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This investigation has been performed by order and for the account of the Food and Consumer Product Safety Authority and the Ministry of Health, Welfare and Sport, within the framework of Pesticidal and Biocidal Products

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# Abstract

**Aggregate exposure to chemicals**

The risks to public health posed by exposure to and use of a specific chemical are difficult to assess when the chemical risk assessment has also to consider the combined exposure to a chemical from multiple routes and products (aggregate exposure). This difficulty is primarily due to a lack of relevant exposure data. For example, information is often not available on whether or not chemicals are present in products and, if present, on their concentrations. This is the conclusion drawn by the RIVM based on four case studies. The research was performed by order of the Food and Consumer Products Safety Authority and the Ministry of Health, Welfare and Sport of the Netherlands. The increasing demand for aggregate risk assessments by regulatory authorities necessitates further refinement of risk exposure assessments.

The four case studies were carried out by the RIVM to explore the feasibilities and limitations of current aggregate risk assessments. An aggregate exposure assessment based on worst case deterministic exposure estimates is, in some cases, sufficient to indicate the absence of a human health risk. To the contrary, in other cases, an aggregate exposure assessment can indicate that the current maximum allowed amounts of substances in products (norms) may not provide adequate protection to the consumer.

The RIVM recommends the development of a guidance for dealing with possible health risks. One approach could be to reduce the maximum allowed amounts of a chemical in a product. Further, more realistic data on the use of products and the possible exposure routes to chemicals could be used to refine risk assessments. In recent years, probabilistic methods have been used for this purpose. Additional measurements on specific substances and products may also be needed to improve the risk assessment.

Key words:
chemicals, aggregate exposure, risk assessment, consumer products

# Rapport in het kort

**Geaggregeerde blootstelling aan chemische stoffen**

Het risico van een chemische stof is lastig te beoordelen als mensen via verschillende routes en producten aan deze stof staan blootgesteld (geaggregeerde blootstelling). Dat komt meestal doordat relevante blootstellingsgegevens ontbreken. Het kan bijvoorbeeld onbekend zijn in welke producten de stoffen voorkomen en in welke concentratie. Dit is de conclusie van het RIVM op basis van studies naar vier stoffen. Het onderzoek is uitgevoerd in opdracht van de Voedsel en Waren Autoriteit (VWA) en het ministerie van VWS. Vanwege de toenemende vraag van de regelgevende instanties naar geaggregeerde risicoschatting is het noodzakelijk om de blootstellingsschatting verder te ontwikkelen.

In de vier casestudies zijn de huidige mogelijkheden en beperkingen van een geaggregeerde risicoschatting uitgewerkt. Soms kan op basis van grove blootstellingsschattingen worden aangetoond dat er geen gezondheidsrisico is. Anderzijds kan blijken dat de huidige normen consumenten onvoldoende bescherming bieden.

Aanbevolen wordt om een leidraad te ontwikkelen hoe met mogelijke gezondheidsrisico's om te gaan. Dat kan bijvoorbeeld door de toegestane hoeveelheid van een stof in een product te verlagen. Ook kunnen realistischere gegevens over gebruik en blootstelling worden gebruikt om de risicoschattingen te verfijnen. De laatste jaren worden hiervoor zogeheten probabilistische methoden ingezet. Soms zijn extra metingen nodig van stoffen in producten.

Trefwoorden:
chemische stoffen, geaggregeerde blootstelling, risicobeoordeling, consumentenproducten

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# Summary

A single chemical may be used in a variety of products. Therefore, people may be exposed to the same compound via several products and routes. Within the EU, this is commonly defined as 'aggregated exposure'. Today, several regulatory frameworks, e.g. REACH, the Biocide Products Directive and the Pesticide Directive require that the aggregate exposure to a chemical from all identified sources is considered. However, presently no harmonized methodology is available on the European level for this type of risk assessment.
In the current report, four case studies (i.e. triclosan, permethrin, carvone and calcium) are analyzed to illustrate the importance of aggregated exposure. Each case study aimed to make an inventory of the relative contribution of each source of the chemical in the total exposure of the consumer and to assess whether the aggregate exposure may pose a risk to human health. The study was carried out to explore the current possibilities and limitations of an aggregate risk assessment, with respect to data collection, availability of data, methods of exposure calculation et cetera.
Some specific conclusions on the cases can be drawn. The aggregate exposure and risk assessment for permethrin indicates no health concern, even though the exposure estimates can be considered rather worst case. For triclosan, the present risk assessment raises concern on its use in skin care products (body lotion), and indicates that it may be worthwhile to reconsider the use of triclosan in oral hygiene products (mouth wash), and in sun care cosmetics (if present at all). For carvone, a (worst case) aggregate exposure indicates a health concern, mainly from its use as a food additive, and it is advised to refine the exposure estimates, although this also requires measurements of carvone in various products. The calcium case study indicates that the risk of overexposure to this nutrient due to habitual intake and consumption of dietary supplements is probably limited. There are not enough data available to determine the prevalence of insufficient calcium intake in the population.
The current case studies confirm the notion that aggregate exposure to single substances from various sources is reality. In general, data on the toxicological profile of a chemical are not the largest obstacle for an aggregate risk assessment. However, data on the presence of chemicals in products are often difficult to obtain. It is concluded that the availability of exposure data in general is a bottleneck in the aggregate exposure and risk assessment of chemicals. The present study shows that sometimes an aggregate exposure assessment based on simple worst case deterministic exposure assessments is sufficient to indicate the absence of concerns. In that case no further actions are required. On the other hand in some cases the current maximum allowed amounts of substances in products may not be protective enough when considered in a (worst case) aggregate exposure assessment. If concerns cannot be excluded, refinement of the exposure assessment, preferably using probabilistic methods, is the first priority. However, this refinement is often limited due to the absence of relevant exposure data. Therefore, additional measurements on specific substances and products will be needed to improve the risk assessment. Such additional measurements can possibly be incorporated in enforcement monitoring programs or should be separately addressed.
To be able to deal with the increasing regulatory demands for aggregate exposure, further development of exposure models will be necessary. A joint action between public and private parties may be the most efficient way forward.
It is conceivable that exposure within a single framework is safe (eg for use as biocide and for use in cosmetics) but aggregation over different frameworks gives reason to concern. This requires a policy decision on risk management options within one or more frameworks. A stakeholder dialogue should be started to bring this issue forward.

# Samenvatting

Een chemische stof kan worden toegepast in verschillende producten. Mensen kunnen dus aan een bepaalde stof worden blootgesteld via verschillende producten en routes. In de EU wordt dit 'geaggregeerde blootstelling' genoemd. In verschillende beoordelingskaders in de EU, zoals REACH, de Biocidenwetgeving en de Bestrijdingsmiddelenwetgeving, is vereist dat de geaggregeerde blootstelling aan een chemische stof uit alle geïdentificeerde bronnen in beschouwing wordt genomen. Op dit moment is er echter in de EU geen geharmoniseerde methodologie voor dit type risicobeoordeling beschikbaar.

In het huidige rapport zijn vier casestudies (triclosan, permethrin, carvon en calcium) onderzocht om het belang van geaggregeerde blootstelling te illustreren. Elke casestudie had tot doel om de relatieve bijdrage van iedere blootstellingbron van een chemische stof aan de totale blootstelling in kaart te brengen, en om te bepalen of de geaggregeerde blootstelling een gezondheidsrisico oplevert. De studie werd uitgevoerd teneinde de huidige mogelijkheden en beperkingen van een geaggregeerde risicoschatting te onderzoeken met betrekking tot gegevensverzameling, beschikbaarheid van gegevens, methodes van blootstellingsschatting et cetera.

Voor de vier cases kunnen enkele specifieke conclusies worden getrokken. De geaggregeerde blootstellings- en risicobeoordeling voor permethrin geeft geen gezondheidsrisico aan, ook al zijn de blootstellingsschattingen conservatief. Voor triclosan geeft de huidige risicobeoordeling aan dat voor huidverzorgingsproducten (bodylotion) gezondheidsrisico's niet uit te sluiten zijn en dat het gebruik van triclosan in mondhygiëneproducten en zonnebrandproducten (mocht het daarin gebruikt worden) heroverwogen dient te worden. Voor carvon geeft de (conservatieve) geaggregeerde blootstelling aan dat een gezondheidsrisico niet kan worden uitgesloten, voornamelijk als gevolg van het gebruik als voedseladditief. Het wordt aanbevolen om de blootstellingsschattingen voor carvon te verfijnen, hoewel hiervoor aanvullende metingen van carvon in producten nodig zijn. De casestudie van calcium geeft aan dat er waarschijnlijk slechts een klein risico is op overmatige blootstelling aan deze stof als gevolg van de dagelijkse inname via voedsel of voedingssupplementen. Er zijn te weinig gegevens beschikbaar om vast te stellen welk deel van de bevolking te weinig calcium binnenkrijgt.

De vier casestudies geven aan dat geaggregeerde blootstelling aan een enkele stof uit verschillende producten een reëel probleem kan zijn. In het algemeen is de beschikbaarheid van toxiciteitsgegevens over een chemische stof afdoende om een geaggregeerde risicoschatting uit te voeren. Echter, het is vaak moeilijk om gegevens over de aanwezigheid van chemische stoffen in producten te verkrijgen. Er kan worden geconcludeerd dat de beschikbaarheid van blootstellingsgegevens een knelpunt in de geaggregeerde blootstellings- en risicoschatting van stoffen vormt. De huidige studie toont aan dat soms een geaggregeerde blootstellingsschatting gebaseerd op simpele conservatieve deterministische blootstellingsschattingen voldoende is om aan te tonen dat er geen gezondheidsrisico is. Anderzijds geven sommige casestudies aan dat de huidige maximaal toegestane hoeveelheden van stoffen in producten de gezondheid van de consument niet afdoende waarborgt in een (conservatieve) geaggregeerde blootstellingsschatting. Als een gezondheidsrisico niet kan worden uitgesloten dient eerst een verfijnde blootstellingsschatting te worden uitgevoerd, bij voorkeur met probabilistische methodes. Echter, deze verfijning wordt veelal beperkt door het ontbreken van relevante blootstellingsgegevens. Daarom zijn additionele metingen naar specifieke stoffen in producten nodig om de risicobeoordeling te verbeteren. Deze additionele metingen kunnen mogelijk worden geïncorporeerd in monitoringprogramma's, of afzonderlijk worden uitgevoerd.

Teneinde aan de toenemende vraag van regelgevende instanties naar geaggregeerde blootstelling te kunnen voldoen is verdere ontwikkeling van blootstellingsmodellen nodig. Hiertoe kan door publieke en private partijen gezamenlijk actie worden ondernomen.

Het is mogelijk dat blootstelling binnen een bepaald kader (bijvoorbeeld gebruik als biocide of in cosmetica) veilig is, maar dat geaggregeerde blootstelling over verschillende kaders een reden tot zorg geeft. In die gevallen zullen beleidskeuzes gemaakt moeten worden over de risicomanagementopties

binnen een of meer kaders. Hiertoe dient een dialoog tussen de belanghebbende partijen te worden gestart.

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# 1 Introduction

Public health risk assessment of chemicals is performed within the scope of several regulatory frameworks. However, the approaches followed in these frameworks are variable. For some of these frameworks, a safety assessment is considered sufficient without a detailed exposure and risk assessment (e.g. food additives). For other frameworks, a detailed risk assessment including a hazard assessment (toxicological effects), an exposure assessment (sometimes according to the prescribed use of the product) and a risk assessment/characterization (combining hazard and exposure), is necessary before a substance is allowed on the market (e.g. plant protection products or biocides). Despite differences in the level of detail, a common characteristic of most frameworks is to consider the safety of a chemical only within the scope of that specific framework for the intended specific use, which is often a single use. For example, the safety of a plant protection product is considered primarily for its use as a plant protection product according to its intended application. In this example the safety is assessed within the regulatory framework of Plant Protection Products (EG 91/414). However, this compound is also used in other products with other types of application, e.g. as an insect killer in the residential area (biocide) or as an anti-flea product on pets (veterinary product). Therefore, people may be exposed to the same compound via several routes and products. Within the EU, this is commonly defined as 'aggregated exposure'.
Such aggregated exposure is normally not covered in the various chemical frameworks although examples exist. In the former EU existing chemicals regulation (replaced by REACH) total aggregated exposure was included on a robust level (EUSES software tool). Today, under the REACH regulation it is required to consider the aggregate exposure to a chemical from all identified sources. Also, the Biocide Product Directive (98/8/EG) and Pesticide Directive (91/414/EC) state that in the risk assessment of a biocide or pesticide aggregate (one chemical, multi-source) and cumulative (different chemicals, same mode of action) exposure should be considered. However, presently no harmonized methodology is available on the European level for this type of risk assessment. For non-food products in particular, little is known on the aggregate exposure to substances [1, 2].
Currently, there is increased awareness that aggregated exposure may be an important issue in the risk assessment of chemicals. Delmaar and Van Engelen [3] published a report on the principles and methodologies for aggregating human exposure to chemicals. Recently, Schuur et al. [1] presented some examples of (partly) aggregate exposure scenarios. In the current report, a few but very different type of case studies are analyzed to illustrate the importance of aggregated exposure. In four case studies an aggregate exposure and risk assessment for triclosan, permethrin, carvone and calcium were performed. Triclosan and permethrin are synthetic substances with, among others, biocidal activity which are used in a variety of products. Both compounds are considered as typical examples of substances with multiple uses in a wide range of product types. Carvone and calcium are substances of natural origin. Carvone is used as a flavouring and fragrance agent, as well as a plant growth regulator. Calcium is a natural chemical element and an essential nutrient and as such is used as a food supplement and in food fortification. However, it is recognized that too high intakes of calcium may cause adverse health effects. As is the case with many essential elements, the margin between the adequate intake of calcium and an intake level causing adverse effects is only small. In view of this, risk assessment of these essential nutrients differs from that of other chemicals.

The study was carried out to explore the current possibilities and limitations of an aggregate risk assessment, with respect to data collection, availability of data, methods of exposure calculation et cetera. The results of these case studies indicate policy implications and some recommendation can be given for a future direction.

# 2 Approach and methods

## 2.1 General approach

For four substances an aggregate exposure and risk assessment was performed. Data on use, exposure and toxicological profile were obtained from all available public sources. Web searches were performed using the substance name (e.g. triclosan) or trade name (e.g. Irgasan) and (combinations of) other search terms e.g. exposure, level, concentration, cosmetic, plastic, oral hygiene, toxicity, kinetic, absorption et cetera.

## 2.2 Selection of case studies

Triclosan and permethrin were selected for the present aggregate exposure and risk assessment since their use on food and non-food products is wide spread.
Triclosan has not been notified as a biocide (yet). However, it has antimicrobial action, and this 'biocidal' property is the reason why it is used in a wide variety of products such as cosmetics, oral hygiene products, textiles and plastics. It has been marketed for decades and this substance is considered as a typical example for the present study.
Permethrin has been in use in the European Union predominantly as a pesticide. In 2001 its use as a pesticide has been withdrawn by the EC. However, the use of permethrin as a pesticide is still allowed outside the EU. Thus food products imported into the EU may still be a source of permethrin exposure. Moreover, permethrin is present in a variety of other products such as fabric, pet care products, wood preservatives, et cetera.
Carvone is a natural substance, occurring in herbs, citrus fruits, guava, beetroot, cabbage and celery. It is presently used as a food additive in a variety of products, and in personal care products. Furthermore it is used as a plant growth regulator on potatoes.
Calcium is an essential nutrient which is present in a wide range of food stuffs. In addition it is used in dietary supplements. Calcium was chosen as a case study for aggregate risk assessment in view of its narrow range between the daily requirement and its toxic effect level. Accordingly, risk assessment for such an essential nutrient differs from that of other chemicals.

## 2.3 Exposure estimates

Exposure estimates were made preferably on the basis of actual data on levels of the substance in products and actual exposure data. Where data on levels in products were lacking it was assumed that the product would contain the substance at the maximal allowed concentration. If actual exposure data were lacking, exposure estimates were based on default assumptions as described in for instance the RIVM Cosmetics fact sheet [19], or exposure models as described e.g. in the User guidance of the TNsG (2002). For carvone used as food additive, the exposure was based on the annual production data. For each exposure calculation the methods or models that are used are indicated.

# 3 Case study 1 - triclosan

## 3.1 Description of the case

Triclosan (2,4,4'-trichloro-2'-hydroxy-diphenylether, CAS no. 3380-34-5) is a broad-spectrum antimicrobial agent. It has antibacterial and to a lesser extent antifungal and antiviral properties. It is used in a variety of products, e.g. dental care products, cosmetics, soaps, detergents, medicinal products, food contact materials, textiles and plastics [4, 5, 6].
Triclosan is regulated in the Cosmetic Directive 76/768/EEC, Annex VI, part 1, reference no.25 [7], and can be used as a preservative up to a maximum concentration of 0.3 % in the finished cosmetic products. In Annex VI, triclosan is marked (+) and therefore may be added to cosmetic products in concentrations other than those laid down in this Annex for other specific purposes apparent from the presentation of the product. A search on the internet indicates that triclosan is used in solid and liquid soaps at a concentration of 0.5 %.
Although triclosan is reported to be used in a vast range of products, it is difficult to get information on the products in which it is actually used and data on the levels of triclosan in these products. In a study by the Stichting Natuur en Milieu and Milieudefensie [8] performed in 2005 in the Netherlands, triclosan was found in 1 (toothpaste) out of 55 cosmetic products. It is not clear whether this incidence is representative for all cosmetic products marketed in the Netherlands. A survey in Denmark indicated that cosmetics are the largest contributors of triclosan on the Danish market: 99 % of the total amount reported in the survey could be attributed to cosmetics [9]. Within this product group the largest amount is found in products for dental hygiene, including toothpaste. The survey also showed that the amount of triclosan used in products in Denmark had decreased from 3.9 tonnes in 2000 to 1.8 tonnes in 2004.

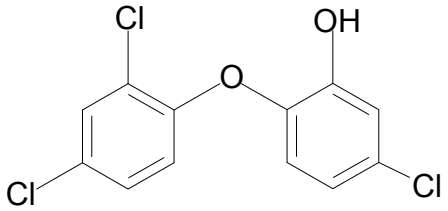


**Figure 3.1: Chemical structure of triclosan**

**Note 1**: During the final stages of the preparation of this report, an opinion on triclosan of the Scientific Committee on Consumer Products (SCCP) was published (January 2009) [10]. This opinion was based on information provided by Industry, and contains much more information on the toxicology of triclosan and on its use in consumer products.
Since the triclosan aggregated risk assessment had been finalized at the time of the publication of the SCCP opinion and since the present case can be considered exemplary for risk assessment of chemicals, we chose not to include the information from the SCCP opinion on our aggregated exposure and risk assessment of triclosan. However, the findings of our assessment will be discussed in the light of the findings of the SCCP.
**Note 2:** In 2008 US-EPA performed an aggregated risk assessment for triclosan on the basis of biological monitoring data, conversing spot urine concentrations to doses [11]. US-EPA considered the population based biological monitoring data a more accurate predictor of triclosan exposure than determining aggregate exposure from individual regulated uses. On the basis of the conversion of

monitoring data to doses US-EPA concluded that the aggregated exposure to triclosan poses no health concern. Although the US-EPA exposure assessment may give an indication of the actual aggregated exposure to triclosan in the USA it is considered not of use for the present aggregated risk assessment of triclosan, which aims to describe the situation in the Netherlands.

## 3.2 Toxicological profile and limit values

The present description of the toxicological profile of triclosan is mainly based on a report of the Norwegian Scientific Committee for Food Safety [12] dated 2004 and 2 reports of Ciba-Geigy from 2003 [13, 14] . Additional sources are indicated in the text. The toxicity of triclosan has been investigated in toxicokinetic, acute-, subchronic- and chronic studies, carcinogenicity and mutagenicity studies and studies of reproductive and developmental toxicity.

### 3.2.1 Kinetics

The kinetics of triclosan was studied in mice, rats, hamsters and humans. Maximum concentrations (*presumably in blood*) were reached after 4h. In rats and mice excretion was almost complete after 48h, with the majority (*presumably of radioactive label*) being excreted in the feces, and a minor part being excreted in the urine as sulphate and glucuronide conjugates. In hamsters, excretion (*presumably of radioactive label*) was complete after 7 days, with the majority excreted in urine. In humans exposed to triclosan in drinking water or in toothpaste for 21 days a steady state in plasma was reached after 7 days. After cessation of treatment most of the triclosan was excreted within 4-5 days.
The dermal absorption of triclosan in an alcoholic solution was investigated *in vivo* in the rat and *in vitro* in rat and human skin [15]. The *in vitro* studies indicated that the dermal absorption through skin in rats is 3-4 times higher than in humans (23 % vs 6.3 %). In an *in vivo* study in the rat, with triclosan, applied at an area dose of 0.19 mg/cm$^2$, after 24h exposure a dermal absorption of 21 % was measured (excreta + carcass), with 30 % remaining in the stratum corneum and 26 % being rinsed off. Since 23 % of radioactivity was unaccounted for (total recovery was only 77 %) actual absorption may be higher (up to 44 %). Furthermore it should be noted that dermal absorption of triclosan, present in the stratum corneum, after the 24h exposure period was not measured. It is also not known what the effect of vehicle on the percentage dermal absorption is.
In the Norwegian risk assessment [12] a dermal absorption percentage of 10 % for rinse-off products and 25 % for eye products and non rinse-off products was used, with a reference to a Ciba report (not available for the present case study).

In humans, triclosan was found in 3 out of 5 samples of human milk, at a level of 60-300 µg/kg lipid weight (it is not stated how this relates to the level in milk (w/w)) [16]. Triclosan was also detected in human maternal and cord blood [17]. Triclosan was found in about half of the samples in both maternal and cord blood (detection limit <0.1ng/g serum). Levels in cord blood (0.5-5.0 ng/g serum) were higher than in maternal blood (0.1-1.3 ng/g serum). Based on lipid content the maternal blood levels were 15-199 µg/kg lipid weight, which is comparable to the levels in milk in the study of Adolffson-Erici et al. [16].

### 3.2.2 Toxicodynamics

The acute oral LD50 is > 5000 mg/kg bw, based on studies in several species (rat, mouse, rabbit, dog). Triclosan is moderately irritating to eye and skin. Triclosan is not irritating formulated as a consumer product. Triclosan is not sensitizing or phototoxic/photoallergenic.

The subchronic toxicity of triclosan was tested in rats, mice, rabbits, dogs, baboons and rhesus monkeys. The primary effects of triclosan were hepatic, and minimal renal and haemopoietic toxicity. In a 90-day dietary study in rats the NOAEL was 1000 ppm, equal to 65 mg/kg bw/day, based on decreased body weight gain and alterations in the liver (not specified) at doses of 3000 ppm (equal to 203 mg/kg bw/day) and higher. In an additional 90-day dietary study in mice, hepatic hypertrophy, inflammation and necrosis were observed at doses of 75 mg/kg bw/day and higher. Dose-related decreases in mean erythrocyte count and hemoglobine levels were reported at 25 mg/kg bw/day (lowest dose tested) in males and at 75 mg/kg bw/day and higher in females. The extent of the effects is not reported. In a 90-day dermal toxicity study in rats no systemic effects were observed at doses up to and including 80 mg/kg bw/day (highest dose tested. Local dermal irritation (erythema and edema, hyperplasia/hyperkeratosis, sebaceous gland hyperplasia, dermal inflammation, focal epidermal necrosis and exudate) was observed at all doses (i.e. 10 mg/kg bw/day and higher). It is not clear what the concentration of triclosan/$cm^2$ skin was.

In a 1-year oral study in baboons, in which the animals received triclosan in capsules, the NOAEL was 30 mg/kg bw/day on the basis of intermittent diarrhoea at 100 and 300 mg/kg bw/day. In this study no effects on haematology or blood chemistry were found.

In a 2-year toxicity/carcinogenicity study in rats dose-related reductions in mean body weight gain, and select haematology, clinical chemistry and urinalysis parameters were observed, mainly in the 3000 and 6000 ppm dosage groups. Reportedly also centrilobular hypertrophy and associated clinical chemistry changes were found. The NOAEL was 1000 ppm, equal to 52 mg/kg bw/day.

Triclosan is not genotoxic.

Reproductive and developmental toxicity was tested in mice, rats and rabbits.
Pregnant rats exposed to triclosan by gavage showed changes in food consumption in the dams and retarded ossification in the foetuses. The NOAEL for maternal and embryo/fetotoxicity was 50 mg/kg bw/day.
In a developmental toxicity study with rabbits the NOAEL for maternal toxicity was 50 mg/kg bw/day on the basis of reduced body weight gain and food consumption. The NOAEL for embryo/fetotoxicity was 150 mg/kg bw/day, i.e. the highest dose tested.
In a developmental toxicity study in mice the NOAEL for maternal toxicity was 25 mg/kg bw/day, on the basis of increased liver weight and necrosis. The NOAEL for embryo/fetotoxicity was 25 mg/kg bw/day on the basis of reduced fetal weight and delayed ossification.

In a 2-generation study of reproductive toxicity in rats the NOAEL for reproductive effects was 150 mg/kg bw/day, i.e. the highest dose tested. The NOAEL for offspring toxicity was 50 mg/kg bw/day, on the basis of reduced F1 body weight gain between days 14-21 of lactation. The NOAEL for parental toxicity was not reported.

### 3.2.3 Conclusion on toxicology

For the present case study an oral dermal and inhalation absorption of respectively 100, 25 and 100 % is assumed. Based on the effects observed in the developmental toxicity study in mice (increased liver weights and necrosis in dams, reduced fetal weight and delayed ossification in fetuses), the overall NOAEL for effects of triclosan is 25 mg/kg bw/day. This is the same NOAEL as used by the Norwegian Scientific Committee for Food Safety Norway [12].

## 3.3 Exposure assessment

No data on exposure of the general population or subgroups to triclosan are available. However, it is known that triclosan may be present in cosmetics, medicinal products, food contact materials, fabrics, plastics etcetera [4, 5, 6].

### 3.3.1 Exposure sources

#### 3.3.1.1 Cosmetics and oral hygiene products.

Triclosan is regulated in the Cosmetic Directive 76/768/EEC, Annex VI, part 1, reference no.25, and can be used as a preservative up to a maximum concentration of 0.3 % in the finished cosmetic products. In Annex VI, triclosan is marked (+) and therefore may be added to cosmetic products in concentrations other than those laid down in this Annex for other specific purposes apparent from the presentation of the product.
Triclosan is used as a preservative in cosmetic products at a concentration limit of 0.3 % in the finished product. In solid and liquid soaps it is used at concentrations up to 0.5 %. However, little data is available on the products that actually contain triclosan and levels therein. A search on the internet reveals that triclosan may be used in for instance lipstick, lipgloss, antiperspirant/deodorant, facial cleanser, liquid hand soap, acne treatment, facial moisturizer [4, 6].

#### 3.3.1.2 Cleaning products

Apart from its use in personal care products, triclosan is known to be used in dishwashing liquids [4]. It is assumed that triclosan is used in dishwashing liquids in concentrations up to 0.5 %.

#### 3.3.1.3 Textiles and plastics.

Triclosan is used to impregnate fibres which may be used in clothes, mattresses etc, and in polymers which may be used in plastic toys etc. A person may be exposed through wearing clothes or sleeping on a mattress made from these textiles. No data from public sources were available on the levels of triclosan in the treated products and the leaching from the product.
It is reasonable to assume that the antimicrobial action of triclosan from textiles and plastics is due to the slow release from the fabric. This suggests that the release of triclosan from plastic products is low, especially for materials in which triclosan is incorporated in the material during the manufacturing process.

#### 3.3.1.4 Food contact materials.

Triclosan is intended to be used to improve hygienic conditions of plastic articles made from e.g. PP, HDPE, PVC, coming into contact with food during holding and transportation (e.g. plastic containers) or during preparation (e.g. cutting boards). The recommended level of triclosan in these polymers is 0.3-1 % [5]. The EU Scientific Committee on Food classified triclosan in SCF_List 3 (substances for which an ADI or TDI could not be accepted, but where the present use could be accepted) with a restriction 5 mg/kg of food [18].
However, on the basis of migration data from PP, HDPE and thin PVC films the Scientific Committee on Food [18] concluded that migration from food contact materials could exceed 5 mg/kg food.

### 3.3.2 Exposure estimates.

Above, three possible sources of triclosan exposure have been identified; 1. cosmetics and oral hygiene products, 2. textiles and plastics, and 3. food contact materials. In addition exposure of infants to triclosan can occur through breast feeding. Below the potential exposure to triclosan from these sources is calculated.

Data on the presence of triclosan in products and the concentration are scarce. In order to calculate the potential exposure to triclosan for each product type it is assumed that triclosan is present at the maximally allowed concentration. The exposure estimates are based on data from the RIVM cosmetics fact sheet [19] and cleaning product fact sheet [20]. The exposure estimates can be considered reasonable worst case.
Acute toxicity of triclosan is low. Therefore, an acute exposure assessment using very worst case exposure assumptions was not performed.
For calculation of the systemic exposure oral and dermal absorption of 100 and 25 % respectively are assumed. As the difference between oral absorption and dermal absorption is only 4-fold, the additional contribution to the systemic exposure due to hand-to-mouth transfer from dermal exposure is considered negligible.

#### 3.3.2.1 Cosmetics and oral hygiene products

The exposure to triclosan is estimated based on the amount of product applied, frequency of application and retention factor. For instance, for the use of bath foam the daily amount of product used (4.8 g) is based on the amount of product used in a bath (17 g) and the frequency of application (104 times/year) [19].
It should be noted that some products are used only during certain periods of the year. For instance, it is assumed that sun care products are used daily, but for a period of only 25 days/year [20]. Since the overall NOAEL is based on a developmental toxicity study, with a short period of triclosan exposure, it was considered appropriate to compare the daily exposure to triclosan due to this short period of sun cream use to this short-term NOAEL.

Furthermore the exposure estimates are based on the following assumption

- Due to the use of rinse-off products (e.g. soap) or leave on products (e.g. sun care cream) a residue of triclosan may be left behind on the skin. For rinse-off products, except bath foam, a retention factor of 10 % is used [21]. For leave on products the retention factor is 100 %.
- Since triclosan is rather lipophylic (Log kow = 4.76), with a relatively low molecular weight (MW = 289.5) it is assumed, worst case, that when a person takes a bath with bath foam all triclosan is retained on the skin. It is noted that this assumption will lead to a very worst case exposure estimate.

For cosmetic and oral hygiene products, the following product types are identified:

- oral hygiene products: tooth paste, mouth wash
- rinse-off products:
    - hair care: shampoo, conditioner
    - bathing, showering: shower gel, bath foam
    - soap: bar and liquid
- skin care:
    - face cream, hand cream, body lotion
    - make-up: eye make up, mascara, eyeliner, lipstick, lipgloss, make-up remover
    - deodorant: stick, roller
    - sun care cosmetics: sunscreen lotion

#### 3.3.2.2 Cleaning products

For cleaning products, the use of triclosan in dishwashing detergent is identified as a main source of exposure. For loading of dish washer detergent a retention factor of 100 % is assumed. For washing the dishes a retention factor of 20 % is assumed.

The estimated exposure to triclosan from cosmetics, oral hygiene products and cleaning products is presented in Table 3.1.

#### 3.3.2.3 Textiles and plastics

Exposure through plastics

No data were available on the levels of triclosan in the treated products neither on leaching from the product. However, it is claimed that the effect of the treatment is long lasting. This suggests that the release of triclosan from plastic products per unit of time is small, especially for materials in which triclosan is incorporated in the material during the manufacturing process. Plastic products used for storage or cutting of food products should comply with the limit of 5 mg/kg food for food contact materials, set by the Scientific Committee on Food (see below).

Exposure through textiles

It is reasonable to assume that the antimicrobial action of triclosan from textiles is due to the slow release from the fabric. A person may be exposed through wearing clothes or sleeping on a mattress made from these textiles. Unfortunately, also in this case no data are available on concentrations or leaching levels. A simple calculation gives an indication of the amount of daily exposure that can be expected. For example, it can be assumed that the cover of a mattress, weighing 2 kg, is impregnated with 1 % triclosan in order to give it an antimicrobial protection for 5 years. Accordingly, the cover would contain 20g of triclosan. Furthermore, it is assumed that a person sleeping on this mattress will be dermally exposed to 10 % of triclosan released from this mattress, i.e. 2g of triclosan. A constant release of this amount of triclosan over 5 years would lead to a total daily dermal exposure to triclosan of 2g/(365*5)= 1.1 mg/day. Assuming a body weight of 60 kg and a dermal absorption of 25 % the daily systemic exposure would be 0.005 mg/kg bw/day. For a child of 2.5 years of age, weighing 12.5kg a dermal exposure of 1.1 mg/dag would lead to a daily systemic exposure of 0.02 mg/kg bw/day. It is noted this exposure estimate can be considered very conservative. For instance, over a period of 5 years the body weight of a 2.5 year old child will increase considerably, resulting in a lower exposure per kg bodyweight.

#### 3.3.2.4 Food contact materials

As is the case with plastics and textiles, it is likely that the release of triclosan from food contact materials (e.g. plastic containers and cutting boards) is very slow. Furthermore, it can be assumed that the area of a food product that comes in contact with these food contact materials, is relatively small in comparison to the total food volume. The EU Scientific Committee on Food classified triclosan in SCF_List 3 with a restriction of 5 mg/kg food [18],

For a person weighing 60 kg daily consumption of 1 kg of food containing 5 mg of triclosan would result in an exposure of 0.08 mg/kg bw/day.

On the basis of migration data from PP, HDPE and thin PVC films the Scientific Committee on Food [18] concluded that migration from food contact materials could exceed 5 mg/kg food. Thus, daily exposure might exceed 0.08 mg/kg bw/day. However, the assumption that a person would consume 1 kg of food containing (more than) 5 mg triclosan on a daily basis seems to be unrealistic.

**Table 3.1. Calculation of daily exposure using cosmetics factsheet [19] and cleaning products factsheet [20].**

| | | | | | | Adult | | | Child (2.5 years) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Product | Triclosan level in product (%) | % absorption | Product amount (g)/ event | Frequency of use | Retention factor (%) | Daily exposure to product (g) | External exposure to triclosan (mg) | Estimate of systemic triclosan exposure/day | Daily exposure to product (g) | External exposure to triclosan (mg) | Estimate of systemic triclosan exposure/day |
| Oral hygiene [A] | 0.3 | 100 | | | | | | | | | |
| - toothpaste | | | 1.4 | 2/day | 6 | 0.16 | 0.48 | 0.48 | 1.06[1] | 3.2 | 3.2 |
| - mouthwash | | | 10 | 4/day | 10 | 4.0 | 12 | 12 | - | - | - |
| Hair care [A] | 0.5 | 25 | | | | | | | | | |
| - shampoo | | | 20 | 260/y | 10 | 1.4 | 7 | 1.75 | 0.47[6] | 2.3 | 0.58 |
| - conditioner | | | 14 | 104/y | 10 | 0.4 | 2 | 0.5 | - | - | - |
| Bathing/showering [A] | 0.5 | 25 | | | | | | | | | |
| - shower gel | | | 8.7 | 329/y | 10 | 0.78 | 3.9 | 1.0 | 0.26[6] | 1.3 | 0.32 |
| - bath foam | | | 17 | 104/y | 100[4] | 4.8 | 24.2 | 6.1 | 1.6[6] | 8 | 2 |
| Skin care [A] | 0.3 | 25 | | | | | | | | | |
| - face cream | | | 0.8 | 2/day | 100 | 1.6 | 4.8 | 1.2 | - | - | - |
| - hand cream | | | 1.7 | 2/day | 100 | 3.4 | 10.2 | 2.6 | - | - | - |
| - body lotion | | | 8 | 2/day | 100 | 16.0 | 48 | 12.0 | 2.7[3,5] | 8.1 | 2 |
| Make-up [A] | 0.3 | 25 | | | | | | | | | |
| - eye make-up | | | 0.01 | 2/day | 100 | 0.02 | 0.06 | 0.015 | - | - | - |
| - mascara | | | 0.025 | 1/day | 100 | 0.025 | 0.075 | 0.019 | - | - | - |
| - eye liner | | | 0.005 | 1/day | 100 | 0.005 | 0.015 | 0.004 | - | - | - |
| - lipstick/lipsalve | | | 0.01 | 4/day | 100 | 0.04 | 0.12 | 0.03 | - | - | - |
| - make-up remover | | | 0.5 | 1/day | 100 | 0.5 | 1.5 | 0.38 | - | - | - |
| Deodorant stick/roller [A] | 0.3 | 25 | 0.5 | 1/day | 100 | 0.5 | 1.5 | 0.38 | - | - | - |
| Sun care cosmetics [A] | 0.3 | 25 | 10 | 3/day [2] | 100 | 30.0 | 90 | 22.5 | 10[3] | 30 | 7.5 |
| Soap (liquid) for washing hands [A] | 0.5 | 25 | 1 | 5/day | 10 | 0.5 | 2.5 | 0.65 | 0.5 | 2.5 | 0.65 |
| Dish washing detergent [B] | 0.5 | 25 | | | | | | | | | |
| - loading | | | 0.01 | 426/y | 100 | 0.01 | 0.05 | 0.013 | - | - | - |
| - washing up | | | 8.6 | 426/y | 20 | 2 | 10 | 2.5 | - | - | - |

[A] Data from RIVM Cosmetics fact sheet [19].
[B] Data from RIVM Cleaning product fact sheet [20].

[1] Amount ingested by a child per event: 0.53g. Frequency of use: 2/day [19].

[2] Sun care cosmetics may be used daily during short periods. It is assumed that it is used during 25 days/year in summer [19]. Since the overall NOAEL is based on a developmental toxicity study, with a short period of triclosan exposure, it was considered appropriate to compare the daily exposure to triclosan due to this short period of sun cream use to the short-term NOAEL.
[3] For sun care cosmetics and body lotion the amount used per event for a child is 1/3 of that for an adult, since the total body surface of a child is also about 1/3 that of an adult.
[4] Since triclosan is rather lipophylic fat soluble (Log kow = 4.76) with a relatively low molecular weight (MW = 289.5) it is assumed, worst case, that when a person takes a bath with bath salt/foam/oil all triclosan is retained on the skin.
[5] It is assumed that a child may be exposed to body lotion once per day.
[6] It is assumed that for a child the amount of shampoo, shower gel and bath foam used per event is 1/3 that used by an adult.

#### 3.3.2.5 Infant: breast feeding and cosmetic products

Exposure through breast feeding

Mothers exposed to triclosan, in particular those using personal care products containing triclosan will excrete part of this chemical in the milk [22]. It was found that milk contained triclosan up to 0.95 ng/g fresh weight. Assuming a milk intake of 150 ml/kg bw/day [23] this would equal a daily exposure of a neonate of 150 (ml) x 0.95 (ng/g)= 143 ng/kg bw/day.
In another recent study in humans, triclosan was found in 3 out of 5 samples of human milk, at levels of 60-300 µg/kg lipid weight [16]. In a human study, milk fat levels peaked at 3.06 ± 0.21 % (w/v) at 3 weeks after birth in milk from mothers delivering at full term [24]. In milk from mothers delivering prematurely fat levels in milk were higher, peaking at 4.33 ± 0.24 % (w/v) three weeks after birth [24]. Assuming a milk intake of 150 ml/kg bw/day [25] lipid intake through breast feeding for infants is 150 (ml) x 0.0433 (% lipid) = 6.5 g/kg bw/day. On the basis of 300 µg triclosan/kg lipid fat the daily triclosan intake through breast feeding is 300 x 6.5/1000 = 1.9 µg/kg bw/day.
Thus, based on the triclosan levels in milk from both studies, a maximum triclosan intake of 1.9 µg/kg bw/day through breast feeding is estimated.

Exposure through cosmetic products

Infants may be exposed to triclosan through baby oil or baby cream, and through shampoo and soap. For a 4.5 months old infant daily exposure is estimated at 0.54g for baby cream (amount of product/event= 0.27g, frequency= 2/day) and 2.6g for baby oil (amount of product/event= 1.3g, frequency= 2/day) [19]. If these products would contain triclosan at a concentration of 0.3 % the daily exposure to triclosan through baby cream and baby oil would be 1.6 and 7.8 mg/day respectively. Assuming a body weight of 6.21 kg [19] this is equal to 0.26 and 1.26 mg/kg bw/day for baby cream and baby oil respectively. Application of sun care cosmetics on infants would probably lead to similar triclosan exposure levels.

### 3.3.3 Aggregate exposure

In an aggregate exposure assessment the estimated exposure to a compound is based on the summation of the exposure from all possible sources and is based on a derterministic calculation (using high end point estimates instead of distributions). The use of triclosan is allowed in several product categories. However, data on which products actually do contain triclosan, and the levels therein is very scarce. In a study by the Stichting Natuur en Milieu and Milieudefensie [8], performed in 2005 in the Netherlands, triclosan was found in only 1 (toothpaste) out of 55 cosmetic and oral hygiene products. Although it is not clear whether this incidence is representative for all products of this class marketed in the Netherlands, it indicates that the presence of triclosan in these products is limited. A survey in Denmark indicated that cosmetic and oral hygiene products are the largest contributors of triclosan on the Danish market: 99 % of the total amount reported in the survey could be attributed to these products. The data from this survey and the exposure assessment suggest that the potential contribution of other products such as textiles and plastics and food contact materials to the exposure to triclosan is relatively small.
It is likely that in the Netherlands triclosan is predominantly used in cosmetics and oral hygiene products, and that even in these product classes the use of triclosan is limited. It is therefore unrealistic to assume that triclosan is present in all products in which its use is allowed, and to base the aggregate exposure assessment on this assumption.
In view of the above, for the present case study with triclosan, no aggregate exposure calculation is performed. However, the potential health risk due to the use of a single product or several products containing triclosan will be discussed.

## 3.4 Risk assessment

From the exposure assessment it is concluded that exposure to triclosan from textiles and plastics, and food contact materials will not pose a health concern. Significant exposure to triclosan occurs through the use of cosmetics and oral hygiene products. Below the potential health risks posed by these products for adults and children are discussed.

Adult

Table 3.1 indicates that for adults the major exposure to triclosan may occur through exposure to sun care products (22.5 mg/day), body lotion (12mg/day), mouth wash (12mg/day) and bath foam (6.1mg/day). Exposure to triclosan through other cosmetics and oral hygiene products, plastics, textiles and food contact materials is much lower, and as single sources of exposure are unlikely to pose a health concern. For adults a triclosan exposure due to the use of sun care cosmetics alone is calculated to be 22.5 mg/day. Assuming a body weight of 60 kg [26] this is equal to 0.38 mg/kg bw/day. As compared to the NOAEL of 25 mg/kg bw/day the MOS is 66. In risk assessment, generally a MOS of $\geq 100$ is considered adequately protective. Accordingly, based on the MOS value of 66, adverse health effects for an adult as a result to exposure to triclosan through sun care products cannot be excluded, should they contain triclosan at the maximally allowed concentration. The single use on a regular basis of either body lotion, mouth wash or bath foam would probably not be a health concern (MOS values of respectively 125, 125 and 245). It is noted that the estimated exposure to triclosan from the use of bath foam is probably very conservative. However, it is not unrealistic to assume that a person would use mouth wash, sun screen lotion and/or body lotion on a regular basis. Should all of these three products contain triclosan at the maximum allowed level, a daily exposure of 46.5 mg/day is calculated, equal to 0.78 mg/kg bw/day. In that case the MOS to the overall NOAEL is only 32.
The overall NOAEL for triclosan was derived from a developmental toxicity study in mice, based on effects in dams (liver toxicity) and in fetuses (reduced fetal weight and delayed ossification). The present risk assessment indicates that adverse health effects to adults and developing fetuses due to exposure from triclosan from cosmetics and oral hygiene products cannot be excluded. However, as was stated before, it is not clear which products actually contain triclosan, nor is information on the in-use concentrations available. Therefore, more realistic exposure assessment, using realistic in-use concentrations, would needed to be performed.

Child

Table 3.1 shows that for children the major potential contributors to triclosan exposure are sun care cosmetics (7.5 mg/day) and toothpaste (3.2mg/day), and to a lesser extent bath foam and body lotion (2mg/day). Exposure to triclosan through other cosmetics and oral hygiene products, plastics, textiles and food contact materials is much lower, and as single sources of exposure are unlikely to pose a health concern. Assuming a body weight of 12.5 kg for a 2.5 years old child [26] for children the triclosan exposure of 7.5 mg/day due to the use of sun care cosmetics alone is equal to 0.6 mg/kg bw/day. As compared to the NOAEL of 25 mg/kg bw/day the MOS for this exposure is 42. The use of tooth paste may lead to an exposure of 3.2 mg/day, equivalent to 0.26 mg/kg bw/day, resulting in a MOS value of 96. Thus, adverse health effects for a child, due to the use of sun care cosmetics, should they contain triclosan at the maximally allowed level of 0.3 %, cannot be excluded based on the conservative exposure assessment as described above. Furthermore, it is not unrealistic to assume that a child would use sun care cosmetics in combination with tooth paste, or one of these products in combination with for instance bath foam or body lotion on a single day on a regular basis, resulting in even lower MOS values.

Infant; exposure through breast feeding

Exposure of an infant through breast feeding was estimated at 1.9 µg/kg bw/day. As compared to the overall NOAEL of 25 mg/kg bw/day the MOS is >13000. It can be concluded that the exposure to triclosan through breast feeding is negligible.

Exposure of infants to triclosan through baby oil or baby cream is estimated at 1.26 and 0.26 mg/kg bw/day with MOS values of respectively 20 and 96. Clearly, based on this low MOS value for baby oil adverse health effects for infants cannot be excluded in case triclosan is used at the maximum allowed level. Infants may also be exposed to triclosan through other sources, such as shampoo and soap. However, no data are available on the amounts of these products that are used for infants.

## 3.5 Discussion and conclusions

The data from the exposure assessment indicate that the major sources of exposure to triclosan are cosmetics and oral hygiene products. The potential contribution of other products such as textiles and plastics and food contact materials to the exposure to triclosan is relatively small.
In an aggregate exposure assessment the estimated exposure to a compound is based on the summation of the exposure from all possible sources. However, as discussed in section 3.2.3.3, in the Netherlands the presence of triclosan in cosmetics and oral hygiene products is probably limited. Therefore in the case of triclosan it is considered unrealistic and not appropriate to perform an aggregate exposure assessment based on the assumption that all products in which the use of triclosan is allowed actually contain triclosan.
Nevertheless, from the data in Table 3.1 it is clear that for adults significant exposure to triclosan may result from the use of mouth wash alone (MOS: 66). Moreover, adults may be exposed to more than one product containing triclosan on a daily basis. Above it was concluded that the use of mouth wash, sun cream and/or body lotion could lead to an exposure of 0.78 mg/kg bw/day. In that case the MOS to the overall NOAEL is only 32.

The overall NOAEL for triclosan was derived from a developmental toxicity study in mice, based on effects in dams (liver toxicity) and in fetuses (reduced fetal weight and delayed ossification). The present risk assessment indicates that adverse health effects to adults and developing fetuses due to exposure from triclosan from cosmetics and oral hygiene products cannot be excluded, a more refined exposure estimation is needed to better assess this risk.

For children the potentially major contributors to triclosan exposure are toothpaste (MOS: 96) and sun care cosmetics (MOS: 42). For infants potential major sources of triclosan exposure are baby oil (MOS: 20) and baby cream (MOS: 96). Should combinations of these products be used daily on a regular basis even lower MOS-values would be calculated. In risk assessment, generally a MOS of $\geq 100$ is considered adequately protective. Thus, should this specific group of products contain triclosan at the maximally allowed concentration adverse health effects due to exposure to triclosan, derived from single sources or combinations of these products, cannot be excluded.

*Comparison with the recent opinion on triclosan by SCCP*
A number of differences exist between the present risk assessment of triclosan and the recent SCCP opinion on triclosan. The present riwsk assessment was based on limited publicly available information. The opinion on triclosan by SCCP was based on information provided by Industry, and contained more studies and more detailed information on current-use concentrations. Moreover, an internal exposure study was performed in volunteers using triclosan containing products.
Based on the available information SCCP concluded that the overall NOAEL was 12 mg/kg bw/day, observed in a 2 year study in rats. In our evaluation we used a NOAEL of 25 mg/kg bw/day, observed in a developmental toxicity study in mice as the bases for the risk assessment.
In the present case a dermal absorption value of 25 % was used, based on information from a Norwegian evaluation. In the SCCP opinion on triclosan dermal absorption percentages of 7-12 %, based on in vitro human skin studies, were used. Furthermore, the present assessment based the exposure assessment solely on the assumption that a product contained triclosan at the maximally

allowed concentration whereas for the SCCP opinion information from Industry on triclosan levels in products were available. Thus SCCP calculated exposure for both current-use and maximally allowed concentrations. Also, some assumptions on the amount and frequency of product used slightly differed between the present assessment and the SCCP opinion.

Based on its exposure and risk assessment the SCCP concluded that the use of body lotions and mouth washes may result in high exposures and is not recommended. The use of common-use products such as tooth paste, hand soap, body soap/shower gel and deodorant stick was considered safe, whereas exposure to triclosan from face powder and blemish concealer was considered low.
In the present risk assessment it was concluded that the use of body lotion, mouth wash, sun care products and bath foam (latter two product types were not considered by SCCP, as Industry did not indicate these products as products in which triclosan is used) resulted in high exposures. For other triclosan-containing products (other cosmetics, oral hygiene products, plastics, textiles and food contact materials) the calculated exposure was much lower. Thus, although the available data bases and exposure estimates for SCCP and the present risk assessment differed, in both studies similar conclusions on the risk of triclosan-containing products were drawn.

SCCP concluded that exposure of children aged 6-11 to triclosan was lower than in children aged 11-19 years and adults, on the basis of measurements of spot urine concentrations. In the present risk assessment for a child 2.5 years of age high exposures were calculated for sun care cosmetics and tooth paste, and to a lesser extent bath foam and body lotion. Furthermore, in the present risk assessment it was concluded that adverse health effects of the use of certain triclosan-containing products, should they contain triclosan at the maximally allowed concentration, for the developing fetus cannot be excluded. It should be noted that the SCCP did not specifically address exposure and risk for young children and fetuses.

It can be concluded that to a certain extent SCCP and the present aggregate risk assessment come to the same conclusion with respect to safety of the use of triclosan in certain types of products. However, since in the SCCP opinion and the present risk assessment different sub-populations and product types were addressed and different exposure parameters and exposure models were used, the conclusions do not always concur.

**Conclusion**
The present risk assessment for triclosan is hampered by the lack of data. Although it is reported that triclosan may occur in a variety of products, it appeared to be very difficult to obtain data on which products actually do contain triclosan, and on actual the levels of triclosan in the product. A survey in the Netherlands indicates that the use of triclosan in cosmetic and oral hygiene products is limited. The use of triclosan in other product types is probably even less. Therefore, an aggregate exposure estimation based on the assumption that triclosan is present at the maximally allowed concentration in every product in which its use is allowed is very unrealistic. Nevertheless, the exposure estimate does indicate that the daily use of certain products by themselves (i.e. mouth wash, tooth paste, sun care cosmetics and skin care products), should they contain triclosan at the maximally allowed concentration, may lead to a significant exposure to triclosan in adults as well as children and infants. Under those circumstances adverse health effects due to the use of these products can not be excluded. The present aggregated risk assessment based on limited information, and the SCCP opinion on triclosan, which was based on an extensive data base, to a certain extent came to the same conclusion on the health risks of certain types of products containing triclosan. However, the comparison between the two risk assessments also indicates that the outcome may depend on, for instance, the choice of sub-populations, product types, exposure parameters and exposure models.
The biological monitoring data on triclosan, performed by US-EPA [11] indicated that the actual aggregate exposure to triclosan in the USA is no cause for concern. This suggests that the determination of aggregate exposure performed in the present report and by SCCP may be overly

conservative. However, whether triclosan levels in products and their use by the sampled population in the US-EPA study are comparable to the theoretical exposure estimates in the present study and the SCCP opinion is not clear.

It can be concluded that, based on the present conservative exposure estimates and risk assessment, it may be worthwhile to reconsider the use of triclosan in oral hygiene products (mouth wash), and in skin care (body lotion) and sun care cosmetics (if used at all). To get a better estimate of this risk, additional exposure data (e.g. on the actual in-use level of triclosan in the various products) are needed.

# 4 Case study 2 – permethrin

## 4.1 Description of the case

Permethrin (CAS No 52645-53-1) is a type I synthetic pyrethroid. It is an insecticide effective against a wide range of pests in agriculture, animal husbandry, forestry, the private area, and public health. It is also used as a preservative for wood and fabrics. The insecticidal action of permethrin is due to its interaction with ion channels on axons of the nervous system of target species. The binding of permethrin to sodium channels causes a slowing of the rate of closure, resulting in repetitive firing of nerves, depolarisation and nerve block. Permethrin has good residual action on inert surfaces, is moderately stable in the environment and is nonsystemic in plants [27].
Permethrin is an ester of the dichloro-analogue of chrysanthemic acid, and 3-phenoxybenzyl alcohol, chemically identified as (3-phenoxyphenyl)methyl-(±)-cis-trans-3(2,2-dichloroethyenyl)-2,2-dimethylcyclopropanecarboxylate. The technical-grade materials are racemic mixtures of four stereoisomers (1R-trans, 1R-cis, 1S-trans and 1S-cis), of which the 1R-cis isomer is the most active insecticide, followed by the 1R-trans isomer. The most commonly used cis-trans ratios of permethrin are 25:75, 40:60 and 80:20.

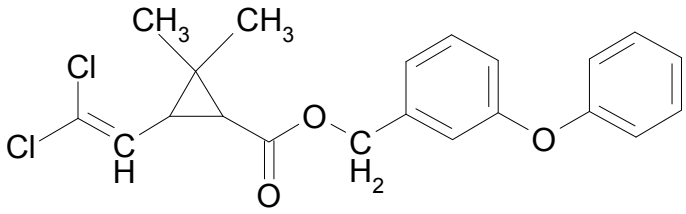


**Figure 4.1: Chemical structure of permethrin**

Permethrin is effective, as an ovicide, larvicide and adulticide against a wide variety of insects: crickets, mites, cockroaches, locusts, grasshoppers, woodboring beetles, silverfish, bed-bugs, ticks, ants, mosquitoes, lice, fleas, blackflies, tsetse fly, domestic flies and other undesirable arthropods. Permethrin is very effective as a direct contact poison or as a residual substance. However, as a lipophilic substance lacking fumigant action it is not usually effective against aphids, systemic parasites and soil pests except by direct contact application [27].
In view of this wide range of pests permethrin is effective to, application of permethrin is widespread and aggregated exposure occurs. Most common route of exposure is oral by consumption of food, as permethrin is used as insecticide on crops and can be used as veterinary medicine (for the control of ectoparasites on animals), both with possible residues in food. Besides exposure from food, consumers can also be exposed to permethrin from the use of permethrin as wood preservative (as non-professional user or from contact with treated wood), as fabric preservative (contact with treated carpets), as insect repellent (treated clothes, mosquito nets, curtains, bed sheets etc.), and as insecticide (use of lice control shampoo, use of sprays to eliminate unwanted insects in and around the house or animal houses). Some applications are for health safety purposes, as mosquitoes or other insects are vectors for pathologic parasites, bacteria and viruses causing illnesses like malaria and dengue fever.

## 4.2 Toxicological profile and limit values

### 4.2.1 Kinetics

Permethrin is readily metabolized with immediate loss of toxicity [28]. Permethrin is readily absorbed from the gastrointestinal tract; by inhalation of dust and spray mist; in non-polar solvents more rapidly than in aqueous solutions [27]. Absorption is minimal through the intact skin (< 2 % in human).

### 4.2.2 Toxicodynamics

Permethrin (25:75 to 40:60 cis:trans isomeric mixtures) has low acute toxicity after oral, dermal and inhalation administration. The toxicity of the racemic mixture varies with the cis/trans ratio and the characteristics of the vehicle used. The cis isomer is the most toxic and non-polar carriers increase the toxicity of both isomers. The oral LD50 values in rats ranged from 6000 mg/kg bw for the 20:80 *cis:trans* isomeric mixture, to 225 mg/kg bw for the 80:20 *cis:trans* isomeric mixture.
The main effects after short-term repeated administration of permethrin to laboratory animals are tremor, hyperexcitability, and changes in body and liver weights. NOAEL values were 5 mg/kg bw/day in a 52-week oral dog study, 1000 mg/kg bw/day in a 21-day study in rabbits treated dermally, and 250 mg/m$^3$ (NOAEC) in a 13-week study in rats exposed by inhalation. It is mildly irritating to the eyes and slightly irritating to skin.
From 5 long-term studies in rats and mice, it was concluded that permethrin has very weak oncogenic potential and that the probability that permethrin has oncogenic potential in humans is very low (IARC classification group 3 [29]). No genotoxic activity was observed in *in vitro* DNA-damage and mutagenicity tests, but there is evidence that permethrin can induce chomosomal aberrations in mammalian cells *in vitro*. Permethrin is not a reproductive or developmental substance.

An ADI of 0.05 mg/kg bw was established for technical-grade permethrin with cis:trans ratios of 25:75 to 40:60 on the basis of a NOAEL of 100 ppm, equivalent to 5 mg/kg bw per day, in the 2-year study in rats, which was based on clinical signs and changes in body and organ weights and blood chemistry at 500 ppm, and the NOAEL of 5 mg/kg bw per day in a 1-year study in dogs based on reduced body weight at 100 mg/kg bw per day, and applying a safety factor of 100 [28, 30]. Establishment of an acute reference dose was not necessary because of the low acute toxicity of permethrin [28].

### 4.2.3 Conclusion on toxicology

For the present case study an oral, dermal and inhalatory absorption of respectively 60, 1 and 100 % is assumed.
For exposure to permethrin from food sources the exposure levels will be compared to the ADI of 0.05 mg/kg bw/day.
For exposure to permethrin from non-food sources a chronic, the systemic exposure to permethrin is compared to a systemic acceptable exposure level (AEL) of 0.03 mg/kg bw/day, as derived from the NOAEL of 100 ppm, equivalent to 5 mg/kg bw per day, based on clinical signs and changes in body and organ weights and blood chemistry in a 2-year study in rats, and the NOAEL of 5 mg/kg bw/day, based on reduced body weight per day in a 1-year study in dogs, applying a safety factor of 100 and an oral absorption of 60 %.

## 4.3 Exposure assessment

### 4.3.1 Exposure sources

Exposure of the general population to permethrin is mainly via dietary residues. Permethrin is used as an insecticide in the culture of corn, soybean, coffee tobacco, oil seed rape, wheat, barley, alfalfa, vegetables and fruits, and as a fog in mushroom houses. In addition to its pre-harvest usage, permethrin can be used in the protection of stored grain.

Permethrin is also used for the control of insects in animal facilities and permethrin might be found as residue in meat, milk and eggs.

Besides the exposure to permethrin as residue in the diet, exposure to permethrin can occur dermally or by inhalation due to use of permethrin in household and forest pest control, in the culture of cotton plants (residues in clothes) and as a wood and fabric preservative. Other applications are in public health, particularly for insect control in aircrafts, treatment of mosquito nets, and human lice control [31].

### 4.3.2 Exposure estimates

#### 4.3.2.1 Exposure due to residues in food

**Pesticide residues**

The consumer can be exposed to permethrin from residues present on crops, as permethrin is used as insecticide on a wide range of food crops, including fruits, nuts, vegetables and grain crops. Due to lack of data to demonstrate that permethrin fully complies with the requirements of directive 91/414/EC, permethrin is not included in Annex 1 of directive 91/414, and consequently in 2001 authorisations for all uses of plant protection products containing permethrin in the EU are withdrawn [32]. As, however, crops can be imported from all over the world, intake of permethrin as residue on crops is still considered here. The last evaluation by JMPR of permethrin intake was conducted in 1999 [28]. The calculated theoretical maximum daily intake (TMDI) with a European diet was 0.963 mg/day. This corresponds to 16.1 µg/kg bw/day for a 60 kg person (32 % of the ADI) [28]. No intake assessment for children was made in the JMPR monograph. For children the intake of permethrin residues via food was estimated using the MRL's from the JMPR 1999, in combination with the Dutch diet for children (1-6 years). The intake was estimated at 716 µg/child/day, which is with a mean of 17 kg bw/child equivalent to daily permethrin intake of 42.6 µg/kg bw/day (84 % of the ADI).
However, monitoring data from the FDA program show that the real intake is much lower, as the population with the highest intake was the group of 6-11 months old infants, for which the average daily intake was determined at 44 ng/kg bw/day, a factor 1000 lower than the worst-case intake calculation based on MRL's. For adults, the intake was about 10 ng/kg bw/day. Monitoring data from the Netherlands for permethrin concentrations in 17906 samples measured between 1997 and 2000 [33], also show that the real exposure is far beneath the calculated worst case intake calculation; dietary modeling of the median intake for permethrin, based on residues measured and using the national diet based on the Dutch food consumption survey (VCP) from 1997-1998 [34], shows a value of 0.3 ng/kg bw/day permethrin (99-percentile: 1 ng/kg bw/day)[1]. Compared with the theoretical maximum daily intake of 16 µg/kg bw/day from the JMPR, the exposure assessment based on Dutch monitoring data yield a mean daily intake value that is a factor 50,000 lower than the intake calculated

[1] 1 Koers, E. (2001) Risk evaluation of the (chronic) cumulative exposure to synthetic pyrethroids through dietary intake. Report of the work placement at RIVM, Bilthoven.

with the worst case assumptions, while based on FDA monitoring data, the intake is more than a factor 1000 lower.

**Veterinary medicine residues**
Permethrin used in veterinary medicines is a racemic mixture of four stereoisomers with cis:trans ratios of 80:20, 40:60 or 25:75. It is used in the form of sprays (including udder sprays), powders, pour-ons or ear-tags for external application to cattle, horses and donkeys for the control of ectoparasites. EMEA estimated a daily intake of 383 µg/ person per day due to residues of permethrin in food products of animal origin [35]. This corresponds to 6.4 µg/kg bw/day for a person weighing 60 kg (12.8 % of the ADI).

#### 4.3.2.2 Exposure due to non-food items

**Residential use**
Permethrin is applied by homeowners for outdoor uses (lawns, gardens, and ornamentals), indoor uses (spraying of surfaces and spaces). Although a lot of products containing permethrin are marketed, it is assumed that only one product is used per household. A product evaluated and still registered in the Netherlands is 'Sprigone', which is used as targeted spot application against crawling insects and wasps, is taken here as an example to estimate the exposure to permethrin due to use of insecticide products in and around the house. The permethrin content of Sprigone is 0.25 % The mean chronic total systemic exposure of the non-professional user was calculated to be 1.02µg/kg bw/day, due to dermal and inhalatory exposure during application [30]. The product was assumed to be applied approximately once every 2 weeks [30]. Re-entry exposure due to this use is negligible as compared to exposure during application and therefore not considered here.
Due to concerns about neurotoxicity of permethrin for young children, use of permethrin-containing biocides to kill insects in and around the house is not allowed for spaces where children up to 4 years of age can go. Exposure of children due to use of permethrin as an insecticide in the house is therefore not taken into account.

**Petcare products**
Permethrin is also used to treat pets that have ectoparasites. Products containing permethrin are powders, shampoos and ampuls, of which the powder is assumed here to yield the worst-case exposure scenario. In the Netherlands, Defencare® powder is registered as a powder to treat pets with fleas and ticks [36]. Permethrin content is 0.94 %. Instructions for use prescribe application of 1 gram powder per kg bodyweight for dogs, and 2 gram/kg bw for cats. It has to be rubbed into the fur, but gloves should be worn, so no dermal exposure takes place. Inhalation of the powder cloud results in exposure of the applicant. In the present calculation, it is assumed that 20 gram powder per treatment (10 kg bw cat, or 20 kg bw dog) is used, and that 10 % of the applied dose ends up in a cloud of powder of 5 m3. The concentration permethrin in the powder cloud will be: 20 g x 9.4 mg/g x 10 % / 5 m3 = 3.76 mg/m3. With an inhalation rate of 34.7 m3/day (light exercise [37]), an assumed duration of treatment of 5 minutes (2 minutes for application, 3 minutes rubbing in), a 100 % absorption by inhalatory exposure, a once a week user frequency, and a bodyweight of 60 kg, the chronic systemic exposure is 3.76 mg/m3 x 34.7 m3/day / 24 h/day / 60 min/h x 5 min x 100 % / 7 days / 60 kg/bw = 1.08 µg/kg bw/day.
After treatment, it is recommended to avoid exposure as much as possible. Young children will however pet their pets. The main exposure will than be the oral exposure hand to mouth contact, they will be exposed orally. To calculate the oral exposure, an EPA formula can be used: PDR = DR * SA * FQ * ET [38].

- PDR is potential dose rate (mg/day)
- DR is dislodgeable residue on pet or floor (mg/cm$^2$ pet or floor)

- SA is surface area of the hands ($cm^2$)
- FQ is frequency of hand-to-mouth activity (events/h)
- ET is exposure duration (hrs/day).

To estimate the dislodgeable residue, it is assumed that there is a one-to-one relationship between the dislodgeable amount on the surface of the pet and on the surface area of the skin after contact [38]. An average pet has a body surface area of 12.3 x body weight 0.65 (in g) [38], which for the 20 kg dog is 7684 cm2. Applied dose was 20 mg per dog, which is 20 mg / 7684 cm2 = 0.0026 mg/cm2. The dislodgeble residue on pet is assumed to be 20 % of concentration on pets skin [38]; 20 % x 0.0026 = 0.00052 mg/cm2 (DR). Surface area of the hands is 350 cm2, but to assume that both hands are totally put into the mouth seems too worst case. In the present calculation, 20 % of the hand area is taken for SA (70 cm2). The hand-to-mouth contact activity for toddlers (3 to 5 years old) equals 1.56 events/h (FQ), exposure duration is 2 hours a day (ET) [38].
The exposure is then calculated as follows: 0.00052 (mg/cm2) x 70 (cm2) x 1.56 (h-1) x 2 (h/day) = 0.114 mg/day. With an internal exposure of 60 % (oral absorption), a mean body weight of 15 kg, and a once in 7 days treatment frequency, the chronic internal exposure due to hand-to-mouth contact is 0.114 (mg/day) x 60 % /15 (kg bw) x 1/7 (day/day) = 0.00065 mg/kg bw/day.
Dermal exposure is 0.0026 mg/cm2 (concentration on hands) x 350 cm2 (surface area hands) = 0.91 mg/day and chronic internal exposure due to dermal uptake is 0.91 (mg/day) x 2 % (dermal absorption) /15 (kg bw) x 1/7 (day/day) = 0.00017 mg/kg bw/day. Total chronic internal exposure of children due to petting permethrin-treated pets is calculated to be 0.00082 mg/kg bw/day.

**Impregnated fabrics**

***Clothes and bed sheets***

Permethrin-impregnated clothing provides protection against mosquitoes, ticks, chiggers, spiders, ants, midges, and flies. Permethrin impregnated battle dress uniforms (BDU's) are used by the U.S. army. An exposure estimate was made [39], using several conversion factors to translate the targeted fabric impregnation concentration, 0.125 $mg/cm^2$, to an internal dose for military personnel through dermal absorption. These factors were the time-weighted-average percentage of permethrin remaining in fabric through 50 washings (26 %), percentage of migration from fabric to skin (0.5 % per day), body contact area (1.5 $m^2$), dermal absorption rate (2 %), and adult body weight (70 kg). To adjust for actual exposure conditions, the U.S. army assumed that military personnel would wear the treated uniforms 18 hr per day for 10 years during a 75-year lifetime. The average lifetime dermal dose for military personnel from wearing permethrin-impregnated BDUs was calculated to be 68 ng/kg bw/day [39]. There is no information to indicate that significant exposure will occur by any route other than dermal absorption.
The exposure calculation of the US army assumed exposure for 10 years during a 75-year life-time. For the present assessment a life-long exposure to permethrin through impregnated fabric is assumed. Accordingly, the average internal dermal exposure is calculated to be 68 x 7.5= 510 ng/kg bw/day. This is the mean exposure in the period of life that exposure actually occurs. As an every day exposure of 18 hours to impregnated fabrics on the skin is a worst case scenario, it will also cover the use of permethrin in impregnated bed sheets and pillows.

***Carpets***

Wool used for carpets is often pretreated with permethrin in a bath before it is processed further. Exposure to permethrin was calculated after measurements of concentration of permethrin in carpet fibers, in the air and in household dust [40]. Residues in carpet fibers were measured with a median value of 37 mg/kg, and a 90 % percentile of 136 mg/kg; in the air a median value of 1.9 ng/m3 and a 90 % percentile of 5.8 ng/m3, and in household dust with a median value of 9.7 mg/kg and a 90 % percentile of 129 mg/kg. It was calculated (using the 90th percentile values) that a child of 15 kg (and 6 m3/day inhalation volume) can be internally exposed to permethrin by inhalation (2.3 ng/kg bw/day),

by mouthing behavior (100 mg of dust: 0.86 µg/kg bw/day) and by dermal contact (0.87 µg/kg bw/day), resulting into a daily permethrin intake of 1.73 µg/kg bw/day [40]. For adults, the exposure is insignificant (<0.1 µg/kg bw/day), as they are only exposed by inhalation.

***Mosquito nets and curtains***
From mosquito nets and from curtains, only inhalatory exposure is expected. Permethrin is not a volatile substance (vapour pressure is 1.3x10-6 Pa) and mosquito nets do not dust. Mosquito nets are treated usually with permethrin at a rate of 500 mg/$m^2$. As inhalatory exposure was measured for permethrin from impregnated carpets [40], extrapolation to mosquito nets is possible from this study when the permethrin concentration per square meter is calculated. Carpet weight is on average 2000 g/$m^2$ and the permethrin level per kg carpet was measured to be 136 mg/kg (90 % percentile), yielding a permethrin concentration of 272 mg/$m^2$ carpet. This is about half the amount of the concentration per square meter as compared to mosquito nets. As is the case for inhalatory exposure from impregnated carpets, the exposure to permethrin from impregnated mosquito nets will be insignificant (<0.1 µg/kg bw/day).

**Application of Impregnation sprays**
By using impregnation spray for re-impregnation of mosquito nets, or to impregnate clothes, the private user can be exposed inhalatory and dermally to the spray solution. By using ConsExpo 4.1 [41] (scenario targeted spot application for pest control products [37]), assuming an application frequency of once a month of a 0.5 % permethrin solution) the total systemic exposure is calculated to be 0.75 µg/kg bw/day.

**Lice control**
Permethrin 1 % lotion is used to treat head lice infections. It has to be used twice, with the recommended second treatment 7-10 days after the first treatment. In an absorption study, 50 ml of an ethanolic solution containing 215 mg permethrin (0.5 % solution; cis/trans: 25/75) was administered to wet hair of the head for 45 min in six volunteers. Absorption was determined measuring urinary excretion of the main metabolite of permethrin and its conjugates for one week. The urinary recovery of the metabolite was on average 0.35 % (permethrin equivalent) of the administered dose, with a maximum of 0.55 %. In an in vitro model with human skin, it was estimated that 0.6 % of the applied dose of radioactive-labeled permethrin was absorbed [42]. In the present calculation, a dermal absorption of 1 % is assumed.

Assuming 430 mg permethrin per treatment (use of 50 ml of a 1 % v/v solution with a relative density of 0.86), and a 1 % dermal absorption, the total internal exposure is 4.3 mg permethrin per person. Using permethrin on children of 15 kg, the internal exposure on the day of application is 0.29 mg/kg bw; when used on adults of 60 kg, the exposure is 0.072 mg/kg bw. Lice infection should be controlled after 1 cure of 2 applications, but reinfestation can occur. Worst case assumption is that each 2 months a new cure (of 2 applications) is necessary, which is tantamount to 1 application in 30 days. The chronic systemic exposure to permethrin in this scenario is 0.0069 mg/kg bw/day for small children, and 0.0023 mg/kg bw/d for adults.

**Wood preservative products**
Permethrin is used as an active substance in a range of wood preservative products. Exposure occurs during application of the product to treat timber (for curing or protection) either by brushing or spraying, and by sanding or handling treated wood. For children, playing on a wooden playground structure or chewing on a piece of wood will expose them. To estimate the exposure of adults and children, scenario's and assumptions described in the 'User Guidance' of the TNsG [43] are used. The percentage dermal absorption is assumed to be 2 %, oral absorption 60 % and inhalatory absorption 100 %.

Inhalation and dermal contact are the most significant routes of exposure of the non-professional user. An exposure scenario is worked out for a spray application to cure wood that is admitted in the Netherlands. The percentage active compound in the product is 0.25 %. The scenario described in the 'User Guidance' for this product is a 40 minutes application [43]. Percentage cloth penetration is assumed to be 50 %. The 75 %-percentile dermal exposure to product is 40 (min) x 120 (mg product /min; spoiling) x 50 % (cloth penetration) = 2400 mg product on the body, and 7040 mg on hands. Total internal dermal exposure is 2400 + 7040 (mg product) x 0,25 % (% a.i. in product) x 2 % (derm. abs) = 0.472 mg permethrin. Internal inhalatory exposure is 40 (min) x 0.021 ($m^3$/min; inhalation rate) x 115 (mg/$m^3$; concentration of product in air) x 0.25 % (% a.i. in product) x 100 % (inh. abs) = 0.243 mg permethrin. The systemic exposure (total of dermal and inhalation) is calculated to be (0.472 + 0.243) (mg) / 60 (kg bw)= 0.0119 mg/kg bw per event. Assuming a 2 times per year frequency of wood treatment, the chronic systemic exposure is 0.0119 (mg/kg bw) x 2 / 365 (days) = 0.000065 mg/kg bw/day.

Indirect exposure as a result of use of treated wood may also occur. For adults, this exposure should be added to the exposure resulting from application of the wood preservative. In the 'User Guidance' a scenario is described that a person is sanding wooden posts for one hour. The posts have been treated with 2 % wood preservative solution by double vacuum process (chronic reference scenario 1) [43]. The internal exposure from inhalation of wood pieces from the air after a worst case duration of 6 hours sanding is: 0.008 ($cm^3$ wood dust inhalation/hour) x 6 (hours) x 1.0 (mg permethrin/$cm^3$ wood) / 60 kg bw x 100 % = 0.0008 mg permethrin/kg bw. The internal exposure from dermal contact with the treated wood is: 84 (mg permethrin on hands) x 2 % (absorption) / 60 kg bw = 0.028 mg/kg bw/event. As it is assumed that the non-professional user will not sand or handle impregnated wood more than once a month, the chronic exposure is calculated: 0.028 + 0.0008 mg/kg bw/day = 0.0288, divided by 30 (1 exposure in 30 days) = 0.00096 mg/kg bw/day. The inhalatory exposure as a result of inhalation of volatilised residues from treated wood installed (chronic reference scenario 2) is negligible, as permethrin is not a volatile substance (vapour pressure is 1.3x10-6 Pa).
The total chronic systemic exposure of adults is the sum of exposure due to primary exposure by application and the secondary exposure due to sanding and handling of treated wood: 0.000065 + 0.00096 = 0.0010 mg/kg bw/day.

For children, the worst case scenario is that they play on a playground structure (dermal exposure), and that they mouth weathered surfaces (oral exposure) (chronic reference scenario 4, [43]), resulting in a systemic exposure of: 0.4 (mg permethrin on hands) x 2 % (derm. abs) + 0.5 (mg permethrin ingested) x 60 % (oral abs.) = 0.31 mg permethrin per day. Assuming a child's weight of 15 kg and children playing once in two days at the playground structure, the exposure is: 0.31 (mg/day) / 15 (kg bw) x 1/2 (day/day) = 0.020 mg/kg bw/day.

### 4.3.3 Aggregate exposure

The aggregated exposure is the sum of exposures from different sources and routes (see Table 4.1, 4.2 and 4.3). For residues of permethrin in food, the exposure is confined to the oral route, and thus can be compared with the ADI of 0.05 mg/kg bw. The exposure due to non-food product is compared with the chronic systemic acceptable exposure level (AEL) of 0.03 mg/kg bw/day (see section 3.2.2).

## 4.4 Risk assessment

Exposure to permethrin may derive from food and non-food sources. In Tables 4.1 and 4.2 the exposure to permethrin, expressed as percentage of the ADI is presented. Although in Table 4.1 the calculated

exposure of children through food, based on the TMDI, exceeds the ADI, it is clear from the actual monitoring data on permethrin residues in food that the exposure of adults and children to permethrin in food products is less than 0.1 % of the ADI.
In Table 4.3 the aggregate exposure to permethrin from non-food sources is presented. The summed non-food exposure for adults and children is lower than the derived AEL (0.03 mg/kg bw/day permethrin). It is reasonable to assume that the actual exposure to permethrin from non-food sources will be well below the 'worst case' exposure estimate from the aggregate exposure assessment. Based on the exposure assessment and the toxicological reference values it can be concluded that it is unlikely that people will experience adverse health effects due to chronically exposure to permethrin from food and non-food sources.

**Table 4.1: Sum of theoretical maximum daily intakes (TMDIs) of permethrin due to consumption of food**

| | **Chronic oral exposure level (mg/kg bw/day)** | | **ADI** | **Percentage of ADI (%)** | |
|---|---|---|---|---|---|
| **Source** | **Adults** | **Children** | | **Adults** | **Children** |
| Pesticide residual levels (TMDI) | 0.0161 | 0.042 | 0.05 | 32.1 | 85.2 |
| Residual levels of veterinary medicine | 0.0064 | 0.0167[1] | 0.05 | 12.8 | 33.4 |
| **Total calculated oral exposure** | | | | **45 %** | **118.6 %** |

[1] For exposure to permethrin residues due to veterinary use the same adult-child ratio as for pesticide residues is assumed.

**Table 4.2: Sum of daily intake of permethrin due to consumption of food based on monitoring data**

| | **Chronic oral exposure level (mg/kg bw/day)** | | **ADI** | **Percentage of ADI (%)** | |
|---|---|---|---|---|---|
| **Source** | **Adults** | **Children** | | **Adults** | **Children** |
| **Total residue intake (based on monitoring data)** | 0.00003 | 0.000044 | 0.05 | **<0.01 %** | **0.09 %** |

**Table 4.3: Sum of exposures for permethrin from non-food sources**

| | **Chronic total systemic exposure level (mg/kg bw/day)** | | **AEL** | **Percentage of AEL (%)** | |
|---|---|---|---|---|---|
| **Source** | **Adults** | **Children** | | **Adults** | **Children** |
| Residential use as insecticide | 0.0010 | - | 0.03 | 3.4 % | - |
| Pet care product | 0.0011 | 0.00082 | 0.03 | 3.6 % | 2.7 % |
| Textile (clothing, bedsheets) | 0.00051 | | 0.03 | 1.7 % | |
| Carpets | < 0.0001 | 0.0017 | 0.03 | <0.3 % | 5.8 % |
| Mosquito nets | < 0.0001 | | 0.03 | <0.3 % | |
| Textile impregnation spray | 0.00075 | - | 0.03 | 2.5 % | - |
| Lice control | 0.0023 | 0.0096 | 0.03 | 8.0 % | 32 % |
| Wood preservative | 0.0010 | 0.010 | 0.03 | 3.4 % | 34 % |
| **Total exposure by non-food use** | | | | **23 %** | **76 %** |

## 4.5 Discussion and conclusions

The main potential exposure to permethrin is of non-food origin. The estimate of exposure to permethrin via food, on the basis of monitoring data indicates that exposure through food is negligible. Even the ‘worst-case’ aggregate exposure assessment indicates that exposure to permethrin from various sources will not lead to a health concern in adults and children.

# 5 Case study 3 – carvone

## 5.1 Description of the case

Carvone is a terpenoid ketone, which has been found in two stereoisomeric forms, i.e. d-(+)-carvone and l-(-)-carvone (CAS-numbers: 99-49-0 (general); 2244-16-8 (d-isomer); and 6485-40-1 (l-isomer). Its synonyms are carvol or *p*-mentha-6,8-dien-2-one. In nature, both optical forms have been reported to occur. The presence of l-carvone has been reported in the oils of *Mentha* (spearmint) in concentration range of 55-75 %. D-carvone (20-75 %) has been found in *Carum* (caraway) and *Anethum* (dill) [44, 45]. Next to their presence in herbs, carvone (d/l) has also been found in citrus peels, citrus fruit/juices (to lesser extend than reported in citrus peels), guava, beetroot, cabbage, and celery.

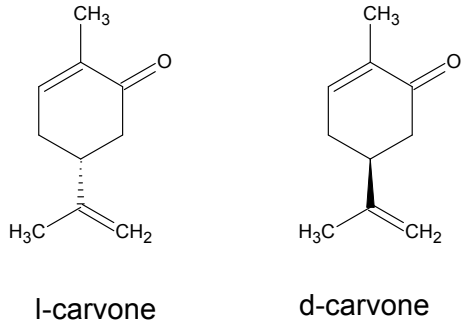


**Figure 5.1: Chemical structures of l-carvone and d-carvone.**

To produce carvone, etheric oil is isolated from the commodities by steam distillation. By fractional distillation carvone is extracted from the etheric oils.

Carvone (d/l) is used in several food stuffs as a flavouring agent. L-carvone is added to chewing gum and d-carvone has been added to food stuff such as biscuits, candies, bread, and meat. Further, d-carvone has been found in (non-)alcoholic beverages [46]. In addition, carvone (d/l) is used in non-food products. Carvone (d/l) is used in personal care products as a flavour and fragrance agent in toothpaste, mouthwash, soap, and perfume. D-carvone also proved a successful plant growth regulator and pesticide on potato crops.
Its use as food additive is considered safe, given the GRAS (Generally Regarded as Safe) status by the US-FDA [47] and latest JECFA (Joint FAO/WHO Expert Committee on Food Additives) evaluation in 1998 [44]. Carvone is authorized in the EU as a pesticide/plant growth regulator in 2008.

Exposure to carvone (d/l) can occur via traditional foods (natural occurrence), via consumption of food stuff resulting from food additive and/or pesticide use, and via the use of personal care products in which carvone was added as flavour and fragrance agent. The different exposures may not pose a health concern, when looked at separately. However, given the nature of the products carvone is used in, the products are used on a daily basis, it is very well possible that consumers are exposed to carvone via multiple products (both food and non-food), and that this aggregate exposure may reach levels of concern. Therefore an aggregate exposure assessment is performed.

## 5.2 Toxicological profile and limit values

JECFA has evaluated the use of carvone as food additive on several occasions and latest in 1998 [44], where at first unspecified carvone and later on d-carvone were considered in their evaluations.

### 5.2.1 Kinetics

In the JECFA 1998 evaluation no information on the absorption of carvone is presented. It is reported that carvone has been detected unchanged in the urine of humans, presumably arising from its dietary intake. Hydroxylation of the ring is the primary metabolic pathway.

### 5.2.2 Toxicodynamics

On acute toxicity, JECFA reported three LD50 studies where carvone was tested by gavage. The LD50s observed in two rat studies were 1640 (unspecified) and 3710 (d-carvone) mg/kgBW, the LD50 observed in the guinea pig was 766 (unspecified) mg/kgBW. Carvone does not seem to have specific acute toxic properties, given the findings in the studies described above.
For (semi-)chronic toxicity, JECFA described three rat studies (16 weeks, 27-28 weeks, and 1 year study) where Osborne-Mendel rats were maintained on diets containing carvone (unspecified). Rats exposed to 500 mg/kg bw/day for 16 weeks showed growth depression and testicular atrophy. Exposures to 50 mg/kg bw/day (27-28 weeks) and 125 mg/kg bw/day did not display any adverse effects [Hagan et al., 1967, cited in 48].
In addition, NTP studies were described where B6C3F1 mice were exposed for 16 days, 13 weeks, or 2 years to d-carvone by gavage [49]. Toxic effects observed during these studies were neurotoxicological effects (such as ataxia, body tremors, and impaired reflexes), and increased relative liver and thymus weight (in 16-day study at 723 mg/kg d-carvone, 13-week study at 750 mg/kg d-carvone, and 2-year study at 375 mg/kg d-carvone). During the two year study, nasal cavity lesions were observed in all dose groups. The NOAELs for these studies were 328 mg/kg and 375 mg/kg for the 16-day and 13-week study, respectively, and not determined for the two year study.
Concurrently with mice studies, NTP carried out 16 day and 13 week studies with F334/N rats. In both studies rats were exposed to d-carvone by gavage. Toxic effects observed were increased relative liver, kidney, and thymus weights and lower body weights (in both studies), and increased relative weight in right testis and lungs/bronchi (males) and decreased relative weight of brains in females [48]. A NOAEL of 150 mg/kg bw/day and 93 mg/kg bw/day could be determined for the 16 day and 13 week study, respectively. A two-year study in rats was aborted, because NTP considered that the ‘collective performance of the contract laboratory was inadequate’.
Carvone (d/l) and d-carvone was tested negative in two Ames tests and d-carvone was found equivocal in two Chinese Hamster Ovary studies, i.e. sister chromatid exchange and chromosome aberration tests [44, 49].
For its use as pesticide/plant growth regulator, an EU monograph (not officially published) was drafted by the Netherlands. Additional studies with d-carvone described herein included an *in vivo* UDS test in rat liver (negative), a two generation reproductive toxicity study and developmental study in rats were both found negative, and a 90 day gavage study in rats. The latter study indicated a NOAEL, which was determined at 5 mg/kg bw/day. Relative increased liver and kidney weights and decrease in absolute and relative thymus weight were observed in female rats at a higher dose groups in this study.

*Acceptable Daily Intake (ADI)*
During the 37th meeting of JECFA it was determined that the stereoisomers should be evaluated separately, because the Committee considered that stereoisomers should not per se be regarded as toxicologically identical. JECFA established an ADI of 0-1 mg/kg bw/day for d-carvone, based on the

NOAEL of 93 mg/kg bw/day observed in the 13 week rat study by NTP. For l-carvone, however, there is insufficient toxicological data to establish an ADI [48].
In the unpublished EU monograph an ADI of 0.025 mg/kg bw/day for d-carvone was derived based on the NOAEL of 5 mg/kg bw/day observed in the 90-day study in rats. Uncertainty factors for intra- and interspecies extrapolation (both factor 10) and an additional factor for sub-chronic to chronic extrapolation (factor 2) were applied to derive the ADI of 0.025 mg/kg bw/day.

### 5.2.3 Conclusions on toxicology

Since this proposed ADI from the unpublished EU monograph is based on a more extensive dataset, this ADI will be taken forward in the calculations. As no ADI could be derived for l-carvone, the ADI for d-carvone will be used in the calculations.

## 5.3 Exposure assessment

### 5.3.1 Exposure sources

Carvone (d/l) is present in various natural food products. It is found in spearmint and caraway (herbs) and citrus products, guava, beetroot, cabbage, and celery. Carvone is used as a food additive e.g. in chewing gums and alcoholic and non-alcoholic beverages, biscuits, candies, bread, and meat. Carvone is also used in personal care products such as toothpaste, mouthwash, soap, and perfume, carvone can be used as a pesticide/plant growth regulator (e.g d-carvone is used as an anti-sprout agent on potatoes after harvesting [50].

### 5.3.2 Exposure estimates.

#### 5.3.2.1 Exposure to carvone; natural occurrence

To determine exposure from food stuff the National Food Consumption Survey in the Netherlands [51] was advised in the (unpublished) EU monograph. Determining the exposure to carvone (d/l) from natural sources is difficult, because consumption data concerning herbs are not recorded in the National Food Consumption Survey [51]. Further, carvone is found in citrus peels, but citrus peels are generally not consumed. To estimate the exposure to carvone (d/l) its presence in citrus fruits, guava, beetroot, cabbage, and celery was combined with the food consumption data. The exposure for the Dutch population was estimated at 0.027 mg/person/day (unpublished data). Assuming an average weight of 70 kg per person provides: 0.0004 mg/kg bw/day.
It is noted that this estimation does not take into account the contribution of herbs to the exposure to carvone (d/l), because no intake data on herbs is recorded. This may have resulted in significant underestimation of the exposure, because herbs (e.g. spearmint and caraway) may contribute significantly to the exposure to carvone (d/l) from natural sources as could be observed in Stofberg and Grundschober [1987, cited in 44].

#### 5.3.2.2 Exposure to carvone; use as food additive

As mentioned above, l-carvone is used in chewing gums whereas d-carvone is used in beverages (alcoholic and non-alcoholic), biscuits, candies, bread, and meat as food additive.
On a European scale Clark [52] estimated the total annual production of carvone (d/l; natural and synthetic) to be 515,000 kg. Of this amount it is estimated that 51,500 kg l-carvone (assumed 10 % of total production volume) and 25,750 kg d-carvone (assumed 5 % of total production volume) is used as food additive on the European market. The daily intake per capita can be estimated using the poundage

method. The annual poundage (in kg) is divided by the population (375 mln European inhabitants), days per year, a default correction factor for underreporting (0.6), and where it is assumed that 10 % of the population consumes carvone. Using this equation provides daily intake of in total 9.4 mg/person/day (0.13 mg/kg bw/day) for carvone (d/l) (6.3 mg/person/day for l-carvone and 3.1 mg/person/day for d-carvone).
From several surveys in Europe a daily intake of carvone (unspecified) per capita was estimated to be 2.8 mg/person/day (0.04 mg/kg bw/day, body weight set at 70 kg) based on 15,000 kg annual consumption [44]. The annual consumption is based on data from the International Organisation of the Flavour Industry (IOFI) from 1995 [cited in 44]. An earlier estimation from IOFI on the annual consumption of carvone (d/l) from food additives was 20,700 kg (unspecified isomer; geographical area and population unknown) as reported by Stofberg and Grundschober [1987, cited in 44].
The data by Clark [52] display higher tonnage levels than data provided by WHO [44], but are based on data from 1989 or earlier and partly on assumptions. It may well be that the production of carvone (d/l) has decreased in time, but this cannot be concluded from the data above. However, it does seem like the estimation made by Clark [52] is a worst case assumption, because the two estimations by IOFI are much lower even though the estimation of 20,700 kg was not linked to a geographical area. Because of considerations stated above, the exposure estimation of 0.04 mg/kg bw/day based on data from IOFI [1995, cited in 44] is used for further calculations.

#### 5.3.2.3 Exposure to carvone; use as pesticide

The consumer can also be exposed to d-carvone from residues present on crops from its use as pesticide/plant growth regulator. D-carvone is used as an anti-sprout agent on potatoes after harvesting [50]. In a study by Oosterhaven et al., [50] potatoes were exposed to carvone to test its potential as sprout inhibitor. In addition, residual levels were measured after the experiment. It is unknown whether the experiment resembles real life use of d-carvone. Measured residual levels on potatoes were found in highest concentrations in the peel and sprout. About 90 % residual content was associated with the peel fraction. The concentration of d-carvone in a peeled potato (tuber fraction) did not exceed 0.5 mg/kg fresh weight [50]. An intact tuber contained about 5 mg/kg fresh weight of d-carvone. In general, potatoes consumed in the Netherlands are peeled, but a ratio between peeled/unpeeled consumption is unknown. A worst-case assumption is made where consumption of unpeeled potatoes by the entire population is assumed. From the Food Consumption Survey [51] a potato consumption of 172.56 g/person/day is taken into account. The estimated exposure to d-carvone from residue on potatoes is 0.012 mg/kg bw/day. This exposure level will be put forward in aggregate exposure assessment.

#### 5.3.2.4 Exposure to carvone; use in personal care products

Carvone (d/l) is used in toothpaste, mouthwash, soap, and perfume. From Clark [46] an annual tonnage of 435,800 kg l-carvone was estimated for the use in toothpaste and mouthwash. For d-carvone, only 0.6 % of the total carvone (d/l) production is used in personal care products, i.e. 3,100 kg. Reasonably, it can be assumed that everybody will use toothpaste, but this assumption will not hold for mouthwash, soaps, and perfumes. Further, Clark [46] does not specify the annual tonnage per product. Thus, providing an exposure estimate based on the data above is not possible.
In the EU monograph, the notifier provided data on the use of carvone in these products. Assumptions were made concerning the concentrations of flavours in personal care products, carvone's presence in those products, oral and dermal uptakes, and 'consumption' of the product. A daily intake per capita was estimated based on above data. The daily intake for carvone (d/l) was calculated to be 0.0425 mg/person/day (0.0006 mg/kg bw/day).

### 5.3.3 Aggregate exposure

The aggregated exposure is the sum of exposures from different sources and routes. From the exposure assessments described above it is clear that, theoretically, the main source of human exposure is food, where carvone may present as a result of its use as a food additive (flavouring) or as a residu due to its use as a pesticide (see also Table 5.1).

**Table 5.1 : Sum of exposures for carvone (d/l).**

| Source | Exposure level (mg/kg bw/day) | Percentage of ADI (%) |
|---|---|---|
| **Acceptable Daily Intake** | **0.025** | - |
| Natural occurrence | 0.0004 | 1.6 |
| Food additives | 0.04 | 160 |
| Pesticide residual levels | 0.012 | 48 |
| Personal care products | 0.0006 | 2.4 |
| **Total** | **0.053** | **212** |

## 5.4 Risk assessment

As shown in Table 5.1 the aggregated exposure to carvone (0.053 mg/kg bw/day) exceeds the ADI of 0.025 mg/kg bw/day (212 % of the ADI). Carvone (d/l) used as food additive is the main cause of this exceeding. The exposure to carvone from is use as food additive alone exceeds the ADI. Although relatively small in comparison to its use as food additive, the use as pesticide also attributes significantly (48 % of ADI) to the total exposure. The other two remaining sources do not have a significant contribution to the total exposure to carvone. For this reason, the logical proceeding would be to focus more on the major sources of the exposure, i.e. the use as food additive and as pesticide.

## 5.5 Discussion and conclusions

*Exposure to carvone; use as food additive*
The major part of exposure to carvone originates from its use as food additive. Based on the deterministic exposure assessment the exposure exceeds the ADI derived for d-carvone, and accordingly adverse health effects cannot be excluded.
The deterministic exposure assessment is a rather crude approach, and refinement of the exposure assessment is desirable. The food additives committee stated that the stereoisomers should be regarded separately, but also indicated that there is insufficient data for l-carvone to derive an ADI [44]. One could question whether this kind of refinement is useful in exposure assessment when toxicological effects are not necessarily different. In addition, the data from IOFI does not discriminate between the optical isomers, and thus separate evaluation of the risk is impossible with the provide data.

Refinement can come from more specified data concerning the use of carvone (d/l) as food additive in several products and consumer behaviour. In the deterministic approach the poundage method was used, which is a rather crude method. Underlying assumption is complete consumption of carvone (d/l) by the assumed population (10 % of total). Refinement can be achieved when concentrations of carvone are measured in food products and linked to the Dutch Food Consumption Survey data. The

main advantage of this approach is that certain food products with high concentrations of carvone or much consumed products (with carvone) can be identified. Non-consumers are taken into account which makes assumptions about target population unnecessary. Another advantage is that the final product is considered, thereby not including carvone which may be lost during manufacturing. The requirement of more specific data increases when this kind of refinement is aimed for. At this moment, such specific data on carvone concentrations in food products is not available. For this reason, refinement of exposure to carvone from its use as food additive is not possible.

It is noted that the suggested refinement for food additives leads to a similar approach as was drawn up for the use of carvone as pesticide in a deterministic approach. Nevertheless, the suggested approach is considered a refinement of the deterministic approach for food additives. Hence, refinement of exposure can be achieved at several degrees, dependent on the case (starting point) and available data.

*Exposure to carvone; use as pesticide*
A suggestion for refinement in exposure assessment, concerning the use of d-carvone as pesticide is to discriminate between the consumption of peeled and unpeeled potatoes. As mentioned before, the peel contains the larger fraction of d-carvone after use. However, data on the manner of potato consumption is not available.
For reasons of demonstration it is assumed that the ratio peeled versus unpeeled potato consumption is 3 : 2. Using the residue level in peeled potatoes (0.5 mg/kg fresh weight) and in unpeeled potatoes (5 mg/kg fresh weight) and the average potato consumption (172.56 g/person/day) in the Dutch population provides: 0.0057 mg/kg bw/day d-carvone. Of this figure 0.0007 mg/kg bw/day results from consuming peeled potatoes, while the consumption of unpeeled potatoes result in 0.0049 mg/kg bw/day exposure. Together the exposure corresponds to 23 % of the ADI.
One must bear in mind that the underlying assumption is made that all potatoes consumed are treated with d-carvone during storage. Whether treatment with d-carvone is a normal procedure in the Netherlands is unknown to us. Other uncertainties about the exposure assessment result from the origin of consumed potatoes in the Netherlands. Other methods or pesticides may be used in other countries, indicating that export and import of potatoes may play a role in exposure assessment.

*Other refinement suggestions*
Other kinds of refinement for calculations of the exposure to carvone could be the division in adult and child population. Children may consume lesser amounts of food stuff, but relative to their weight the exposure may be higher. This form of refinement can be applied to the other sources as well.
The amount of consumption can vary between individuals and from day to day. Not everybody consumes potatoes and one subject consumes more than the other. The Dutch Food Consumption Survey provides a range of potato consumption in the range of 35 to 300 g/person/day [51]. Hereby, the outcome of the exposure assessment will be a range of exposure concentrations.

*Probabilistic exposure assessment*
A next step for refinement could be to perform a probabilistic exposure assessment. Hereby, insight is created in the uncertainty and variability of the exposure to carvone. However, in order to be able to perform a probabilistic exposure assessment the data requirements will increase. For instance, specific data on variability in measured concentrations or food consumption are required, but are not available. Below, suggestions on how to perform a probabilistic exposure assessment with data needs for the specific utilities of carvone are given.

*Exposure to carvone; use as pesticide*
After refinement of the exposure, the ratio between unpeeled and peeled consumption is included in the exposure assessment. In addition a range of potato consumption was suggested to refine the exposure. A range already provides insight on the variety of exposure to carvone from potato consumption. However, it does not conclude on whether certain consumption levels occur more often than other

levels (the probability). More detailed information on the potato consumption distribution of the population could provide insight whether certain findings are commonly observed or not.
The residual concentration on the potato originates from several measurements of which the reported value was never exceeded. Instead of using that point value, a distribution of the measurements can be made (data not reported in the article by Oosterhaven et al., [50]), since not every potato will have the same residual concentration. This can be conducted for the peeled and unpeeled potatoes. Combining both distributions may provide a wide distribution of the exposure to which the population can be exposed to. With this distribution a comparison can be made with the ADI.

*Exposure to carvone; use as food additive*
A similar exercise can be performed for its use as food additive for the different food stuffs carvone (d/l) is used in. Concentration distributions in the different food stuffs combined with the Food Consumption Survey data provides a distribution of the exposure for the specific food stuff on a population level. These distributions can then be added up to obtain a distribution of the total exposure to carvone from its use as food additive. As mentioned above, this exercise would require large amounts of product specific data.

**Conclusion**
Carvone was selected as a case study for aggregate exposure, because of the use as pesticide and food additive. Next to these utilities other sources, i.e. natural and personal care products, were identified. Carvone is approved as a food additive and currently authorization is requested for its use as pesticide. Concern was raised whether the aggregated exposure from these sources may become too high, since the exposures from these sources are inextricably bound up together.
A deterministic exposure assessment was carried out for carvone, taking the stereoisomers together in the assessment. This resulted in a gross exceeding of the ADI. The exceeding was mainly caused by the use of carvone as food additive and to a smaller extent its use as pesticide. The other two sources were minor contributors. Therefore, for refinement the focus was on the major contributors. A simple refinement for pesticide usage where a ratio was assumed between peeled and unpeeled potato consumption already lowered the exposure. For the use as food additive separation between the stereoisomers was suggested by WHO food committee, but was not deemed successful. Other refinement methods included assessing the exposure on product level instead of tonnage level. However, information required to perform this kind of refinement is not available. It can be concluded that the calculated exposure will decrease, because used or underlying assumptions in the deterministic approach are (reasonable) worst case.
With a probabilistic approach a distribution of the exposure can be obtained. It can be expected that the outcome of the deterministic approach is found in the upper part of the distribution. The data requirement is even higher to perform a probabilistic exposure assessment, and thus was not performed. Because the use of carvone as food additive alone is already responsible for the exceeding of the ADI, efforts should be made to refine the exposure assessment to identify which food products are major contributors of exposure to carvone. A probabilistic exposure assessment would provide a distribution of the exposure to carvone from the use of food additive on a population basis. This provides insight on to what degree the ADI is exceeded by the exposure of the population.

It appears that authorization of d-carvone for the use as pesticide will lead to a higher exceeding of the ADI. Based on this, the allowance of an additional source on the market would not seem logical. On the other hand, the exposure resulting from its use as food additive alone exceeds the ADI, whereas the use of pesticide alone does not. The use of carvone as food additive was considered of no concern by the US FDA and WHO, however this conclusion was based on a different (and higher) ADI than used in this case study. The results of the aggregated exposure assessment impose that measures should be taken to reduce the exposure and possible risks. Disregarding the decision whether d-carvone is allowed as pesticide or not, fact remains that exposure from the use of food additive is too high.

For these reasons, a reevaluation of carvone (d/l) as food additive is advised. During the reevaluation, the WHO Food Additive committee should consider the 90-day rat study from which the ADI of 0.025 mg/kg bw/day was derived. Risk reduction measures should be taken to reduce the exposure to carvone from the use as food additive.

# 6 Case study 4 – calcium

## 6.1 Description of the case

Calcium is the fifth most abundant element in the human body. The calcium content increases from 25-30 g at birth to 900-1300 g in adult men. Calcium is needed in the body for e.g. the skeleton, teeth, blood coagulation, neurotransmitter release, muscle contraction, activation of enzymes, release and activation of hormones, growth and development of cells [53, 54, 55].
Sufficient amounts of calcium should be ingested to fulfill the demand of the human body. Foods vary widely in their calcium content and besides not all foods contain well-absorbable calcium. A well-known good source of calcium are dairy products[54, 55]. Besides food, calcium can be ingested via drinking water and dietary supplements. Next to natural food sources, calcium may be added to foods for substitution, restoration, or fortification. In case of fortification, the total present calcium content of a reasonable daily portion should be at least 15 % and maximal 100 % of the recommended daily intake [56]. Dietary supplements are permitted to contain calcium, if the quantity is not harmful for public health [57]. Currently, European regulations for setting maximum and minimum amounts for vitamins and minerals in foods and dietary supplements are under discussion [58, 59].

## 6.2 Toxicological profile and limit values

### 6.2.1 Kinetics

There are two ways to absorb calcium in the intestinal tract; active transport and passive diffusion. The active transport is regulated by the need of the body and is a saturable process. This transport is inverse associated with dietary calcium intake and is mediated by the parathyroid hormone and 1,25-dihydroxycholecalciferol. Decrease of serum calcium (e.g. due to low intakes) stimulates the secretion of parathyroid hormone and 1,25-dihydroxycholecalciferol. In addition, the expression of the gene for calbindin is stimulated. The latter causes enhancement of the calcium absorption in the intestinal tract, whereas the first two will result in increased renal reabsorption and bone resorption. This will finally result in normalisation of serum calcium levels [54, 55, 56].
The passive diffusion of calcium mostly depends on the concentration of calcium in the gut lumen. It is important that calcium is in soluble form. About 8-23 % of the total absorbed calcium is taken up by passive diffusion [54, 55, 56]. At high calcium intakes, the uptake via passive diffusion is more important than the active transport [56].
Bone is a dynamic tissue, osteoclasts constantly resorb bone, whereas osteoblasts constantly form bone. The balance between the resorption and formation of bone depends whether there is net bone formation, maintenance, or loss [56].
In the body calcium is mainly present as hydroxyapatite (i.e. calcium-phosphate $Ca_{10}(PO_4)6(OH)_2$) in bones and teeth. The remaining 1 % of body calcium is present in blood, other body fluids, muscles, and soft tissues. The bone mineral provides the body structure and strength and is a very important calcium reservoir that will be used to maintain blood calcium homeostasis (calcium is blood is kept between 2.25 and 2.75 mmol/L) [54, 55, 56].

### 6.2.2 Toxicodynamics

***Calcium requirements***

In 2000 the Health Council of the Netherlands published dietary reference intakes for calcium (Table 6.1) [55]. Several factors influence the calcium requirement like bioavailability (influenced by several nutritional factors such as vitamin D), physical activity, age, gender, ethnic background, pregnancy, and lactation. At the age of 25-30 the bone mass is maximal (peak bone mass). After this age, the bone mass will constantly decrease. How long it will take till there will be an increased risk on bone fractures depends on the peak bone mass and the rate of degradation. Although the peak bone mass is genetically fixed for 60-80 %, calcium intake is another important factor. Inadequate calcium intake will result in a non-optimal peak bone mass. For persons up to 30 years old the intake is defined as adequate if an increased intake will not increase the bone mass anymore. For persons older than 30 years, the intake is defined as adequate if a further increase in intake will not influence the rate of bone loss or the fracture risk [55].

In adults, acute effects of low calcium intakes are rare, however in infants this can cause tetany [55]. Calcium deficiency can be caused by low dietary intake, low absorption, or excessive losses. Long term inadequate calcium intakes will harm the skeletal development and maintenance and is associated with osteoporosis and bone fractures. A calcium decrease in extracellular fluid stimulates the secretion of parathyroid hormone, so calcium from bone can be mobilised to maintain calcium levels, as described above. However, parathyroid hormone also increases the calcium concentration intracellular in several cell types (e.g. blood cells, adipocytes, hepatocytes, osteoblasts, renal tubular cells). An increase in intracellular calcium turns off a number of reactions for instance the permeability of the plasma membrane, (de-)activation of enzymes, cytoskeletal rearrangement, gene transcription. This has been called the calcium paradox disease, because the release of parathyroid hormone is needed to increase the extracellular calcium level, but the adverse effect is the increase of the intracellular calcium. Diseases like hypertension, arteriosclerosis, Alzheimer's disease, muscular dystrophy, diabetes mellitus, and malignancies may result by the calcium paradox disease [54]. It is therefore suggested that high intakes of calcium are associated with lower blood pressure and a lower risk of colon cancer [54, 55, 56].

There is no biochemical assay that reflects the nutritional calcium status. Because of the tight regulation, blood calcium concentration is in general no good indicator. Bone mineral content and density both strongly predict the risk of fractures, especially in adults because of the stable skeletal size [56].

***Tolerable upper intake level***

Recently the Scientific Committee on Food (SCF) set an European tolerable upper intake level (UL) for calcium [54]. Earlier, the Institute of Medicine (IOM, USA, 1997)) and the Health Council of the Netherlands (2000) also set an UL for calcium [55, 56]. Too high intakes of calcium are mainly associated with three effects: 1) nephrolithiasis (kidney stone formation), 2) syndrome of hypercalcemia and renal insufficiency (formerly called milk-alkali-syndrome), and 3) interaction of calcium with the absorption of other essential minerals [54, 55, 56].

Based on available studies, both IOM and the Health Council of the Netherlands set a LOAEL for milk-alkali-syndrome at 5 grams calcium per day. It is known that at intakes of 2.4 grams per day this syndrome will not appear. Both institutes use an uncertainty factor of 2 and come at an UL of 2.5 grams per day for adults. For children the chance to develop milk-alkali-syndrome is smaller, but the risk of decreased absorption of zinc and iron is little higher. Due to lack of data specific for children IOM and the Health Council of the Netherlands recommend the same UL as for adults. Perhaps this is for children a bit conservative, because in preparation of puberty rates of bone formation and calcium absorption start to rise [55, 56].

The SCF based the UL on several long duration interventional studies in adults, in which 2500 mg calcium a day was tolerated well without adverse effects. They conclude that there are not enough data

to really set an UL for children and adolescents; however with the current (extreme) intakes of calcium no appreciable risk has been identified [54].

**Table 6.1. Dietary reference intakes and tolerable upper intake level of calcium**

| Age-group (year) | | Adequate intake (mg/day)[55] | Tolerable upper intake level (mg/day) [54, 55, 56] |
|---|---|---|---|
| | 1-3 | 500 | 2500b |
| | 4-8 | 700 | 2500b |
| boys | 9-18 | 1200 | 2500b |
| girls | 9-18 | 1100 | 2500b |
| | 19-30 | 1000 | 2500 |
| | 31-50 | 1000 | 2500 |
| | 51-70 | 1100 | 2500 |
| | > 70 | 1200 | 2500 |
| pregnancy[a] | | 1000 | 2500 |
| lactation[a] | | 1000 | 2500 |

[a] Dutch Health Council did not set dietary reference intakes for women < 19 years being pregnant or lactating

[b] EFSA was not able to propose age-dependent ULs for children and adolescents, because of insufficient data. The Health council of the Netherlands and IOM set an UL for children that is the same as for adults.

### 6.2.3 Conclusions on toxicology

For the present case study the estimates of the exposure to calcium will be compared to the adequate intake levels (AI) (Table 6.1) as proposed by the Dutch Health Council and to the UL of 2500 mg calcium/day as set by the IOM, the Dutch Health Council, and EFSA.
It is important to note that a quantitative judgement of the proportion of the population with too low intakes can only be made using the Estimated Average Requirement (EAR) under the condition that the requirement distribution in the population is a normal distribution. For calcium no EAR could be set. The level of the EAR is expected to satisfy the need of 50 % of the population in a specific age group. An other reference value is the recommended daily allowance (RDA), which is the level of a nutrient sufficient to meet the requirement of nearly all healthy individuals in each age-gender group. To set a RDA specific detailed data is required. If such detailed data is not available sometimes an adequate intake (AI) is estimated. Often the value is higher than the RDA would be. The AI cannot be used to determine the proportion of inadequate intakes in a population. Only a qualitative statement can be made; if the mean (or median) intake is at or above the AI it is generally assumed that there is a low prevalence of inadequate intakes. However if the mean (or median) intake is below the AI no assumptions can be made. For the estimation of the proportion with too high intakes the UL can be used as a cut-point [60].

## 6.3 Exposure assessment

### 6.3.1 Exposure sources

For human health risk assessment the oral exposure to calcium is relevant. The main sources of calcium are food, drinking water and dietary supplements. To illustrate this case study calculations and results

described below are based on data and methods available in 2006. More recent data and methods were not taken into account.

### 6.3.2 Exposure estimates

Using the Dutch National Food Consumption Surveys (DNFCS) the exposure to calcium from food and drinking water can be estimated. The estimation of calcium intake from dietary supplements is more complicated, because of the small number of subjects using dietary supplements in the DNFCSs, the incomplete dietary supplement composition data, and the infrequent use.
The last DNFCS for the whole population (DNFCS-3, subjects 1-97 yr, N=6250) was carried out in 1997-1998 [61]. A two-day dietary record method was used to collect data on two consecutive days. More recently a DNFCS was executed among a special group; young adults. This DNFCS among young adults was executed in 2003 (19-30 yr, N=750), data was collected with a two-day 24-hour recall method [62, 63].
Below the exposure to calcium from the different sources will be described separately for the combination food and drinking water, and for dietary supplements. Subsequently an estimation of total calcium intake by aggregation of the intake from food, drinking water, and dietary supplements will be made.

#### 6.3.2.1 Food and drinking water

The total acute (observed) intake of calcium was calculated by combining the DNFCS data with food composition data for each food item separately (i.e. consumed amount * concentration Ca) and thereafter summation over all food items (incl. drinking water) consumed per day per subject. Because the harmful effects of a too low or excessive intake of calcium are long term rather than acute effects, the habitual intake of calcium (long-term) was estimated from the observed intake (acute) by statistical correction for the within-person (i.e. day-to-day) variation using the ISU-method [64]. The habitual intake distribution was calculated for different age-groups (Table 6.2).
In general, men had a higher intake than women (Table 6.2). Results for DNFCS-3 (19-50 yr) and DNFCS-2003 (19-30 yr) were comparable. For children the intake increased with age and boys had a higher intake than girls. In all age groups the calcium intake was below the UL. The median calcium intake was above the AI for young children (1-8 yr), and adult men (19-50 yr) indicating a low prevalence of inadequatre intakes. For the other age groups median calcium intake was below the AI and no statement about the proportion with inadequate intakes can be made.

**Table 6.2. Habitual intake of calcium from diet and drinking water in the Dutch population, percentiles (mg/day)**

| Age-group (years) | | N | P10 | P25 | P50 median | P75 | P90 | P99 |
|---|---|---|---|---|---|---|---|---|
| boys | 1-3[a] | 135 | 595 | 714 | 852 | 1003 | 1158 | 1487 |
| girls | 1-3[a] | 119 | 532 | 639 | 773 | 923 | 1073 | 1365 |
| boys | 4-8[a] | 207 | 543 | 684 | 856 | 1045 | 1229 | 1574 |
| girls | 4-8[a] | 224 | 562 | 696 | 858 | 1033 | 1201 | 1516 |
| boys | 9-13[a] | 197 | 600 | 776 | 996 | 1240 | 1481 | 1941 |
| girls | 9-13[a] | 212 | 619 | 750 | 907 | 1074 | 1233 | 1526 |
| boys | 14-18[a] | 229 | 685 | 845 | 1042 | 1263 | 1490 | 1962 |
| girls | 14-18[a] | 216 | 545 | 702 | 904 | 1135 | 1370 | 1837 |
| men | 19-30[b] | 352 | 740 | 918 | 1142 | 1396 | 1651 | 2151 |
| women | 19-30[b] | 398 | 587 | 739 | 937 | 1170 | 1411 | 1905 |
| men | 19-50[a] | 1437 | 636 | 816 | 1047 | 1310 | 1581 | 2163 |
| women | 19-50[a] | 1636 | 597 | 749 | 938 | 1154 | 1374 | 1828 |
| men | 51-70[a] | 511 | 681 | 854 | 1076 | 1330 | 1588 | 2101 |
| women | 51-70[a] | 602 | 583 | 745 | 953 | 1195 | 1451 | 1999 |
| men | > 70[a] | 169 | 546 | 729 | 969 | 1247 | 1532 | 2103 |
| women | > 70[a] | 287 | 551 | 717 | 933 | 1184 | 1443 | 1962 |

[a] data from DNFCS-3 (1997-1998), [b] data from DNFCS-2003

### 6.3.2.2 Dietary supplements

The intake of dietary supplements can only be estimated based on the data about micronutrient content in supplements present in the supplement database of the Dutch Consumers' association (i.e. in Dutch 'Consumentenbond') [65], which was complemented by the RIVM with more recent supplement data and data about mineral supplements, and the intake of supplements observed in the DNFCS-2003. In Table 6.3 more detailed information about the calcium content in dietary supplements available on the market are presented. Based on the names of the dietary supplements (e.g. 'child', 'junior') some were selected as specific for children (N=18), the range of the calcium content of these dietary supplements was 3.75-250 mg. The 90th and 95th percentile of calcium content in the dietary supplements specifically for children is 215 and 250 mg per supplement respectively. About 83 % of the calcium containing dietary supplements for children had a calcium content below 150 mg per supplement. The remaining dietary supplements (N=232) had adults, whole families or other specific groups (no children) as target. The calcium content of these remaining dietary supplements ranged from 1.2-1200 mg. The 90th and 95th percentile of calcium content in these dietary supplements is 660 and 1000 mg per supplement respectively. About 46 % of these dietary supplements had a calcium content at or above 150 mg per supplement, 7 % had even a content at or above 1000 mg per supplement.

**Table 6.3. Percentile distribution of the calcium content in calcium containing dietary supplements (mg/supplement)**

| Target group | N | P25 | P50 | P75 | max |
|---|---|---|---|---|---|
| Specifically for children | 18 | 16 | 28 | 109 | 250 |
| Remaining e.g. adults/family | 232 | 75 | 125 | 330 | 1200 |

The data from DNFCS-2003 is the only dataset available to get insight in the use of dietary supplements among young adults. As described above this may *not* give a representative picture due to the small sample size and the infrequent use of dietary supplements. During DNFCS-2003 123 (out of

750) subjects used dietary supplements containing calcium, 63 subjects used these dietary supplements on both observed days whereas 60 subjects used them only one of the two observed days. In Table 6.4 the observed calcium intake from dietary supplements of users of calcium containing supplements is shown. The maximum observed mean intake over two days was 1500 mg, which is below the UL.

**Table 6.4. Distribution of observed intake of calcium, mean over two days (mg/day) from supplements, calcium containing supplement users only (n=123), young adults 19-30 (DNFCS-2003)**

| | N | P25 | P50 | P75 | max |
|---|---|---|---|---|---|
| Mean both days | 123 | 21 | 60 | 120 | 1500 |

### 6.3.3 Aggregate exposure

In order to compare the calcium intake with the adequate intake and the UL, the total intake from all sources is of interest. With a *deterministic* (reasonable worst-case) approach total calcium intake can be estimated by adding up the 95th percentile of habitual intake from food and drinking water and the 95th percentile of calcium content in dietary supplements available on the market. It should be noted that summation of two 95th percentiles is a reasonable worst-case assumption. Subjects with high intake from one source are supposed to also have high intake from the other source. However, when the intake remains below the UL it is expected that there are hardly excessive intakes. This method is therefore useful as a first indicator.

For children up to 13 years old the dietary supplement intake was based on the data of supplements specifically for children, whereas for adolescents and adults the intake of dietary supplements was based on the remaining supplements; resulting in an intake of 250 and 1000 mg from dietary supplements respectively for children and adults (incl. adolescents) (Table 6.3). In this case the adolescents and adults will have intakes *higher than the UL*, with a maximum of 2816 mg/day for man aged 19-30 years old (Table 6.5). For the children the intake is below the UL (maximum is 1884 mg/day in boys aged 9-13 years old) (Table 6.5).

To *refine* this estimation, the habitual total calcium intake for users of calcium-containing dietary supplements can be calculated also with a deterministic approach. In this approach the individual dietary habits will be taken better into account. At this moment such calculation can only be performed for the very small group of supplement users in the DNFCS-2003 and it should be noted that it is not known whether the observed intake from supplements is for instance daily or less frequent. Therefore it is only possible to perform this calculation under the assumption that subjects who used supplements on both days use them daily, that subjects who used supplements on one of the two observed days use them every other day, and that observed zero intake on both days corresponds to non-users of supplements. In Table 6.6 the distribution of habitual total calcium intake distribution of subjects using calcium-containing dietary supplements (N=123) is presented. It can be seen that the distribution did not exceed the UL. Further the median calcium intake is above the AI, indicating a low prevalence of inadequate intakes. Besides, the habitual total calcium intake distribution of the total population of the DNFCS-2003 is presented (N=750). For the total population also the habitual total calcium distribution of the total population did not exceed the UL. And the median calcium intake was above the AI, indicating a low prevalence of inadequate intakes.

**Table 6.5. Deterministic exposure assessment to compare total calcium intake with the UL, in bold total intake that exceed the UL**

| | | | | Deterministic | |
|---|---|---|---|---|---|
| Age-group (years) | | N | P95 habitual calcium Food & drinking water (mg/day) | P95 calcium content in calcium-containing supplements (mg/day) | Total calcium (mg/day) |
| boys | 1-3[a] | 135 | 1263 | 250 | 1513 |
| girls | 1-3[a] | 119 | 1169 | 250 | 1419 |
| boys | 4-8[a] | 207 | 1345 | 250 | 1595 |
| girls | 4-8[a] | 224 | 1307 | 250 | 1557 |
| boys | 9-13[a] | 197 | 1634 | 250 | 1884[c] |
| girls | 9-13[a] | 212 | 1332 | 250 | 1582 |
| boys | 14-18[a] | 229 | 1641 | 1000 | **2641** |
| girls | 14-18[a] | 216 | 1523 | 1000 | **2523** |
| men | 19-30[b] | 352 | 1816 | 1000 | **2816** |
| women | 19-30[b] | 398 | 1571 | 1000 | **2571** |
| men | 19-50[a] | 1437 | 1764 | 1000 | **2764** |
| women | 19-50[a] | 1636 | 1521 | 1000 | **2521** |
| men | 51-70[a] | 511 | 1756 | 1000 | **2756** |
| women | 51-70[a] | 602 | 1625 | 1000 | **2625** |
| men | > 70[a] | 169 | 1718 | 1000 | **2718** |
| women | > 70[a] | 287 | 1612 | 1000 | **2612** |

[a] data from DNFCS-3 (1997-1998), [b] data from DNFCS-2003, [c] with P90 of remaining dietary supplements this would exceed the UL also

**Table 6.6. Distribution of habitual total calcium intake (mg/day), users of calcium containing supplements only, young adults 19-30 yr (DFCS-2003), bold exceeds the UL, italics is inadequate intake**

| | N | P10 | P25 | P50 median | P75 | P99 |
|---|---|---|---|---|---|---|
| Supplement users | | | | | | |
| habitual | 123 | 846 | 1009 | 1213 | 1445 | 2141 |
| Total population | | | | | | |
| habitual | 750 | 664 | 835 | 1057 | 1315 | 2119 |

## 6.4 Risk assessment

Exposure to calcium through food and drinking water indicates that, in general, men had a higher intake than women (Table 6.2). For children the intake increased with age and boys had a higher intake than girls. Comparison of the habitual intake from diet and drinking water only with the AI (Table 6.1) shows that for young children (1-8) and adult men (19-50) the median calcium intake was above AI what indicates a low prevalence of inadequate intakes. For the other age groups the median intake was below AI and no quantitative nor qualitative statement about the prevalence of too low intakes can be made. To be able to make quantitative statements about the prevalence of too low intakes in the Dutch population an EAR should be set. At this moment data is not sufficient to set an EAR for calcium. As can be seen in Table 6.2, in none of the age-groups the 99th percentile of intake exceeded the UL (i.e. 2500 mg/day).

The inventory of dietary supplements showed that none of the calcium containing supplements present in the database had a calcium content that exceeded the UL. However it remains possible that some subjects will take more than one (daily) dose of dietary supplements or combinations of different supplements, resulting in higher intakes. The maximum calculated intake (2816 mg/day) on one observed day in DNFCS-2003 was above UL, however the maximum observed mean intake (over two days) from dietary supplements was 1500 mg, which is below the UL.
Aggregated exposure using a *deterministic* reasonable worst-case approach by adding up the 95th percentile of habitual intake from food and drinking water and the 95th percentile of calcium content in on the market available calcium containing dietary supplements, resulted in a total calcium intake above the UL for adolescents and adults. For the children the intake remained below the UL, even if the intake from dietary supplements would be 1000 mg (like adults and adolescents) instead of 250 mg, except for boys aged 9-13 years old.

In a refined deterministic exposure estimate, the habitual total calcium intake for the total population and users of calcium-containing dietary supplements was calculated for young adults. For the total population, the median calcium intake was above AI and this indicates a low prevalence of too low intakes. The habitual total calcium distribution of the total population young adults did not exceed the UL.

## 6.5 Discussion and conclusions

For a micronutrient like calcium it is important to estimate the aggregated intake as precise as possible, as there are small margins between the adequate intake (500-1200 mg/day, depending on age), the tolerable upper limit of intake (2500 mg/day) and the LOAEL for adverse effects (5000 mg/day).
In the deterministic reasonable worst-case approach, at the 95th percentile all adults and adolescents were estimated to have intakes above the UL. However in the more refined approach aggregrated intakes did not exceed the UL. Besides comparison with the UL for safety reasons at current intakes, this comparison is also used to get insight in the possibilities to increase the market of enriched foods. In case of the latter it is important that the estimated intakes are not too much overestimated, even if they stay below the UL.
Due to lacking data, it is not possible to immediately calculate representative habitual total calcium intakes from food, drinking water and dietary supplements. For food and drinking water combined habitual intakes can be calculated using DNFCS data. For dietary supplement intake, composition data is not always available since sample sizes of DNFCSs are too small to get good insight in the intake. Besides, dietary supplements are often infrequently used, which can be measured by subsequent 'supplement frequency questionnaires'. At this moment this data is not available but it is proposed that in the future extra attention is paid to infrequently consumed products (dietary supplements, functional foods) [66, 67].
Unfortunately, there is not enough data available to set an EAR. Therefore no quantitative statements about the prevalence of too low intakes can be made. Especially for older children, adult women and elderly, where the median calcium intake is below AI, the EAR is needed to estimate the prevalence of too low intakes. When a good estimation of the prevalence of too low calcium intakes can be estimated the health benefit reducing this proportion in the population should be weighed against the health risk of excessive calcium intake, before starting interventions.

# 7 General discussion

Chemical substances may be used in various products simultaneously. For instance, a substance that is used as a biocide may also be used as a pesticide or veterinary medicine. Hence, consumers may be exposed to a chemical from a variety of sources. Thus it seems logical that in the risk assessment of a chemical, aggregate (single chemical, multiple routes) exposure should be considered. However, at present no harmonized methodology is available to perform such aggregate exposure assessments in Europe.
The present aggregate exposure and risk assessments for triclosan, permethrin, carvone and calcium were performed to explore the current possibilities and limitations of an aggregate risk assessment for chemicals. Triclosan and permethrin are synthetic chemicals used as biocide and/or insecticide. Carvone is a chemical which naturally occurs in a variety of plants and which is used as a flavouring and fragrance agent and as a plant growth regulator. Calcium is a natural element and an essential nutrient with, as many other essential elements, a small window between the adequate intake and an intake level causing adverse effects. An exposure and risk assessment of nutrients may reveal concerns about insufficient or excessive intake in parts of the human population. Each case study aimed to make an inventory of the relative contribution of each source of the chemical in the total exposure of the consumer and to assess whether the aggregate exposure may pose a risk to human health.
The present pilot study yielded valuable insight in, on the one hand, the problems that can be encountered with respect to data collection and the availability of data and, on the other hand, the possibilities of the exposure and risk assessment based on the available data. The limitations and possibilities of the aggregate exposure and risk assessment of chemicals are discussed below.

## 7.1 Data collection

Data on the toxicological profiles were retrieved from well known sources and additional searches. Although sometimes adequate toxicological data matching the relevant routes and durations of exposure are lacking, generally, the toxicological profile is not the largest obstacle for an aggregate risk assessment.
Instead, data on the presence or application of the presently addressed 4 compounds in various products were more difficult to obtain. Information was retrieved from public sources, predominantly through searches on the internet using the name of the substance or product names in combination with other search terms. Although the internet searches for both substances produced numerous ‘hits’ it appeared that of all this information only a limited number of sites yielded data that was of use for the purpose of the exposure and risk assessment.
In particular for triclosan and carvone, information on which products contain this substance and at which levels were lacking. For permethrin, more data were available, for instance levels in products and monitoring data on levels in food. For calcium the main natural sources and their calcium content are reasonably well known and habitual intake can be estimated on the basis of food consumption data. For calcium intake through dietary supplements data on product composition is often, but not always available. However data on intake of food supplements is limited. The present study indicates that the availability of exposure data in general is a bottleneck in the aggregate exposure and risk assessment of chemicals. Similar indications were reported in Wijnhoven et al. [2] and Schuur et al. [1].

## 7.2 Available data and exposure assessment

The present case studies on triclosan and carvone demonstrate that for some chemicals only limited data on actual exposure are available. The case studies on permethrin and carvone show the importance of actual data to perform a realistic (aggregate) exposure and risk assessment.

For instance, due to the lack of data, the present exposure assessment of triclosan was entirely based on the assumption that products contain triclosan at the maximal allowed concentration. Based on this assumption, and using reasonable worst case exposure scenarios, exposure estimates for adults children and infants were made. Since it is not known which products actually contain the substance and to what extent we cannot determine how realistic these exposure estimates are. Furthermore, since the number of products that contain triclosan is probably rather limited it is not meaningful to perform an aggregate exposure estimate based on the assumption that all products in which the use of triclosan is allowed actually do contain triclosan.

Also for carvone actual data on exposure are lacking. In the deterministic assessment of exposure to carvone used as food additive the poundage method was used. The underlying assumption is complete consumption of carvone (d/l) by the assumed population of consumers (10 % of total). It is noted that the poundage method is a rather crude approach to estimate the exposure.
Carvone is also used as a plant growth regulator on potatoes. The exposure assessment for this use of carvone was based on the assumption that the entire population consumes unpeeled potatoes which are all treated with carvone. Although the percentage of potatoes treated with d-carvone and the proportion of the population (if any) that consumes only unpeeled potatoes is unknown, these assumptions will probably lead to an overestimation of the actual exposure to carvone by its use on potatoes. However, again the extent of the overestimation is not known.

The importance of the availability of actual data is demonstrated by the assessment of permethrin exposure from food sources. On the basis of the theoretically maximum daily intake (TMDI) data exposure to permethrin for adults through food fills up a considerable part of the ADI and for children even exceeds the ADI. However, when the exposure calculation is based on existing monitoring data from the Netherlands or the FDA, much lower exposures to permethrin through food are calculated. As compared to the exposure estimate based on the TMDI, the Dutch and FDA monitoring data yield exposure that are respectively 50000 and > 1000 times lower. Whereas the theoretical exposure estimate suggests there may be a health concern, the actual exposure data show that the occurrence of adverse health effects due to residues of permethrin on food is unlikely.

The case of calcium indicates that calcium intake can be reasonably well estimated, although with respect to intake from dietary supplements there are still uncertainties due to the small number of subjects using supplements in the food surveys, the infrequent use of supplements and incomplete dietary supplement composition data. However, in contrast to the other three chemicals studied, calcium is an essential element, with an adequate intake ranging from 500 – 1200 mg/day, depending on age and gender, and a tolerable upper level of 2500 mg/day, which is based on adverse effects observed at 5000 mg/day. Thus, in view of the narrow ranges between the daily requirement, tolerable upper level and toxic level, for calcium it is very important to get an accurate aggregate exposure estimate in order to perform a reliable risk assessment.

## 7.3 Refinement of the exposure assessment

The case studies of triclosan and carvone used exposure estimates that are rather crude and probably conservative. Thus, the aggregated risk assessment for these substances would be greatly advanced if

the exposure assessment could be refined. Preferably such refinements should be done by using probabilistic approaches [e.g. see 68, 69]. However, the necessary input data for such an approach are often lacking.
For triclosan it was concluded that, at present, a refinement of the exposure assessment was not possible. To perform such a refinement would require information on, for instance, which products contain triclosan, on the levels of triclosan in these products, on the use of triclosan-containing products in the population, etcetera.

For carvone refinement can be achieved when concentrations of this substance are measured in food products and linked to the Dutch Food Consumption Survey data providing a probabilistic approach. The main advantage of this approach is that certain food products with high concentrations of carvone or much consumed products containing carvone can be identified. Non-consumers are taken into account which makes assumptions about target population unnecessary. Furthermore, a distinction could be made between adults and children, using distribution data from the DNFCS. Another advantage is that the final product is considered, thereby not including carvone which may be lost during manufacturing or processing. For instance, distribution data on the consumption of unpeeled and peeled potatoes will refine the exposure estimate for carvone used as pesticide.
The requirement of more specific data increases when this kind of refinement is aimed for. At this moment, such specific data on carvone concentrations in food products is not available. For this reason, refinement of exposure to carvone from its use as food additive or pesticide is not possible.

In the case of calcium the initial reasonable worst-case deterministic exposure assessment, that indicated a calcium intake over the UL for dietary supplement users, could be refined by calculating their habitual total calcium intake, based on data in the DNFCS-2003 (young adults only). The refined aggregate exposure estimate indicated that the total calcium distribution in this population or in the subpopulation of supplement users did not exceed the UL. It was noted that in the DNFCS-2003 only data on a small number of supplement users were available, and no information on the frequency of supplement intake was available.

*Probabilistic exposure assessment*
A further step for refinement could be to perform a probabilistic exposure assessment. Hereby, insight is created in the uncertainty and variability of the exposure to a chemical, although it remains difficult to separate these two elements in exposure assessment.
Concentration distributions of, for instance, carvone in the food stuffs combined with Food Consumption Survey data provides a distribution of the exposure for the specific food stuff on a population level. Similarly, concentration data of a chemical such as triclosan in consumer products combined with data on distributions of their use by the population and use frequency, can be used in a probabilistic exposure assessment. However, such an exercise would require large amounts of product specific data, which are presently not available. Also for calcium a probabilistic approach could be an extra refinement. In such an approach information of the frequency of supplement use (eventually from another source) could be taken into account.

## 7.4 Risk assessments

For the four chemicals addressed in the present report risk assessments were performed. When the data were adequate, the risk of aggregated exposure was assessed.
For permethrin the exposure estimate for residues on food was based on monitoring data, since this was considered more reliable than using the Theoretical Daily Maximum Intakes. The aggregate exposure estimates for permethrin from food and non-food sources, indicates that adverse health effects for the

human population due to exposure to permethrin are not expected. It should be noted that the aggregated exposure estimates are based on the assumption that adults or children are simultaneously exposed to permethrin from a variety of sources, often using 90 percentiles of exposure data and reasonable worst case exposure scenarios. The actual exposure to permethrin is likely to be considerably lower. Since this rather worst case aggregate exposure estimate does not indicate a health concern, refinement of the exposure estimates is considered not necessary. On the other hand, discussions on the cumulative effects of various pyrethroids is still ongoing. However, this aspect is not covered in this report.

For triclosan there are indications that its use in products is limited. As was indicated above, it is therefore not realistic to perform an aggregate exposure estimate for triclosan based on the assumption that this substance occurs in all products in which its use is allowed. Interestingly however, for certain product types the exposure and risk assessments indicate that considerable exposure to triclosan may occur through the use of single products. For instance, the use of a sun care product containing 0.3 % triclosan for a short period may lead to systemic exposures in adults and children with MOS values of respectively 66 and 42 as compared to the overall NOAEL for toxicity. For infants treated with baby oil a MOS value of 20 is calculated. Since in risk assessment a MOS value $\geq 100$ is generally considered adequately protective, it can be concluded that for the use of these products, adverse health effects cannot be excluded following the current approach. For an adult or child using a combination of triclosan-containing oral hygiene and skin and sun care products the MOS values would be even more unfavourable, especially since skin care products are sometimes used in relatively large amounts.

In addition it should be noted that the overall NOAEL for triclosan was based on effects in dams (liver toxicity) and in fetuses (reduced fetal weight and delayed ossification) in a developmental toxicity study in mice. Thus, it cannot be excluded that the exposure of pregnant women to some triclosan containing products may have adverse health effects for the developing fetus.
It is worth mentioning that for this particular compound, the skin is an important route of exposure. In this report a dermal absorption of 25 % was used for triclosan. Based on the limited data available, the dermal absorption may be a factor 2-3 lower. For instance, the recent SCCP opinion on triclosan [7] used dermal absorption percentages of 7-12 %, based on information from in vitro absorption studies using human skin. This indicates that the present exposure assement for dermal products may be conservative.
Nevertheless, the current approach as well as the SCCP evaluation, to certain extent came to the same conclusion on the health risks of certain types of products containing triclosan. However, certain conclusions also differed, e.g. with respect to the risk for children, due to differences in the choice of sub-populations, product types, exposure parameters and exposure models.
A recent US-EPA study [11], using biological monitoring data, indicated that in the USA aggregated exposure to triclosan may not pose a health concern. This may suggest that the assumptions made in the present case study on triclosan and the SCCP opinion on triclosan may over estimate actual exposure to this chemical. However, the current use concentrations in the USA may have been lower than the maximal concentration limits as were used in the exposure assessment in the present report.

The aggregate exposure to carvone was calculated at 212 % of the ADI. Exposure to carvone (d/l) used as food additive (160 % of the ADI), and to a lesser extent the use as a pesticide (48 % of the ADI), are the main causes of the exceeding of the ADI. Thus, it could be concluded that adverse health effects due to exposure to carvone cannot be excluded. It should be noted that the exposure to carvone as a food additive was estimated by the poundage method, which gives a very rough estimate. Furthermore, for the use of carvone as a pesticide, the underlying assumption was that all potatoes consumed are treated with d-carvone during storage. Obviously, these exposure estimates are a gross simplification of the actual exposure situation and, at least for the use of carvone on potatoes, is likely to overestimate the exposure. Nevertheless, based on this crude risk assessment it can be concluded that exposure to carvone may pose a health concern.

The exposure assessment for calcium was based on data from the Dutch National Food Consumption Surveys (DNFCS). A deterministic exposure assessment indicated that part of the population that uses dietary supplements may be exposed to levels above the tolerable upper level. However, the refined aggregate exposure estimate indicates that there is no concern for exposure above the UL in young adults. It is noted that the refined exposure assessment was based on a limited base.
No quantitative statement could be made regarding the proportion of the population with too low calcium intakes. In the age groups of young children (1-8 yr) and adult men (19-50 yr) the prevalence of too low calcium intakes seem low, however in the other age groups no statement can be made.

## 7.5 Policy implications

In the present case studies aggregate exposure and risk assessments were performed for four chemicals. The aim of the present report was not to define problems of the four specific case studies but rather to identify common issues that are relevant for an aggregate exposure approach in general. Nevertheless, some specific conclusions on the cases can be drawn next to the more general conclusions.

### Case specific implications

The aggregate exposure and risk assessment for permethrin indicates no health concern, even though the exposure estimates can be considered rather worst case. Thus, for permethrin no further action would be required. However, discussion on the cumulative exposure to pyrethroids is ongoing.

For triclosan its use in skin care products raises concerns. The present risk assessment indicates that it may be worthwhile to reconsider the use of triclosan in oral hygiene products, and in skin care and sun care cosmetics.

The carvone case study indicates that (worst case) aggregate exposure to carvone may exceed the ADI (mainly from its use as a food additive). Refinement of the exposure estimates is advised although this also requires measurements of carvone in various products.

The calcium case study indicates that the risk of excessive intake of this nutrient due to habitual intake and consumption of dietary supplements is probably limited. However trends of increasing food fortification and use of dietary supplement use may in future increase the calcium consumption and thereby increase the risk of excessive intakes. So monitoring of such trends and the intake is warranted.

### Methodological implications

In general terms, as can also be learned from the current case studies, aggregate exposure to the same substance from various sources is reality. The human population is exposed to many substances from many sources. Aggregate exposure is therefore a relevant factor to consider.
In some case (e.g. triclosan case), the current maximal allowed amounts of substances in products may not be protective enough when considered in a (worst case) aggregate exposure assessment. Guidance should be developed how to deal with this condition. Policy decisions are needed to define whether this should be done by an approach on reducing allowed amounts or by using more realistic exposure assessments.

For most cases, the limitations of aggregate risk assessment are found in the exposure assessment.

When aggregate exposure is taken into account, sometimes simple worst case deterministic exposure assessments are sufficient to indicate the absence of concerns. In that case no further actions are required.

If concerns cannot be excluded, refinement of the exposure assessment is the first priority. However, this refinement is often limited due to the absence of relevant exposure data. Therefore, additional measurements on specific substances and products will be needed to improve the risk assessment. Such additional measurements can possibly be incorporated in enforcement monitoring programs or should be separately addressed.

To be able to deal with the increasing regulatory demands for aggregate risk assessment, further development of exposure models will be necessary. A joint action between public and private parties may be the most efficient way forward.

It is conceivable that exposure within a single framework is safe (eg for use as biocide and for use in cosmetics) but aggregation over different frameworks gives reason to concern. This requires a policy decision on risk management options within one or more frameworks. A stakeholder dialogue should be started to bring this issue forward.

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