tests can rarely be quantified.

Haptens are low molecular weight substances which are not in themselves capable of inducing an immune response, but which when bound to a larger molecule, for example. a protein, can be a sensitizer.

Prohaptens are substances that can only activate the immune system following metabolism and bioactivation in the skin.

Patch test (epicutaneous test) is a well-established method for diagnosing allergic contact dermatitis. In the test the patient is exposed to one or more allergens that can provoke an eczema reaction if the person is sensitized. Test substances applied to the skin under occlusion (sealed) by means of the exposure chamber and patches, or "ready-to-use" test formulations or a low concentration of the allergen is placed on the back. After 2 days, the patch is removed, and the individual may then see if there has been a reaction on the skin within a week where the test patch test reactions assessed by an international standard and a response can be classified as arising from questionable (+ / -), weak (+), strong (+ +) or extreme positive (+ + +).

Clinical relevance of contact allergy to a substance is defined as contact dermatitis caused or worsened by a probable/proven exposure to the allergen. Contact eczema must wholly or partially be explained by exposure to the allergen in terms of temporal correlation, localization and intensity of exposure, ideal concentration of allergen.

Sources: National Allergy Research Centre; ECHA, 2010; AMI, 2004; Dansk Kontaktdermatitis Gruppe, 2005

5.2 Review of existing knowledge on contact allergy associated with exposure to thiourea compounds

Much of the available literature on contact dermatitis caused by thiourea compounds in chloroprene rubber is derived from case reports demonstrating the allergic effects of the substances in people exposed to various product types that involve close skin contact, including rubber gloves, knee braces/supports, waders, wetsuits , wrist supports for computer keyboards, running shoes and rubber straps (Gudi *et al.*, 2004; Kroft and Valk, 2007; Liipo *et al.*, 2011; Martínez-González *et al.*, 2009; Aplin *et al.*, 2001; Alcántara *et al.*, 2000; Buus *et al.*, 2002; Mizuno *et al.*, 2011; Munoz *et al.*, 2008; Woo *et al.*, 2004; Yokota *et al.*, 2007; Friis *et al.*, 2011).

In addition, retrospective analysis of patch test results from large groups of patients suspected of allergy to rubber chemicals in North America, Sweden, Germany and Finland are reported.

The larger retrospective studies of sizeable patient groups often distinguish between occupational relevance of allergy and general clinical relevance.

The analyses from the North American Contact Dermatitis Group (NACDG) are from the periods 1998-2000 and 1992-2004. MDTU (mixed dialkyl thiourea = DETU + DBTU) has been a regular part of the NACDG standard test package since 1991. In the package, the substances are tested in 1% petrolatum. MDTU was chosen as the allergen of the year in 2009 by NACDG because it had the highest rate of clinical relevance among the substances in NACDG's standard battery. From 1998 to 2000, among 5,807 patients patch tested by NACDG with MDTU, 1.1% positive reactions were found. Of these clinical relevance was considered certain for 15.6%, probable for 25% and possible for 42.2%. (Anderson, 2009; Comfere *et al.*, 2005)

NACDG's results for 22,025 patients with allergies tested in the period 1992 to 2004 showed that 225, or 1%, responded positively to MDTU. Of these, 173 (76.9%) were currently relevant and 29 (17.1%) were occupationally relevant. Positive reactions with other rubber chemicals were significantly higher in the patients who responded positively to MDTU compared with those who did not react. Furthermore, it is concluded that it is necessary to involve several individual thiourea compounds in the future to better characterize the epidemiology, cross-reactivity, sources and clinical significance of allergic contact dermatitis from thiourea compounds. (Warshaw, *et al.*, 2008)

Results from the period January 1999 - June 2001 from the contact dermatitis group of the Mayo Clinic (USA) showed a 1.2% positive reaction rate among 1321 patients tested. The same criteria for evaluation of test results were used throughout the test period. During the period 2001 to 2002, results from NACDG showed a rate of 0.8% among 4,897 patients. Of these, 18.4% were considered certain in terms of relevance, which at the time placed MDTU in second place after the epoxy resin. Footwear was considered the main cause, together with gloves used in occupational settings. The results led to a recommendation that MDTU should be considered in the context of screening tests. It is estimated that screening with MDTU captures up to 75% of relevant thiourea reactions (McCleskey and Swerlick, 2001).

In a Swedish study 28 (1.1%) out of 2,491 routinely tested patients at University Hospital in Lund had positive allergic reactions to DPTU. The substance was tested as the department of occupational dermatology of the hospital had used a tape for patch test for many years, which proved to contain DPTU. Fifteen of the 28 DPTU-positives were further tested with dilutions of DPTU, and 11 were tested with dilutions of phenylisothiocyanate (PITC). One patient reacted down to a concentration of 0.000001% DPTU. All patients who responded to DPTU also reacted to PITC. It is not clear whether the patients are sensitized to both substances or only one of them, since both substances are contaminated with the other. It is also not known if sensitization is caused by other substances which are present together with substances, or are formed in the skin. The concentrations of DPTU in the tape was lower than the 0.3-0.5% (w/w) which was added to the PVC material, as the substance is decomposed by heat during the production process. But the small remaining amount was sufficient to elicit the patients' reactions. Most patients responded to concentrations down to 0.001%(DPTU 0.4×10^{-4} mol/L, PITC 0.8×10^{-4} mol/L). (Fregert *et al.*, 1982)

In a German study, 67,188 patients who were suspected of having rubber glove allergy were patch tested in 1995-2001 in dermatology departments belonging to the "Information Network of Departments of Dermatology" at the universities of Göttingen and Erlangen. Among patients with occupational glove allergy, 1,443 were patch tested with DPTU and 1,436 with DBTU. Positive reactions were observed in 0.3 and 0.1% of patients, respectively. Reactions to other chemicals (including thiuram) in the rubber gloves were observed in a greater proportion of patients and concluded that thiourea compounds play a minor role in rubber gloves and contact allergy. (Geier *et al.*, 2003)

A Finnish study included 15,100 patients, which were patch tested at five different hospitals with a mixture of DETU (0.5%), DBTU (0.5%) and DPTU (0.5%). One thousand five hundred reacted positively to the mixture. Among 1,500 suspected of having rubber allergy, 59 (0.39% of all patients studied) patients demonstrated positive reactions to thiourea mixture, thirty-five of these with a response of 2+. The individual thiourea compounds were tested on 33 of these 59 who reacted positively to the mixture. DETU gave a positive response in 24 (73%), DPTU in 5 (15%) and only 1 (3%) responded to DBTU. Men with contact allergy to thiourea compounds were on average 10.5 years younger than women, a result that is expected to be caused by more extensive contact with sports equipment. Positive reaction to the mixture but not the individual thiourea compounds was seen in four individuals. The authors expect that a higher concentration of the substances (1%) could have triggered a positive response. (Liippo *et al.*, 2010)

Cross-reactivity between the individual thiourea compounds has not been reported in any of the above studies, and is considered to be unusual (Liippo *et al.,* 2010).

To clarify the mechanism of contact allergy induced by DPTU, Samuelsson *et al.* (2010) have investigated whether DPTU is a prohapten which is activated by metabolism in the skin. Metabolites formed in the treatment of liver microsomes and P450 enzymes included 1-diphenylurea (DPU), phenylisothiocyanate (PITC) and phenyl isocyanate (PIC). These metabolites, along with DPTU tested in the local lymph node assay in mice (LLNA) where two out of three metabolites were strong skin sensitizers whereas DPTU itself, as previously known, gave no positive result. This may explain why patch testing with DPTU may give false negative reactions when skin permeability is limited and hence the concentration becomes very small. In addition, the authors suggest that the metabolites should be included in the screening tests to increase the probability of diagnosing contact allergy to DPTU. This recommendation appears in much of the referenced literature since underdiagnosis of contact allergy to thiourea compounds in rubber using the standard screening series is an acknowledged problem.

Allergic contact dermatitis caused by thiourea is generally expected to be under-reported, since thiourea compounds has not been a part of the standard patch test series used in the clinics. Meanwhile, the use of chloroprene rubber, among other protective gear for the ageing population, has increased in recent years and may lead to a change in the perception of thiourea-sensitization as an uncommon cause of rubber allergy (Sakata *et al.*, 2006).

With regard to the induction and likely elicitation levels, the literature is somewhat limited. Based on a single case report, it is stated in a study from the Danish Allergy Research Centre that shoes containing DETU at 192-251 ppm triggered an allergic reaction (Friis *et al.*, 2011). In another case, an allergic reaction to a content of DETU of 76-77 ppm in work gloves was observed. It is emphasised, however, that it must be confirmed in further studies whether these levels are representative of elicitation concentrations for chloroprene rubber products (Friis *et al.*, 2011).

In the Swedish study, the content of DPTU in the tape which caused allergic reactions was 0.005 to 0.10% (w/w). In most of test samples the content was in the range of 0.01-0.02% (w/w) (100-200 ppm. The content of the PITC was 0.001 to 0.003% (w/w). In the patch tests, the patients reacted at dilutions of DPTU and PICT down to 0.001% (10 ppm) (Fregert *et al.*, 1981).

Reported levels of thiourea compounds in the test devices for the patch test was 0.5-1% (w/w) in the Finnish study.

Based on the available literature, it is difficult to distinguish precisely between induction levels and elicitation levels because it is not always clear what is causing the sensitization.

A summary of results of the retrospective studies from the referenced literature are shown in Table 5.1.

Substance	Number of tested	Products	Type of investigation	Results
DETU+DBTU	5,807 (patients patch tested to a standard battery)	Not known/reported	Retrospective analyses (NACDG/USA) 1998 – 2000	1.1% positive.Of these 15.6% certain, 25% probable and 42.2% possible.
DETU+DBTU	21,898 (patients patch tested to a standard battery)	Footwear (20%) and gloves (12.9%) most frequent cause of the allergy	Retrospective analyses (NACDG/USA) 1992 – 2004	1% positive (225) 76.9% (173) Clinical relevant and 17.1% (29) occupational relevant. 25% of 173 'positive' reacted positive on other rubber chemicals
DETU, DBTU, DPTU, ETU	1,368 (patients patch tested to a standard battery)	Not known/reported	Retrospective analyses (Mayo Clinic/USA) 1999 – 2001	2.4% positive day 5. Of these 42% clinical relevant reactions.
DPTU, PITC[1)]	2,491 (patients patch tested to 1% DPTU in petrolatum and 0.1% in acetone)	Tape backing used for patch test, Leukoflex® same batch: 0.005-0.10% (w/w) but most samples with 0.01-0.02% PITC: 0.001-0.003%, In DPTU used for the test: 0.007%	Patient investigations. Lund. University Hospital	28 positive to DPTU in conc, below 1% All tested patients sensitive to DPTU did not react to PITC. Not clear if the patients reacted to both DPTU and PITC – or one of them. Both are contaminated with the other. 0.3-0.5% (w/w) DPTU in PVC

Substance	Number of tested	Products	Type of investigation	Results
		PITC I PITC used for tests: 0.19% DPTU,		and 0.5-1.5% in rubber. I PITC used for tests and exposed to light the content of DPTU increased from 0.007% to 1% over two weeks.
DPTU, DBTU	67,188 patients patch tested during the period 1995-2001 by Information Network Departments of Dermatology (IVDK) 9,551 had occupational contact dermatitis, 3,786 were tested for glove allergy, 2047 met both criteria	Rubber gloves	Retrospective analyses (Information Network Departments of Dermatology (IVDK) Germany) 1995 – 2001	Positive response by patients with occupational contact allergy and glove allergy to DPTU: 0.3% of 1443 and DBTU: 0.1% of 1436. Thiourea and 1-DPG seem to play a minor role in rubber glove allergy Rubber glove allergy is most frequent seen in health workers and secondly in cleaning staff.
DBTU, DETU, DPTU	15,100 patients patch tested during the period 2002 to 2007 at five Finnish dermatological clinics. 1,500 with suspected rubber allergy were tested to the rubber chemicals series.	Knee supports, footwear, sports and angling equipment and rubber handle	Patient investigations (Dermatological clinics. Finland) Concentration of TU compounds in mixture: 0.5% Concentration of individual substances: 1%	The mixture: 59 positive (0.39%). By 35/59 the reaction was 2+ or more. DETU positive in 33/59 (73%). DPTU positive in 5/33 (33%). DBTU positive in 1/33 (3%). 4/59 did not react on the individual thiourea-compound, men three reacted on thiuram. Cross reactions between individual thiourea compounds were not observed Thiourea sensitization by 0.30% of the Finnish dermatology patients with suspected contact allergy. In a similar U.S. study 1% reacted positively to thiourea. Clinical relevance was slightly lower in the U.S. survey. Men were on average 10.5 years younger a women (explained by sports equipment)

A summary of the results of case reports discussed in the referenced literature are shown in Table 5.2.

TABLE 5.1
SUMMARY OF CASE REPORTS OF CONTACT ALLERGY TO THIOUREA COMPOUNDS (SEE REFERENCES IN THE DESCRIPTION ABOVE

Substance	Number of tested	Products	Type of investigation	Result
DBTU, DETU, DPTU	1 woman, 51 years, previous history of asthma and rhinitis	Slimming suit	Case report, patch test: Test of product, DPTU, DETU, DBTU. Positive for DPTU.	Itchy erythema-vesicular eruption on the thighs and buttocks. No cross-reaction to DETU and DBTU
DBTU, DETU, DPTU	1 woman, 55 years, no previous history of allergy or atopy.	Orthopaedic hip support with chloroprene rubber edges containing DPTU.	Case report, patch test:	Itchy erythema-vesicular eruption on the thighs and waist. Patch test: positive reaction for DPTU. Tested 2 months after the initial reaction. Cross-reactions discussed.
DETU, DBTU	1 man, 29 years	Wetsuit	Patch test	Eczema on the neck, back and body. Positive DBTU of (+) and DETU (+ + +) and the wet suit.
EBTU, DETU, DBTU	1 man, 59 years	Knee support	Patch test	Itchy dermatitis, which spread from the knee to the whole body. Strong reaction to DETU, DBTU and EBTU and the knee support.
TUs	1 woman, 50 years	Breast implant	Patch test	Granulomatous reaction. ACD from thiourea and thiuram and may be under-diagnosed, since they are not part of the standard test.
DETU	1 man, 22 years 1 man, 44 years	1) wetsuit/running shoe Content in shoe: 192-251 ppm DETU. 2) Arm support / chloroprene rubber glove Content in gloves: 76-77 ppm DETU	Patch test	1) Positive response to DETU and the chloroprene rubber part of the shoe 2) Positive response to DETU and gloves Likely elicitation levels: Contents of shoes: 192-251 ppm DETU. Contents of gloves: 76-77 ppm DETU. To be further investigated whether this is the general levels for elicitation
DPTU, DBTU	1 woman, 28 years	Wetsuit	Patch test with the standard rubber and textile series	Itching and erythema around the body 4 hours after use of the wetsuit. Positive reaction to DBTU and wetsuit in the patch tests after 48 and 96 hours. No cross reaction to other thiourea compounds.

Substance	Number of tested	Products	Type of investigation	Result
MDTU, DBTU, DETU	1 man, 55 years	Rubber products, tourniquet	Patch test standard	Itchy eczema on the hands and eyelids. Positive reaction to MDTU, DBTU and DETU. Standard patch tests do not capture 25% of thiourea allergies. Recommends the use of the R-1000 series, especially for health workers.
MDTU, DETU	1) Woman, 43 years 2) Woman, 19 years	Chloroprene rubber handle of a dolly	Patch test	1) dermatitis on the palms, fingers, wrists and arms 2) the palms and fingers Positive reaction from both to MTDU and DETU History of childhood eczema
DETU	1 man, 41 years, atopic dermatitis during childhood	Chloroprene rubber waders	Patch test	Itchy erythema on the lower legs spread to the back and arms. Positive reaction to DETU in patch test.
DBTU, DETU, DPTU	1) Woman, 60 years 2) Man, 49 years 3) Woman, 68 years	Knee support Wrist support for keyboard oxygen mask (rubber strap)	Patch test	1) Positive DETU 2) Positive TU and DETU 3) Positive DETU and DBTU.
MDTU	1 man, 62 years	Leg prosthesis	Patch test	Positive response in patch test to MDTU
MTDU, DETU	1 woman, 66 years	Knee support	Patch test	Allergic reaction after 18 months. Positive reaction in patch tests to MDTU, DETU and knee
MDTU	1 Woman, 29 years	Computer wrist support	Patch test	1) Positive MDTU (+++)

In a local lymph node assay in mice (LLNA - murine local lymph node assay), in which four thiourea compounds (diphenylthiourea (DPTU), dibutylthiourea (DBTU), dilaurylthiourea (DLTU) and diethylthiourea (DETU)) were tested, only DLTU was classified a sensitizer. In the more sensitive SLNA test (sensitive mouse lymph node assay), all four substances were found to be sensitizing.

The results of in vivo animal tests and ranking of the potency of sensitization are listed in Table 5.3 (Ikarashi *et al.*, 1994).

TABLE 5.2
RESULTS OF POTENCY ASSESSMENT OF FOUR THIOUREA COMPOUNDS IN GPMT- AND SLNA-TESTS, RESPECTIVELY

Substance	Type af investigation	Result
MDTU, DBTU, DETU	LLNA (murine local lymph node assay) SLNA (sensitive mouse lymph node assay) Comparison with GPMT (Guinea Pig Maximization Test)	Sensitization - potency of GMPt: DPTU> DBTU> DLTU> DETU Comparable results using SLNA: DPTU> DLTU> DBTU> DETU

5.3 Other critical effects of the thiourea compounds of relevance for the risk assessment

It may be relevant to involve other critical effects than allergy in the risk assessment, among these long-term effects like cancer, toxicity to reproduction and endocrine disrupting effects.

This is relevant for thiourea (TU) and ethylene thiourea (ETU).

TU is suspected of causing cancer and reproductive harm (classified as carcinogenic and toxic to reproduction category 2 (Carc. 2 and Repr. 2 of CLP - suspected to cause cancer and harm the unborn child). It should be emphasized that no information indicating that thiourea was used in chloroprene rubber has been identified, and the substance is not detected in the investigated samples.

ETU is toxic for reproduction and classified as such in the category Repr.1B (CLP). ETU is included in the EU's list of potential endocrine disruptors. ETU is not detected in the analyzed samples, but is known be used to some extent for the production of chloroprene rubber products.

An examination of review documents from WHO Environmental Health Criteria (1988), IRIS (Accessed January 2011 - online, last revised 2003) and IARC (2001) for ETU and WHO Concise International Chemical Assessment Document 49 (2003) for TU gave the following overview of the impact areas.

Thiourea

The critical effect of TU is the inhibition of the thyroid gland function, as has been demonstrated in both humans and animals (WHO, 2003). TU was previously used as a thyroid-inhibiting agent against raised metabolic rate (hyperthyroidism), where doses of <0.2 mg/kg bw/day did not result in inhibition, while doses of 1.0 mg/kg bw/day resulted in improvement of the condition (WHO, 2003).

TU has been investigated in a number of carcinogenicity studies in mice and rats. Most studies demonstrated effects on the thyroid and the influence on body weight. TU has not been tested in the standard guideline tests in rodents. There are several studies from before the mid-1960s, and they describe the occurrence of tumours in several places other than the thyroid, but the distribution varies between individual studies. Moreover, essential details for adequate evaluation of the experiments are lacking. There are no reports of cancer caused by exposure to TU in humans (WHO, 2003).

Genotoxicity has been investigated in rats in a micronucleus test whereby the animals were given two successive doses of 20% of the LD_{50} value (350 mg/kg bw). No symptoms of toxicity resulted from the treatment. Other *in vivo* and *in vitro* studies predominantly produced negative, but not consistent, results. TU is not considered to be a genotoxic carcinogen (WHO, 2003).

Tests of reproductive toxicity are also older and typically conducted with one dose. A single male fertility study was carried out with doses ranging from 5 to 500 mg/kg bw, but the actual levels are not provided. It appears that a likely NOAEL is 35 mg/kg bw per day (WHO, 2003). Teratogenic effects in rodents are not reported. However, there are described effects on foetuses *in utero* in sheep and lambs.

Effects on the thyroid gland have also been reported for humans who have been occupationally exposed to TU. In a Russian study, thyroid hyperplasia was reported in 17 of 45 workers exposed to a TU concentrations in air of 0.6 to 12 mg/m^3, corresponding to 0.07 to 1.4 mg TU/kg bw/day (bw = 70 kg ; inhalation: 1 m^3/hr for 8 hours/day and 100% uptake). Details on the exposure type and size in other studies are not reported (WHO, 2003).

TU passes the placental barrier. Experiments in rats at maternally toxic doses (0.25% in drinking water), was toxic to the foetuses of the dams.

There are no available studies to illustrate the long-term effects on the skin.

Ethylene thiourea
The critical effect of ETU is increased cell formation in the thyroid tissue (hyperplasia). ETU is also suspected of being an endocrine disruptor, and is included in the EU list of potential endocrine disruptors.

ETU has been demonstrated to inhibit the formation of the thyroid hormones T4 and T3 in experimental animals, leading to elevated levels of thyroid-stimulating hormone (TSH) in serum. Prolonged and continuous exposure to elevated levels leads to hypertrophy and hyperplasia of thyroid follicular cells in rats, mice, monkeys and dogs, and ultimately to the development of adenomas and carcinomas in rats and mice (Charles *et al.*, 2000).

Experiments with short-term and long-term exposure of rats with different dietary doses indicated that the LOAEL for thyroid hyperplasia was 5 mg/kg bw/day; the NOAEL is therefore below 5 mg/kg bw/day.

In an unpublished 90-day dietary toxicity study from 1985 in rats, the NOAEL was considered to be 1.72 mg/kg bw/day for males and 2.38 mg/kg bw/day for females based on the incidences of thyroid hyperplasia and in females also increased liver weight (IRIS, 2011 - online).

In 2007, the U.S. Environmental Protection Agency (U.S. EPA) published a risk assessment for ETU, occurring as a metabolite from a number of dithiocarbamate group of fungicides (among others, manozeb). The risk assessment is followed by an addendum in April 2011. The assessment refers to subchronic and chronic toxicity studies in rats, mice and dogs, which showed reduced levels of thyroid hormone T4, changes in thyroid hormone T3, increase in levels of thyroid stimulating hormone (TSH), weight gain, thyroid and microscopic changes, mainly in the form of hyperplasia. Obvious necrosis in the liver reported from chronic studies in dogs (EPA, 2007).

In a Danish study (Axelstad *et al.*, 2011), which aimed to assess whether perinatal exposure to manozeb (including the metabolite ETU) would result in development-related neurotoxicity, a dose-dependent reduction of hormone T4 in the dams was observed, but not in the offspring. This study contributes to the suspicion of ETU's endocrine-disrupting effects.

In a chronic toxicity study in rats with oral administration of ETU, thyroid adenomas and carcinomas were observed in the two highest dose groups, which received 250 mg/kg diet and 500 mg/kg diet respectively. Increased incidence of thyroid hyperplasia was observed at all doses down to 5 mg/kg diet (WHO, 1988 and IARC, 2001). Ethylenethiourea would be expected not to be carcinogenic to humans exposed to concentrations that do not lead to alterations in thyroid hormone homeostasis. (IARC, 2001).

ETU has been evaluated by IARC (2001) and classified in group 3 (Not classifiable as to its carcinogenicity to humans due to lack of knowledge).

Studies to elucidate teratogenicity in rats, rabbits, guinea pigs and hamsters showed effects on foetuses at dose levels of 10 mg/kg bw and above. Study of developmental toxicity in rats receiving up to 250 ppm in the diet and mouse receiving up to 1000 ppm gave rise to set a LOAEL for rats of 18.8 mg/kg bw/day and a NOAEL of 6.2 mg/kg bw/day based on decreased survival of offspring, and reduced growth. Same effects in mice indicated a LOAEL of 150 mg/kg bw/day and a NOAEL of 49.5 mg/kg bw/day (IRIS, 2011 online, last revised 2003; WHO, 1988).

U.S. EPA has assessed that the nervous system is the target for ETU in studies to elucidate the developmental toxicity and effects such as exencephaly, dilated ventricles, oedema and atrophy of brain tissue. This has been observed in studies with rats, in which effects were seen at concentrations down to 10 mg/kg bw/day (LOAEL). Based on this, the NOAEL was set at 5 mg/kg bw/day (U.S. EPA, 2007).

A two-generation study in rats with oral administration of 2.5 to 125 ppm ETU in the feed showed no effects on reproduction (U.S. EPA, 2007).

There are no available studies to illustrate the long-term effects by skin contact.

NOAEL values

Table 5.4 shows the lowest NOAEL values found in the referenced review literature on ETU and TU. The values will be included in the determination of the DNELs in the risk assessment.

Table 5.3
NOAEL-values for ETU and TU

Sub-stance	Test	Effect	Exposure route	NOAEL	Source
TU	Chronic toxicity in mice, 2 year	Enlarged thyroid gland and reduction of body weight	Oral (drinking water)	6.88 mg/kg bw/d	WHO, 2003
ETU	90-day food toxicity (Not considered acceptable by U.S. EPA (2007))	Thyroid hyperplasias	Oral	1.72 mg/kg bw/d	IRIS, 2011 (2003)
ETU	Developmental toxicity	Effects on the central nervous system and the brain incl. exencephali	Oral	5 mg/kg bw/d	U.S. EPA, 2007
ETU	Carcinogenicity in rats and rabbits	Thyroid hyperplasias	Oral	5 mg/kg bw/d (NOAEL for all investigated effects)	WHO, 1988

5.4 Health risk assessment

5.4.1 Contact allergy

Through the health assessment of the thiourea compounds, no data have been identified which can be used to set the actual threshold levels for the induction and elicitation of allergy by exposure to thiourea compounds. Data from the literature refers primarily to studies of patients who have already developed an allergy. Generally, however, the risk of developing allergies increases with the dose, but the potency of the substance is also crucial. In this context, DETU is found less potent in animal models than DPTU and DBTU.

As shown in the analysis results, only DETU and DBTU could be detected in concentrations above the detection limit in the tested products. The highest concentrations of DETU among the selected products are found in products for children, including wetsuits, where DETU that migrates from the material will be absorbed in the sweat during the period that children use the suit.

The content of DETU in the investigated products was up to 720 mg/kg in slippers for children, which was the product with the highest content of DETU. For comparison, the concentration of DETU in shoes that have been shown to trigger an allergic reaction was 192-251 mg/kg (Friis *et al.*, 2011), which is lower than the concentration found in several of the tested products. In another case an allergic reaction to a DETU at 76-77 mg/kg in work gloves has been demonstrated (Friis *et al.*, 2011).

Migration rates for DETU measured for the five selected product types ranged between 0.9 and 1.8 $\mu g/cm^2$/hour, corresponding to total doses between 5.6 and 10.6 $\mu g/cm^2$ over 6 or 8 hours of exposure time. Table 4.6 shows that the highest migration rate of 1.8 $\mu g/cm^2$/hour been found in tests with full-length wet suits for children. Since there is direct contact between the skin and the products it can be expected that exposure per cm^2 corresponds to migration rates. The dermal dose can be calculated to be between 0.9 and 1.8 $\mu g/cm^2$/hour. There are no data which can be used for the comparison of this estimated dermal dose.

In order to compare the concentrations used in patch tests of the patients, it is assumed that 0.1 mm perspiration built up on the skin beneath a wet suit which are used in air (i.e. a situation of use where there is no water flow through the suit) during an exposure period of 6 hours. With an overall migration of 12.6 $\mu g/cm^2$,it corresponds to a concentration in sweat at 0.126% DETU, which is slightly lower than the levels used in the patch test (typically 0.5-1%), and which have been demonstrated to cause allergic reactions in sensitized individuals. It should be emphasized that reactions also occur at lower levels. With the uncertainty of the calculation of the concentration in sweat, it must be assumed that the concentration may well reach the same concentrations as found in patch tests.

Since no major differences between products in terms of both concentration of the substances in the products and the migration rates, it must be assumed that all the tested products with demonstrated content of DETU or DBTU could trigger an allergic reaction in sensitized individuals.

There are no studies undertaken that show how the migration rates change over time and whether the rate, and thereby the allergy risk, will decrease.

5.4.2 Other effects

Since DETU is not considered to have long-term adverse effects, as TU and ETU are, no further risk assessment of other effects has been undertaken.

Since there TU and ETU have not been demonstrated in the products no risk assessment has been performed of these substances.

5.5 Conclusion

The concentrations of DETU found in 10 of 14 products (33-720 mg/kg) in this study are at the same level as the concentration in products that are demonstrated to result in contact dermatitis, and it is expected that all products containing DETU could trigger an allergic reaction (elicitation) in sensitized individuals. Based on current knowledge, it has not been possible to clarify at what levels sensitization (induction) can be expected to take place.

DETU is found in a variety of products, including those for both water sports and leisure activities (wetsuits, bathing shoes, kayak gloves and hood) and in all the investigated supports. DETU is not found in the two samples of the sandals, but the number of samples is too small to conclude that the substances are not found in this type of product.

No examples of marketing of products of chloroprene rubber in Denmark, where it is specifically mentioned that the products do not contain thiourea compounds, have been identified, and it must be expected that within all application areas for consumer products made of chloroprene rubber that DETU may be found.

ETU is not detected in any of the 14 products, but since it is known that the substance is used to some extent for the preparation of chloroprene rubber, it is not out of the question that ETU may be present in consumer products. It is known that the majority of the substance, just as is the case with the other thiourea compounds, will be degraded into other compounds in the finished material. It is quite possible that the substance has been used for the preparation of some of the products, but that it is degraded to such an extent that it occurs in the final material at a concentration below the detection limit.

6 References

Akrochem. 2011. Accelerator DETU. Akrochem Corp., Akron. http://www.akrochem.com/pdfs/thioureas/X-detu%20flake.pdf

Alcántara M, Martínez-Escribano J, Frías J, García-Sellés FJ. 2000. Allergic contact dermatitis due to diphenylthiourea in a neoprene slimming suit. *Contact Dermatitis*. 43(4):224-225.

AMI. 2004. Dokumentationsrapport om risikofaktorer og forebyggelse af arbejdsbetingede hudlidelser ved vådt arbejde [In Danish: Documentation Report on risk factors and prevention of occupational skin diseases in wet work]. Arbejdsmiljøinstituttet, Copenhagen.

Anderson BE. 2009. Mixed dialkyl thioureas. *Dermatitis*. 20(1):3-5.

Aplin CG, Bower C, Finucane K, Sansom JE. 2001. Contact allergy to IPPD and diphenylthiourea in an orthopaedic brace. *Contact Dermatitis*. 45(5):301-302.

Axelstad M, Boberg J, Nellemann C, Kiersgaard M, Jacobsen PR, Christiansen S, Hougaard KS, Hass U. 2011. Exposure to the widely used fungicide mancozeb causes thyroid hormone disruption in rat dams but no behavioral effects in the offspring. *Toxicol Sci*. 120(2):439-446.

Bergendorff O, Persson CM, Hansson C. 2004. HPLC analysis of alkyl thioureas in an orthopaedic brace and patch testing with pure ethylbutylthiourea. *Contact Dermatitis*. 51(5-6):273-277.

Buus SK, Andersen KE. 2002. Allergisk kontakteksem på grund af diethylthiourea i neoprengummi. [In Danish: Allergic contact dermatitis due to diethylthiourea in neoprene rubber] *Ugeskr Læger*.164(11):1475.

Charles JM, Tobia A, van Ravenzwaay B.2000. Subchronic and chronic toxicological investigations on metiram: the lack of a carcinogenic response in rodents. *Toxicol Sci*. 54(2):481-492.

Comfere NI, Davis MD, Fett DD. 2005. Patch-test reactions to thioureas are frequently relevant. *Dermatitis*. 6(3):121-123.

DuPont. 2008. A Guide to Grades, Compounding and Processing of Neoprene Rubber. DuPont Dow Elastomers technical information. Rev October 8. 20 Februar 2011 på: http://www.dupontelastomers.com/literature/neoprene/49E8B9407D62DB87301F7900D3712585.pdf

DuPont. 2010. DuPont™ Neoprene GW. http://www.dupontelastomers.com/literature/neoprene/D6E85D803ED3EEECD21B7900D3712585.pdf

Dansk Kontaktdermatitis Gruppe. 2005. Retningslinier for vurdering af relevans af kontaktallergi påvist ved lappetest. [In Danish: Guidelines for assessing the relevance of contact allergy detected by patch test] August 2005. http://www.videncenterforallergi.dk/userfiles/files/vejledninger%20og%20guidelines/retningslinjer_vurdering_relevans_af_kontaktallergi08.pdf

ECHA. 2008. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.8 - Characterisation of dose [concentration]-response for human health. European Chemicals Agency (ECHA), May 2008.

ECHA. 2010. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15 - Consumer exposure estimation. European Chemicals Agency (ECHA), version 2, april 2010.

Fregert S, Trulson L, Zimerson E. 1982. Contact allergic reactions to diphenylthiourea and phenylisothiocyanate in PVC adhesive tape. *Contact Dermatitis*. 8(1):38-42.

Friis UF, Johansen JD, Krongaard T, Menné T. 2011. Quantitative assessment of diethylthiourea exposure in two cases of occupational allergic contact dermatitis. *Contact Dermatitis*. 64(2):116-118.

Geier J, Lessmann H, Uter W, Schnuch A. 2003. Occupational rubber glove allergy: results of the Information Network of Departments of Dermatology (IVDK), 1995-2001. *Contact Dermatitis*. 48(1):39-44.

Gudi VS, White MI, Ormerod AD. 2004. Allergic contact dermatitis from dibutylthiourea in a wet suit. *Dermatitis*. 15(1):55-56.

IARC. 2001. Ethylenethiourea. IARC Monographs Volume 79. International Agency of Cancer Research, Lyon.

Ikarashi Y, Ohno K, Momma J, Tsuchiya T and Nakamura A. 1994. Assessment of contact sensitivity of four thiourea rubber accelerators: Comparison of two mouse lymph node assays with the guinea pig maximization test. *Food Chem. Toxicol.* 32(11):1067-72.

IRIS. 2011 (2003). Integrated Risk Information Systems 2011 online version, last revised 2003. U.S. Environmental Protection Agency.

Kroft EB, van der Valk PG. 2007. Allergic contact dermatitis as a result of diethylthiourea. *Contact Dermatitis*. 57(3):194-195.

Liippo J, Ackermann L, Hasan T, Laukkanen A, Rantanen T, Lammintausta K. 2010. Sensitization to thiourea derivatives among Finnish patients with suspected contact dermatitis. *Contact Dermatitis*. 63(1):37-41.

Liippo J, Ackermann L, Lammintausta K. 2011. Occupational allergic contact dermatitis caused by diethylthiourea in a neoprene handle of a cleaning trolley. *Contact Dermatitis*. 64(6):359-360.

Martínez-González MC, Goday-Buján JJ, Almagro M, Fonseca E. 2009. Allergic contact dermatitis to diethylthiourea in a neoprene wader. *Actas Dermosifiliogr*. 100(4):317-320.

McCleskey PE, Swerlick RA. 2001. Clinical review: thioureas and allergic contact dermatitis. Cutis. 68(6):387-396.

Mizuno J, In-Nami H. 2011. [Allergic contact dermatitis to synthetic rubber, neoprene in compression stockings]. *Masui.* 60(1):104-106. [in Japanese with English abstract].

Mueller Sports Medicine. 2011. Hg80 Mueller Mercury. http://www.muellersportsmed.com/hg80products.htm

Munoz CA, Gaspari A, Goldner R. 2008. Contact dermatitis from a prosthesis. *Dermatitis*. 19(2):109-111.

Nilsson N, Pedersen V. 2004. Mapping and release of chemical substances from products made of chloroprene. Survey of Chemical Substances in Consumer Products no. 51, 2004. Danish Technological Institute for the Danish EPA.

NTP. 2011. Ethylene Thiourea. Report on Carcinogens, Twelfth Edition (2011). National Toxicology Program, Department of Health and Human Services.
http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/EthyleneThiourea.pdf

RDC. 2011. PREMIX Products ACCELERATORS. R.D.C. S.r.l., Milano. http://www.rdcsrl.com/database/listpremix.php?Cat=ACCELERATORS

SafeRubber. 2011. A safer alternative replacement for thiourea based acceerators in the production process of chloroprene rubber. http://www.saferubber.eu/contact-info

Sakata S, Cahill J, Nixon R. 2006. Allergic contact dermatitis to thiourea in a neoprene knee brace. *Australas J Dermatol.* 47(1):67-69.

Samuelsson K, Bergström MA, Jonsson CA, Westman G, Karlberg AT. 2011. Diphenylthiourea, a common rubber chemical, is bioactivated to potent skin sensitizers. *Chem Res Toxicol.* 14;24(1):35-44.

Sedo. 2011. Areas of application. Sedo Chemicals Neoprene GmbH, Berlin. http://www.sedochemicals.de/index2.php?sprache=en&id=fields

SpecialChem. 2008. The polychloroprene drama. SpecialChem - Mar 26, 2008. http://www.specialchem4adhesives.com/home/editorial.aspx?id=2230

Terrapin. 2011. About wet suit material. Terrapin Wet Suits, New Braunfels http://www.terrapinwetsuits.com/materials.htm

U.S. EPA. 2002. Child-Specific Exposure Factors Handbook. U.S. Environmental Protection Agency (EPA), September 2002.

U.S. EPA. 2007. Ethylenethiourea (ETU) from EBDCs: Health Effects Division (HED) Human Health Risk Assessment of the Common Metabolite/Degradate ETU. Document ID: EPA-HQ-OPP-2005-0308-0009Document. Unites States Environmental Protection Agency, Offices of Prevention, Pesticides and Toxic Substances

Warshaw EM, Cook JW, Belsito DV, DeLeo VA, Fowler JF Jr, Maibach HI, Marks JG Jr, Mathias CG, Pratt MD, Rietschel RL, Sasseville D, Storrs FJ, Taylor JS, Zug KA; North American Contact Dermatitis Group. 2008. Positive patch-test reactions to mixed dialkyl thioureas: cross-sectional data from the North American Contact Dermatitis Group, 1994 to 2004. *Dermatitis*. 19(4):190-201.

WHO. 1988. Dithiocarbamate pesticides, ethylenethiourea and propylenethiourea: a general introduction. International Programme on Chemical Safety. Environmental Health Criteria 78. World health Organization.

WHO. 2003. Thiourea. WHO Concise International Chemical Assessment Document 49.

WHO. 2008. Skin Sensitization in Chemical Risk Assessment. Harmonization Project Document No. 5. World Health Organization.

Woo DK, Militello G, James WD. 2004. Neoprene. Dermatitis. 15(4):206-209.

Yokota M, Fox LP, Maibach HI. 2007. Bilateral palmar dermatitis possible caused by computer wrist rest. *Contact Dermatitis*. 57(3):192-193.

Annex 1 Analyses results

The results of the analysis of the content of DETU and DBTU are shown in the table below. For the other thiourea compounds the content was below the detection limit. The measured values were within a range of ± 8% of the average value for all measurements.

Table A.1
Analyses results for content of thiourea compounds in chloroprene rubber products

Sample no.	Type af product	Content of DETU (mg/kg)			Content of DBTU (mg/kg)		
		Sample 1	Sample 2	Average *	Sample 1	Sample 2	Average*
1	Full-length wetsuit for kids	619	610	614	< d.l.	< d.l.	< d.g.
2	Short wetsuit for kids	364	332	348	< d.l.	< d.l.	< d.g.
3	Chloroprene rubber T-shirt for kids	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
4	Chloroprene rubber socks shirt for kids	31	35	33	< d.l.	< d.l.	< d.g.
5	Bathing shoes for kids	770	661	715	< d.l.	< d.l.	< d.g.
7	Flip-flop sandals with chloroprene rubber strap for adults	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
8	Sport Sandal with chloroprene rubber linings for adults	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
9	Wrist brace	675	663	669	< d.l.	< d.l.	< d.g.
10	Elbow support	137	134	135	< d.l.	< d.l.	< d.g.
11	Wrist brace	150	143	146	62	59	61
12	Thigh support	476	491	483	< d.l.	< d.l.	< d.g.
13	Kayaking gloves	46	48	47	< d.l.	< d.l.	< d.g.
15	Chloroprene rubber sleeve for I-PAD	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.g.
16	Kayaking hood	149	131	140	< d.l.	< d.l.	< d.g.
Detection limit, mg/kg		6	6		9	9	

< d.l. Below the detection level
* The average is shown as a rounded value in the report

Results of duplicate determination of migration rates are shown in the table below.

Table A.2
Analysis results for migration rates

Sample No.	Type af product	Total migration during extraction, µg/cm²			RSD% *1	Migration rate µg/cm²/hour *2
		Sample 1	Sample 2	Average		
1	Full-length wetsuit for kids	10.7	10.6	10.6	0.7	1.8
2	Short wetsuit for kids	6.0	5.3	5.6	9.1	0.9
5	Bathing shoes for kids	10.4	9.7	10.0	4.9	1.7
9	Wrist brace	9.0	8.1	8.6	7.3	1.1
12	Thigh support	7.0	7.1	7.1	1.7	0.9

*1 Indicates the relative standard deviation of the dublicare determination.

*2 See surface area in table 4.6

Summary
Chloroprene rubber, which is also known as "neoprene", is a synthetic rubber that is used for both technical and consumer products. The main application areas are water sports products and bandages. According to the literature, a number of thiourea compunds are used as accelerators in the manufacturing of chloroprene rubber products. Six of these have been identified as potentially giving rise to allergic reactions. DETU was present in concentrations of 33 to 720 mg/kg in 10 of the 14 examined products and is of the same magnitude as the concentrations in products that are demonstrated to cause contact dermatitis in sensitized individuals.

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