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P.E. Boon | M.I. Bakker | J.D. van Klaveren | C.T.M. van Rossum

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P.E. Boon*
M.I. Bakker
J.D. van Klaveren*
C.T.M. van Rossum

Contact:
P.E. Boon
VCP@rivm.nl

With contributions of:
A.J. Baars
J-D. te Biesebeek
B.G.H. Bokkers
M. van de Bovenkamp
A. de Mul*
M.C. Ocké
E.H.M. Temme*
G. Wolterink

* RIKILT-Institute of Food Safety, Wageningen University and Research Centre

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Abstract

Risk assessment of the dietary exposure to contaminants and pesticide residues in young children in the Netherlands

The diet of children aged 2 to 6 years in the Netherlands is safe regarding the exposure to fumonisin B_1, deoxynivalenol, patulin (toxic compounds produced by fungi), nitrate and organophosphorus pesticides. For dioxins, that are mainly present in animal fats, there is a limited probability that an adverse health effect will occur. For acrylamide, that is present in baked and fried foods, there is also a probability of an adverse health effect occurring in young children. However, the extent to which this could happen is as yet unclear. For aflatoxin B_1 and ochratoxin A (both also toxic compounds produced by fungi) it was not feasible to determine whether or not adverse health effects occur in this age group.

These are the results of a study performed by the National Institute for Public Health and the Environment (RIVM) and the RIKILT-Institute of Food Safety. For acrylamide, aflatoxin B_1, dioxins and ochratoxin A, more research is needed to refine the risk assessment. The most important requirements for this purpose are the generation of representative concentration data for aflatoxin B_1 and ochratoxin A in food and a better understanding of the toxicological effect of acrylamide.

The aim of the study described in this report was to assess the dietary exposure and the related possible health risk, to a selected group of compounds in young children in the Netherlands. For this purpose, food consumption data from the Dutch National Food Consumption Survey 2005/2006-Young children were linked to data on the concentration of these compounds in foods. The health risk of children was subsequently assessed with the help of the available literature regarding the toxicology of these compounds.

Key words: young children, dietary exposure, chemicals, risk assessment, probabilistic modelling

Rapport in het kort

Risicobeoordeling van de blootstelling van jonge kinderen in Nederland aan contaminanten en residuen van bestrijdingsmiddelen via de voeding

De voeding van peuters en kleuters in Nederland is veilig voor wat betreft de blootstelling aan fumonisine B_1, deoxynivalenol, patuline (gifstoffen veroorzaakt door schimmelgroei), nitraat en organofosfor-bestrijdingsmiddelen. Voor dioxines (vooral aanwezig in dierlijke vetten) bestaat er een beperkte kans dat er een negatief gezondheidseffect optreedt. Ook voor acrylamide (aanwezig in gebakken en gefrituurde producten) is er een kans dat een negatief gezondheidseffect optreedt in peuters en kleuters. Echter de grootte van deze kans kan niet worden geschat. Voor aflatoxine B_1 en ochratoxine A (beide ook gifstoffen veroorzaakt door schimmelgroei) kon niet worden beoordeeld of de voeding veilig is voor peuters en kleuters.

Dit blijkt uit onderzoek van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) en het RIKILT-Instituut voor Voedselveiligheid. Voor acrylamide, aflatoxine B_1, dioxines en ochratoxine A is aanvullend onderzoek nodig om de risicobeoordeling te verfijnen. De meest belangrijke elementen hiervoor zijn het genereren van 1) representatieve concentratiedata van aflatoxine B_1 and ochratoxine A in de voeding, en 2) kennis over het schadelijke effect van acrylamide.

Het doel van dit rapport was om de inname van een groep stoffen, en het mogelijk daarmee samenhangende gezondheidsrisico, te berekenen in peuters en kleuters in Nederland. Hiervoor zijn consumptiegegevens van de Voedselconsumptiepeiling onder peuters en kleuters gecombineerd met monitoringgegevens van concentraties van de onderzochte stoffen in producten. Met behulp van de aanwezige literatuur op toxicologiegebied is vervolgens het gezondheidsrisico voor de kinderen geschat.

Trefwoorden: jonge kinderen, blootstelling via de voeding, chemische stoffen, risicobeoordeling, probabilistisch modelleren

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Summary

Prevention of negative health effects due to pesticide residues and contaminants in food is an important world-wide issue. It has been recognized that especially children may be a potentially vulnerable subgroup in this respect due to their higher consumption levels per kg body weight and differences in physiology compared to adults.

The aim of the study described here is to assess the dietary exposure and the related possible health risk to a selected number of compounds. For this recent food consumption data of the Dutch National Food Consumption Survey-Young Children 2005/2006 were linked to recent monitoring concentration data. To assess both acute and chronic dietary exposure, the data were combined using advanced statistical models. The acute dietary exposure was calculated for the group of organophosphorus pesticides, and the chronic dietary exposure for acrylamide, dioxins (including dioxin-like PCBs), a number of mycotoxins (aflatoxin B_1, deoxynivalenol, fumonisin B_1, ochratoxin A and patulin) and nitrate.

For the risk assessment the 99th percentile of exposure was compared to the health based limit value for chronic toxicity for all chemicals, except organophosphorus pesticides. For this group of pesticides, the 99.9th percentile of exposure was compared to the health based limit value for acute toxicity. If the level of exposure exceeded the health based limit value, the percentage of children exceeding this limit was estimated. For acrylamide and aflatoxin B_1, possible carcinogenic compounds with a genotoxic mechanism, a margin of exposure was derived. When the exposure exceeded the health based limit value or resulted in a relatively low margin of exposure, the available toxicity database was further reviewed with special attention to children to re-assess the risk.

The results showed that according to the present findings the diet of young children in the Netherlands is safe regarding the exposure to fumonisin B_1, deoxynivalenol, patulin, nitrate and organophosphorus pesticides present in food. For dioxins, there is a limited probability that an adverse health effect will occur. For acrylamide there is also a probability of an adverse health effect occurring in young children, although the extent to which this could happen is as yet unclear. This is due to inconsistent results from epidemiology studies concerning the carcinogenicity of this compound. For aflatoxin B_1 and ochratoxin A, it was not feasible to determine whether or not an adverse health effect will occur, due to the use of (partly) targeted concentration data for these compounds.

For acrylamide, aflatoxin B_1, dioxins and ochratoxin A, more research is needed to refine the risk assessment. The most important requirements for this purpose are the generation of representative concentration data for aflatoxin B_1 and ochratoxin A and a better understanding of the toxicological effect of acrylamide.

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Glossary

AChE	acetylcholinesterase
ADI	acceptable daily intake
ARfD	acute reference dose
BBN	beta-binomial-normal
BMD	benchmark dose
BMDL	95 % lower confidence limit of the benchmark dose
bw	body weight
CIAA	Confederation of the Food and Drink Industries of the EU
CMG	common mechanism group
CONTAM	EFSA panel on contaminants in the food chain
dioxins	PCDDs, PCDFs, mo-PCBs and no-PCBs
DNFCS	Dutch National Food Consumption Survey
DNFCS-3	Dutch National Food Consumption Survey 1997/1998
DON	deoxynivalenol
EC	European Commission
EFSA	European Food Safety Authority
EWRS	Early Warning & Response System
GfK	Market Research Agency (Growth for Knowledge)
HBLV	health based limit value
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
KAP database	Dutch Quality Agricultural Products database
LOAEL	lowest observed adverse effect level
LOR	limit of reporting
MCRA	Monte Carlo Risk Assessment programme
MOE	margin of exposure
mo-PCBs	mono-ortho polychlorinated biphenyls
NDMA	N-nitrosodimethylamine
NOAEL	no observed adverse effect level
no-PCBs	non-ortho polychlorinated biphenyls, the so-called ‘dioxin-like PCBs’
OPs	organophosphorus pesticides
OTA	ochratoxin A
PBTK model	physiologically-based toxicokinetic model
PCDDs	polychlorinated dibenzo-p-dioxins
PCDFs	polychlorinated dibenzofurans
PPR	EFSA panel on plant protection products and their residues
PMTDI	provisional maximum tolerable daily intake
PTDI	provisional tolerable daily intake
PTMI	provisional tolerable monthly intake
PTWI	provisional tolerable weekly intake
RAC	raw agricultural commodity
RIKILT	RIKILT-Institute of Food Safety, Wageningen UR
RIVM	National Institute for Public Health and the Environment
RPF	relative potency factor
SCF	Scientific Committee on Food
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

TDI	tolerable daily intake
TEF	toxic equivalency factor
TEQ	toxic equivalent
VSD	virtual safe dose
VWA	Dutch Food and Consumer Product Safety Authority

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1 Introduction

1.1 Vulnerability of children

Prevention of health risks due to the presence of chemicals in food is an important world-wide issue, given, for example, the presence of both international and national food safety authorities, such as the European Food Safety Authority (EFSA). It has been recognized that children may be a potentially vulnerable subgroup in this respect. Children consume more food and water compared to adults when expressed per kg body weight (bw) (National Research Council, 1993; Health Council of the Netherlands, 2004; SCF, 1998), resulting in relatively higher exposures to compounds. Also specific dietary patterns of children may contribute to a higher exposure to contaminants present in food.

Apart from having a higher exposure per kg bw, children have a different physiology from that of adults. This has been thoroughly described in a recent report published by the Dutch Food and Consumer Product Safety Authority (VWA) (VWA, 2008). In this report, the development of children in relation to possible toxic effects of food contaminants is discussed. It is concluded that children are more sensitive and vulnerable for many compounds, while for some compounds there is no difference between the sensitivity of children and adults. Especially young children under the age of 1 may be more vulnerable, since their enzymatic activity and therefore their ability to break down chemical compounds is low compared to adults. They may therefore be longer exposed to compounds than adults (VWA, 2008). Due to significant postnatal development of different organ systems during childhood children may be more sensitive to neurotoxic, endocrine and immunological toxic effects up to 4 years of age. This is also true for children aged 5 to 12 years, however to a lesser extent for immunological toxic effects (VWA, 2008). Due to these differences between adults and children regarding exposure and physiology, it is important to address children as a separate subgroup in risk assessments.

1.2 Available exposure assessments in children in the Netherlands

In the past numerous exposure assessments have been performed in Dutch children covering a large variety of chemicals present in food, including acrylamide (Boon et al., 2005; Konings et al., 2003), dioxins (Baars et al., 2004), pesticides (Boon et al., 2004; Boon et al., 2008), brominated flame retardants (Bakker et al., 2008), mycotoxins (Bakker and Pieters, 2002; Pieters et al., 2004), aspartame (RIVM-RIKILT Front Office, 2006) and nitrate (Westenbrink et al., 2005). All these studies show a higher exposure per kg bw in children than in adults.

In the studies mentioned above food consumption data of the Dutch National Food Consumption Survey 1997/1998 (DNFCS-3) were used. Due to changes in dietary habits and introduction of new foods, as well as changes in concentration levels, it is important to continue monitoring the exposure to compounds present in food by young children.

1.3 Aim of this study

Recently, in 2005/2006, a food consumption survey was conducted in the Netherlands among children aged 2 to 6 years, the so-called Dutch National Food Consumption Survey-Young Children 2005/2006 (Ocké et al., 2008). The aim of the study described here is to assess the exposure and the related possible health risk (*i.e.*, probability that an adverse health effect occurs) to a selected number of

compounds using the food consumption data of this study in children and the most recent concentration data available from Dutch monitoring programmes.

The results presented in this report should be seen as a first step in the identification of a possible health risk in young Dutch children related to the compounds studied. If we conclude, based on the results, that there is a possible health risk (or a health risk cannot be determined) recommendations will be given on how to refine the risk assessment of the relevant compounds aiming at a better identification of the possible health risk involved. It is beyond the scope of this report to formulate policy recommendations regarding measures aiming at a reduction in exposure.

The compounds addressed in this report were selected by an expert group based on previous estimates indicating that these compounds may be relevant to children's health, for example, due to their specific dietary patterns or levels present in foods consumed by children. A second selection criterion was the availability of concentration data for the compound in foods, since without sufficient concentration data reliable assessments cannot be made. Based on this last selection criterion, sulphite, T-2/HT-2 and artificial sweeteners, all identified as relevant to children's health, were not addressed in this report. For sulphite, there were only concentration data available for a few foods in which this additive is used. Also the Dutch concentration data of the mycotoxins T-2/HT-2 were not sufficient to perform a dietary exposure assessment, due to a very low number of samples tested positive for these compounds (probably due to a high limit of reporting). For artificial sweeteners, the available concentration data did not cover the foods relevant for children. As a result of this observation, VWA has performed additional analyses on concentrations of artificial sweeteners in foods relevant for young children. The assessment of the intake of these substances will be reported elsewhere.

The compounds addressed in this report were acrylamide, dioxins (including dioxin-like PCBs), a number of mycotoxins (aflatoxin B_1, deoxynivalenol (DON), fumonisin B_1, ochratoxin A (OTA) and patulin), nitrate and organophosphorus pesticides (OPs).

1.4 Outline of the report

The outline of the report is as follows. In chapter 2, first the input data and methodologies used to assess the exposure to the selected compounds will be addressed as well as the procedure of the risk assessment. The results of the exposure calculations are presented per compound (group), including a risk assessment and conclusions, in chapters 3 to 7. Finally conclusions and recommendations are drawn up in chapter 8.

In this report the terms exposure and intake are used alternatively, referring both to the ingestion of compounds via food and drinks.

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2 Methods

2.1 DNFCS-Young Children 2005/2006

In this report the chemical safety of food consumed by young children is assessed using the food consumption data of Dutch National Food Consumption Survey (DNFCS)-Young Children 2005/2006. The aim of this survey was to gain insight in the diet of Dutch children aged 2 to 6 years. Children under the age of 2 were excluded from the survey, because recent food consumption data are available for Dutch infants aged 9, 12 and 18 months (Breedveld and Hulshof, 2003), as well of 8 to 12 months (Boon et al., 2004).

The DNFCS-Young Children 2005/2006 was authorised by the Dutch Ministry of Health, Welfare and Sport and coordinated by the National Institute for Public Health and the Environment (RIVM). An expert committee advised the Ministry on the quality of the survey during planning, data collection, analyses and reporting of the results. For more details, see the report on this survey (Ocké et al., 2008) and/or the website www.rivm.nl/vcp/en or www.voedselconsumptiepeiling.nl.

2.2 Study population

The target population of the DNFCS-Young Children 2005/2006 consisted of boys and girls aged 2 to 6 years living in the Netherlands. Respondents were selected from representative consumer panels of the Market Research Agency GfK. Panel characteristics, such as socio-demographic characteristics, are known to GfK. Persons in these panels participate in all types of surveys and were not specially selected on nutritional characteristics. Institutionalised persons were excluded, as well as children whose parents/carers (from now on in this report indicated with carers) did not have sufficient knowledge of the Dutch language. Per family, only one child was included to avoid correlations in dietary consumption patterns between children of the same family.

In total 1,634 children were invited to participate in the study, of which 1,279 consented (net response of 78 %). The children were equally distributed over the four age-sex strata (Table 2-1). During recruitment, the representativeness of the study population was monitored and, if necessary, the recruitment was adjusted for age and sex, education of the head of the household, level of urbanisation, place of residence and region. The study population was representative regarding socio-demographic characteristics (including region and education of the head of the household), but densely populated areas were slightly underrepresented.

The carer of each child recorded the consumption of food and drinks on two non-consecutive days (separated by about 8 to 13 days). All days of the week were equally represented, but the winter and autumn period were slightly overrepresented compared to the spring and summer period.

Characteristics of the study population are listed in Table 2-1. About half of the children lived in four-person households and for 68 % of the children both carers were employed. Of the children 43 % had at least one carer with a high educational level. In total, 44 % of the children lived in the west of the Netherlands and 42 % in densely populated areas. The Netherlands was the country of origin of most carers in the study population.

Table 2-1. Characteristics of the Dutch children aged 2 to 6 years.

	Total (n=1,279)	2 to 3 years		4 to 6 years	
		Boys (n=327)	Girls (n=313)	Boys (n=(327)	Girls (n=312)
Characteristics	%	%	%	%	%
Size of household					
2 to 3	23	25	27	18	22
4	49	50	50	53	45
5+	28	24	23	29	34
Highest educational level of carer(s)					
Low	11	10	10	13	11
Moderate	43	44	40	41	47
High	43	44	46	43	37
At least for one carer unknown	3	2	4	3	5
Employment status of carers					
Both employed[a]	68	72	70	68	64
At least one carer not employed	28	26	28	28	31
At least for one carer unknown	3	2	2	4	4
Region					
Three largest cities in the west of the Netherlands[b]	13	11	11	15	14
Rest of the west	30	32	30	27	29
North	10	11	11	10	9
East	23	24	24	23	23
South	24	23	24	24	24
Urbanisation					
High	37	35	36	40	35
Moderate	24	24	25	23	23
Low	40	41	39	37	42
Native country of carers					
Both of Dutch origin	93	95	92	91	92
At least one not of Dutch origin	6	5	7	8	6
Unknown for at least one carer	1	1	1	1	2

[a] This category also includes single carers that were employed.
[b] Amsterdam, Rotterdam, The Hague

During the home visit, the child's weight and height were measured and recorded. The mean height was 107.7 cm and weight was 18.8 kg for the total study population (Table 2-2). For more details about the study population, see the previous report (Ocké et al., 2008).

The results on food safety reported in this report were not corrected for socio-demographic factors and season as done for nutrients and foods in the same study population (Ocké et al., 2008), due to the lack of statistical tools for doing so when addressing food safety.

Table 2-2. Mean height (in cm) and weight (in kg) of the Dutch children aged 2 to 6 years.

		2 to 3 years		4 to 6 years	
	Total (n=1,279)	Boys (n=327)	Girls (n=313)	Boys (n=327)	Girls (n=312)
Height (cm)	106.0	97.3	96.2	115.3	115.2
Weight (kg)	18.3	15.7	15.1	21.3	21.2

2.3 Method of dietary assessment

The food consumption data of the DNFCS-Young Children 2005/2006 were collected in the period October 2005 to November 2006. Carers willing to participate were visited at home by a trained employee of GfK. During the home visit survey materials were presented and overall instructions were given. Survey data were collected by means of a written general questionnaire and subsequently through two food records. After completion of the second record, the respondents returned the questionnaire and food diaries to GfK by regular mail.

The questionnaire includes questions on the background of the child and family, the child's daily rhythm and activities (developed by TNO-Quality of Life) and general characteristics of the child's diet. Questions related to eating habits include consumption frequency of certain specific foods, use of dietary supplements, the purchase of organic foods and the volume of cups and glasses used habitually by the child.

The food records were filled in by the carer of each child. The carer recorded in pre-structured diaries the foods and drinks the child had consumed on two non-consecutive days (separated by about 8 to 13 days). The survey dates were determined for each child by GfK.

The diaries were structured according to the food consumption occasion (three meals and three in-between meals). For each food consumption occasion, the carer was asked to indicate the time and place of consumption and to tick each food on the list consumed by the child on that occasion. Ample space was provided to enter additional foods not listed. For each food, the carer was asked to indicate characteristics such as fat content, sugar content, flavour, brand name and preparation method. Portion size of the foods and meals were estimated by using photographs, domestic measures (a small and a large spoon were supplied to standardise estimates), standard units, weight and/or volume. The usual volume of cups and glasses used was measured by the carer. The final section of the diary included questions on the use (specific type and amount) of dietary supplements on the recording day and the use of toothpaste. Furthermore, carers were asked to indicate whether the recording day was special in any way, such as holiday, travelling day or illness.

Dieticians entered the data from the diaries into the EPIC-Soft computer program. EPIC-Soft is developed to process food consumption data derived from highly standardised 24-hour dietary recalls (Slimani et al., 2000), as used in the current ongoing Dutch food consumption survey among the general population (DNFCS-2007-2010; www.voedselconsumptiepeiling.nl). The dieticians were specifically trained in data entry and were given detailed instructions on how to enter specific foods.

2.4 Concentration data

The concentration data for the majority of compounds addressed in this report were derived from monitoring programmes performed in the Netherlands by the VWA. Additional organophosphorus pesticide (OP) residue data were derived from monitoring programmes performed by the Dutch Produce Association (*e.g.,* The Greenery), Bakker Barendrecht B.V. (retail) and Food Compass. The Dutch Produce Association and Bakker Barendrecht also supplied data on nitrate. For dioxins, data were obtained from the Dutch monitoring programme on dioxins of the Dutch Ministry of Agriculture, Nature and Food Quality of 2005-2006. Additional dioxin concentration data on vegetables and fruits sampled in 2005 were extracted from (Traag and Hoogenboom, 2006). All concentration data are stored in the Quality Agricultural Products Database (KAP database[1]) and for OPs also in the Early Warning & Response System (EWRS[2]). For nitrate, which occurs naturally in tap water, concentrations in tap water were used as provided by the Centre of 'Inspectieonderzoek, Milieucalamiteiten en Drinkwater'. Concentrations of ochratoxin A (OTA) in meat were not available in the Netherlands. Therefore, the most recently reported concentrations in pork from Germany (SCOOP, 2002a) and in poultry from Denmark (Jorgensen, 1998) were used. For an overview of the concentrations, see Table 2-3.

In the monitoring programmes samples are reported to contain a chemical at a certain, quantifiable level. However many samples are reported to contain no contaminant or pesticide residue below a certain level (the so-called non-detects). This level is termed here the limit of reporting (LOR) and is just the value below which concentrations are reported as 'less-than' (see also section 2.6). In fact, the LOR reported in the present report may be the limit of detection (the lowest quantity of a substance that can be distinguished from a blank value) or the limit of quantification (the lowest quantity of a substance that can be quantified, which is always higher than the limit of detection), but the available information did not allow for this distinction. See Appendix A for a list of all concentration data included in the exposure assessments per compound.

When the number of food samples was less than five but concentrations above the LOR were reported, these foods were examined more closely. First we tried to create food groups by grouping similar foods to increase the sample size. If this was not possible, and 1) the foods contained low concentrations, and 2) they did not belong to a food group in which the substance was expected, and 3) the consumption of these foods was low, they were excluded from the calculations. For acrylamide, dioxins, nitrate and OPs all foods sampled less than five times were included due to this approach. For mycotoxins some samples were excluded (*e.g.,* for deoxynivalenol (DON) concentrations in blancmange powder and hazelnut spread (n = 1)). Concentration data related to indefinable foods, for example, 'various soups, sauces and -products', 'remaining drinks', 'remaining fresh herbs', 'remaining fruits and nuts', were omitted from the exposure calculations because they cannot be linked to specific consumed foods.

Concentration data of mycotoxins as supplied by VWA are a mixture of both monitoring and survey concentration data. Survey concentration data are obtained from targeted sampling of batches suspected of containing (possible) higher concentrations of mycotoxins. These data are not suitable to estimate a reliable exposure to contaminants. Based on indications received by VWA survey samples were removed from the mycotoxin concentration dataset, including all peanut, fig and raisin samples, as well as all samples of other foods that were analysed more than once on the same day (the so-called duplo and triplo samples). The remaining mycotoxin concentration dataset contained only the results of

[1] www2.rikilt.dlo.nl/kap/index.html

[2] www2.rikilt.dlo.nl/ewrs/index.html

Table 2-3. Overview of the concentration data used to assess the exposure to different compounds in Dutch children aged 2 to 6 years.

Chemical	Years included in the assessment	Source[a]	Representative
Acrylamide	2006-2007[b]	VWA.	Yes
Dioxins	2005-2006[b]	• Dutch monitoring programme LNV. • Data on vegetables and fruits from (Traag and Hoogenboom, 2006).	Yes
Mycotoxins	2002-2006[c]	• VWA (ochratoxin A concentrations of raisins from survey program). • Ochratoxin A levels in pork and poultry were derived from literature (Jorgensen, 1998; SCOOP, 2002a), respectively.	Biased due to (partly) targeted sampling
Nitrate	2002-2006[c]	• VWA, DPA, BB. • Tap water concentrations from Centre of 'Inspectieonderzoek, Milieucalamiteiten en Drinkwater'	Yes
Organophosphorus pesticides	2005-2006[d]	VWA, DPA, BB, Food Compass.	Biased due to (partly) targeted sampling

[a] VWA = Dutch Food and Consumer Product Safety Authority; LNV = Dutch Ministry of Agriculture, Nature and Food Quality; DPA = Dutch Produce Association (*e.g.,* The Greenery); BB = Bakker Barendrecht B.V. (retail).
[b] Only the most recent years were included because of decreasing levels of acrylamide and dioxins in time.
[c] Several years were included in the assessment because adverse effects occur after long-term exposure and there is no decreasing trend in concentrations.
[d] Concerns acute toxic effects. Therefore it is important to include only the most recent years in the assessment.

samples analysed as part of the monitoring programme. Also these samples may not be completely at random, as they are sampled to ensure compliance with legal limits. This is also true for monitoring concentration data of pesticide residues. Because of this the resulting exposure levels of mycotoxins and OPs as reported here are very likely overestimates of the true exposures.

Some of the mycotoxins studied (namely aflatoxin B_1, DON and OTA) may be present in the foods that were removed from the mycotoxin concentration dataset, namely peanuts, raisins and figs. Whereas the consumption of figs by children is negligible (and hence the contribution of figs to the exposure to these mycotoxins can be ignored), the consumption of peanuts (on average 0.1 g/d) and raisins (0.9 g/d) is not. Not addressing these two foods in the assessments may therefore result in an underestimation of the exposure. For this reason, for aflatoxin B_1, DON and OTA the concentrations in nuts (all nuts grouped together because of data scarcity) were assigned to peanuts (Appendix A). Unfortunately, for raisins, which may contain aflatoxin B_1 and OTA, concentrations in comparable foods were not available. When looking at the concentrations in the survey samples of raisins (aflatoxin B_1: 0.01 µg/kg, OTA: 5.3 µg/kg) it was concluded that for aflatoxin B_1 these values could be safely ignored (as the consumption of raisins is fairly low), but for OTA this was not the case. Therefore, to make sure that the exposure to OTA was not underestimated, we included the average OTA concentration of raisins in the exposure assessment, even though this value was derived from targeted survey samples.

Cumulating concentrations
Some of the compounds addressed in this report are groups of compounds with a similar mode of action. To address the risk of exposure to this group of compounds, the individual exposures should be addressed simultaneously. These groups include dioxins, OPs and the mycotoxins aflatoxins and fumonisins.

The collective term ‘dioxins’ encompasses the polychlorinated dibenzo-p-dioxins (PCDDs), the polychlorinated dibenzofurans (PCDFs), and the mono-ortho polychlorinated biphenyls (mo-PCBs) and non-ortho polychlorinated biphenyls (no-PCBs), the so-called ‘dioxin-like PCBs’. It is generally assumed that the toxicity of dioxins is expressed through a common mechanism of action and all compounds act through this mechanism, namely interaction with the cytosolic aryl hydrocarbon receptor protein (Ah receptor). During the last few decades, data from many experimental studies with dioxins are consistent with an additive model, resulting in the development of the toxic equivalency concept during the mid 1980s. This concept uses the relative effect potency determined for individual PCDD, PCDF, and dioxin-like PCB compounds for producing toxic or biological effects relative to a reference compound, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is considered the most toxic dioxin. The total toxic equivalent (TEQ) is operationally defined by the sum of the products of the concentration of each compound and its toxic equivalency factor (TEF) value, and is an estimate of the total TCDD-like activity of the mixture (van den Berg et al., 2006). Thus, in the TEF scheme, a dose of TCDD of, for example, 1 pg TCDD/kg bw/d is considered to be equivalent to a toxic equivalence of 1 pg TEQ (properly addressed as ‘WHO-TEQ’)/kg bw/d. See Appendix B for the used TEFs.

The OPs is also a group of compounds with a similar mode of action, namely inhibition of acetylcholinesterase (AChE) by phosphorylation, resulting in a spectrum of acute cholinergic effects (ILSI, 1999; Mileson et al., 1998; Pope, 1999). Also for this group of compounds an additive model is expected at the low doses to which people are exposed (EFSA, 2008b). To address these compounds relative potency factors (RPFs) were used as previously reported in (Boon et al., 2008), using acephate as index compound (Appendix C). These RPFs were derived from benchmark doses (BMD) at which AChE activity in the brain of female rats was reduced by 10 % compared to background activity (BMD_{10}) as reported by the US Environmental Protection Agency (EPA) (2006). BMDs were available

for eighteen of the OPs addressed in this report. For the remaining OPs RPFs were calculated as the ratio of the no-observed adverse effect level (NOAEL) for AChE inhibition, mainly obtained from JMPR (FAO/WHO Joint Meeting on Pesticide Residues, Appendix C). For more details, see Boon et al. (2008)[3].

In the case of aflatoxins, the concentrations of aflatoxin B_1 and aflatoxin M_1 were summed. The carcinogenic potency of aflatoxin M_1 is probably one or even two orders of magnitude lower than that of aflatoxin B_1 (Bennett and Klich, 2003; EFSA, 2004b; FAO/WHO, 2001b). As a conservative estimate the potency of aflatoxin M_1 was assumed to be 10 % of that of aflatoxin B_1.

Fumonisin B_1 on foodstuffs is generally accompanied by fumonisins B_2 and B_3 in a ratio of about 8:3:1 (Sydenham et al., 1993). The intake of fumonisin B_1 was therefore increased with 50 % to obtain the intake of all three fumonisins, under the assumption that the RPFs of the different fumonisins were equal.

2.5 Link between food consumption data and concentration data

Analyses at the level of raw agricultural commodities
Most of the chemicals addressed in this report are analysed in raw agricultural commodities (RACs), including peels and non-edible parts. This is due to the fact that in legislation the majority of limits for concentrations of, for example, pesticides, dioxins and nitrate are set for RACs. For mycotoxins analyses are performed both at RAC level (*e.g.,* wheat, maize, rye) and food level (*e.g.*, wheat bread, tortilla crisps, biscuits). To model the dietary exposure to chemicals using concentrations analysed at RAC level a link between concentration and food consumption data needs to be established. In that way, also prepared foods (like apple cake, salads, bread) containing RACs as ingredient will be included in the exposure assessment.

At the RIKILT-Institute of Food Safety a food conversion model has been developed in 1995 (van Dooren et al., 1995), which has been regularly updated since then to cover new foods as recorded in later Dutch food consumption surveys, including the present DNFCS-Young Children 2005/2006. This food conversion model is based on the foods as listed in the Dutch food composition database NEVO (NEVO Stichting, 2006). In this model, all NEVO-codes recorded in the children's survey have been converted to their RAC ingredients (and their mass fraction). To establish the weight fraction of RACs present in foods several sources of information were used. These included recipes from cookbooks, food legislation and information from either literature, label of the product, internet or manufacturer, as well as recipes developed and used for the purpose of NEVO (NEVO Stichting, 2006). Furthermore, nutrient concentrations as listed in NEVO were used in the conversion or as check for the amounts deduced from other sources. When there is only little information of a food, ingredient levels of a similar food were used. The type of processing a RAC has undergone before consumption is also recorded. For example, apple juice may be converted to RAC 'apple' with processing type 'juicing', apple eaten peeled to RAC 'apple' and processing type 'peeling', and apple eaten with skin to RAC 'apple' with processing type 'raw'. In this way, the effect of processing on concentrations in RACs before consumption can be taken into account in an exposure assessment. For more details on the food conversion model, see van Dooren et al. (1995).

[3] The RPF approach is identical to the toxic equivalency factor (TEF) approach. Unlike the term TEF, which is applied to groups of substances with a strong degree of toxicological similarity and where TEF values are assumed to encompass all endpoints and exposure routes, the term RPF is applied in situations where the mode of action appears to be similar, but the exact mechanism is complex and maybe not known in detail (EPA, 2000b).

Analyses at food level
Apart from analyses in RACs, all compounds were also (or exclusively as for acrylamide) analysed at the level of foods as eaten. For example, acrylamide was analysed in biscuits, nitrate in frozen spinach, aflatoxin B_1 in peanut butter, patulin in apple juice and pesticides in fruit juices. These foods were either linked directly to foods coded in the food consumption survey (*e.g.*, peanut butter) or by creating food groups. These food groups consisted of comparable foods as those analysed based on chemical concentrations and food characteristics. An exception was made for home made freshly squeezed juices (predominantly orange juice). These drinks were not linked to analyses performed in (commercially produced) fruit juices, but were linked to concentrations analysed in the corresponding RAC using the food conversion model. The reason for this is that in commercially produced fruit juices hardly any pesticides are detected.

For details on linking of analysed foods or RACs to foods coded in the food consumption survey for acrylamide, dioxins and mycotoxins, see Appendix D.

2.6 Dietary exposure calculations

The dietary exposure was calculated using Monte Carlo Risk Assessment programme (MCRA), Release 6.0 and 6.1, available for registered users at the RIKILT website (de Boer and van der Voet, 2007). Release 6.0 was used for most exposure analyses, except for the acute cumulative exposure calculations for OPs following Approach 2 (see below). This approach was not implemented in Release 6.0. All daily consumption patterns (*e.g.,* 2,558 for the total population (2 days × 1,279 individuals)) were multiplied with the (mean) concentration of the substance per consumed food and summed over foods consumed per day per individual. The estimated exposures were adjusted for the individual's body weight. This resulted in an exposure distribution.

All substances studied here, except OPs, are expected to be present at levels for which chronic effects are the relevant health effects. Therefore, only for OPs a short-term intake assessment was performed, and for the other substances a long-term intake assessment. For short-term intake assessments the whole range of analysed concentrations is used to take the variation in concentrations into account. For long-term intake assessments however the mean concentration of the substances in the various foods is used, because fluctuations in daily concentrations are assumed to average out in the long run.

Samples with levels below LOR
For most of the basic exposure analyses non-detects were assumed to contain concentrations equal to ½LOR (middle bound scenario). Whether to assign ½LOR to analysed foods or RACs with only non-detect levels was determined by the possibility that the food or RAC could be contaminated. For this data from literature were used. This possibility was estimated based on information from previous studies and/or expert judgement. For example, no acrylamide concentrations above the LOR were analysed in bread. However, bread can contain some acrylamide (FSA, 2005; Matthys et al., 2005) and is consumed regularly and in large quantities by young children. Therefore, the non-detects of acrylamide in bread were assigned ½LOR. On the other hand, congener concentrations of dioxins or dioxin-like PCBs in vegetables, fruit and cereal samples below LOR were assumed to be zero, because assigning ½LOR to these food groups very likely overestimates true concentrations. Fruits, cereals and vegetables are contaminated with dioxins only via atmospheric deposition which results in far lower concentrations compared to animal products. For pesticides non-detects were assumed to contain no pesticide residue. Because only a part of the commodities will be treated with pesticides, a large part of the non-detects will truly contain no pesticide. Therefore non-detects for pesticides are commonly assumed to be true zero's.

Apart from assigning ½LOR to non-detects, two other scenarios were performed in which either zeros (lower bound scenario) or LORs (upper bound scenario) were assigned to these samples to study the sensitivity of the intake calculations to the concentration assigned to non-detects (except for OPs). Results of these scenarios per compound (group) are presented in Appendix E.

Note that mycotoxins are determined using several methods of analysis, each having its own LOR. Since 2004 VWA has used a screening method with relatively high LORs to screen multiple mycotoxins simultaneously, If a mycotoxin is found to be present using this screening method, the actual level is determined with a method specific for that mycotoxin (already in use before 2004). This specific method has a lower LOR. In the exposure calculations, LORs of the screening method were used to replace the non-detects, since the screening method is used to analyse (most of) the samples first. For the samples of 2002 and 2003 this was not the case. Using the LORs of the screening method will therefore have resulted in a slight overestimation of the mean concentrations. This overestimation is however likely small in comparison to the overestimation due to (partly) targeted sampling (section 2.4).

Long-term intake
A distribution of daily exposures, calculated as described above using mean concentrations per food or food group, includes both the variation between individuals and between the two days of one individual. However, to assess the long-term intake within a population only the former type of variation is of interest, since in the long run the variation between different days of one individual will level out. Therefore, to calculate a long-term distribution, the within-person (between days) variation should first be removed from the distribution of daily exposures using statistical models like the relatively new beta-binomial-normal (BBN) model (de Boer and van der Voet, 2007; Slob, 2006) or the foods model developed at Iowa State University (ISUF model; (Dodd, 1996; Nusser et al., 1996, 1997)). To remove the within-person variation from the daily exposures, the BBN and ISUF models transform the daily exposure distribution into a normal distribution. After removal of the within-person variation, the normal distribution is back-transformed and is now considered a long-term exposure distribution. Both models are incorporated in MCRA. In contrast with the ISUF model, the BBN model can calculate the exposure distribution as a function of age. Since the dietary exposure of children is expected to be a function of age, this is the preferred model for calculation of long-term exposures in the present study.

However, the BBN model has more limited possibilities than the ISUF model to transform the short-term intake distribution to a normal distribution. In both models, daily exposures are transformed first by a logarithmic or power function and, in the case of ISUF, this can be followed by an additional spline transformation to achieve normality. For some substances, the transformation of daily exposure distributions to normality using the logarithmic or power transformation failed due to multimodality of the daily exposure distribution, for example, due to the presence of subgroups in the population with different consumption patterns (*e.g.,* groups that consume apple juice, but no apple sauce, groups that consume both, etc.). In those cases, ISUF was used. However, considerations as described in (de Boer et al., 2009) and preliminary results of simulation studies seem to indicate that also the ISUF model may not be optimal for non-normal (transformed) data. The preliminary results seem to indicate that in these cases ISUF most likely overestimates the true exposure. However, whether this is always true in more complex realistic situations is yet unclear.

Since a model that can always safely handle multimodal intake distributions is lacking, the ISUF model was used for those cases. When using the ISUF model, possible age-dependency of the intake cannot be modelled.

For acrylamide, nitrate, dioxins and DON the BBN model could be used to model long-term exposure and age-dependency was modelled if significant. For the other mycotoxins, including aflatoxin B_1, fumonisin B_1, OTA and patulin, multimodal intake distributions were observed and the ISUF model was therefore used. The reported percentiles of the long-term exposure distribution are P50, P95 and P99.

Acute cumulative dietary exposure to OPs
Acute cumulative dietary exposure modelling using the RPF method is still a fairly new methodology. There are no international guidelines on how to perform these assessments. At the RIKILT-Institute of Food Safety, two approaches have been developed to calculate the acute cumulative exposure using the RPF method. One approach (Approach 1) has already been used in a number of publications (Boon et al., 2008; Boon and van Klaveren, 2003; Caldas et al., 2006; van Klaveren et al., 2006), while a second approach (Approach 2) has been developed only recently. Both approaches were used in this study to calculate the exposure to OPs.

With Approach 1, the acute cumulative dietary exposure to OPs is estimated by linking daily consumption levels of RACs with summed OP levels per relevant RAC expressed in acephate-equivalents. This is done by multiplying the concentration of each OP in a RAC sample by its RPF and adding up the different equivalents to one OP concentration per sample expressed in acephate-equivalents. These samples are consequently used in the acute exposure assessment of OPs. In Approach 2, first the acute dietary exposure distribution per OP is calculated. Using the RPF per compound, the exposure distributions per compound are subsequently added to generate an acute cumulative dietary exposure distribution expressed in acephate-equivalents. For a discussion on these two approaches, see section 7.2.

For the estimation of the acute cumulative dietary exposure 100,000 randomly drawn daily consumption patterns of RACs from the RAC consumption database were multiplied with randomly selected cumulative or compound specific concentrations per RAC. Summing over RACs resulted in an empirical estimate of the acute cumulative exposure distribution for either total OP or the exposure distribution per compound. The exposure calculated per compound was summed using the same sequence of simulated person days in each case. The total acute cumulative exposure per person day was calculated by multiplying each compound specific exposure with the corresponding RPF and summing up these exposures per person day. The reported percentiles of the acute cumulative exposure distribution in both approaches are P50, P95, P97.5, P99 and P99.9.

Processing factors
Processing factors are factors used to calculate the fraction of the substance that is lost from the food/RAC by processes as peeling, cooking, grinding, etc. For example, a processing factor equal to 0.75 means that the concentration of the compound is reduced by 25 %, and a factor one means that the compound concentration is not affected by processing. For several compounds processing factors were included in the calculations to make the assessment as accurate as possible. The compounds for which processing factors were applied include OPs, nitrate and all mycotoxins except patulin. See Appendix F for the processing factors used, as well as for the methodology to include them in the exposure assessments.

Variability factors
When assessing the acute exposure to pesticide residues using concentrations derived from composite samples as analysed in pesticide monitoring programmes variability factors (also referred to as homogeneity factors) should be included in the assessment (EC, 2001b; FAO, 2002). These factors account for the fact that the concentration analysed in a composite sample consisting of, for example,

12 apples can originate from one individual unit of the commodity, and that consumers may be confronted with a concentration in a single unit (*e.g.,* an apple) rather than the average concentration as analysed in composite samples. In the acute cumulative dietary exposure assessment presented in this report, variability factors were included in the assessment. For more details on both the factors and methodology used, see Appendix G.

Uncertainty due to limited number of consumption and concentration data
There are different sources of uncertainty in dietary exposure assessments. Important sources are sampling uncertainty, under- or over reporting of foods, linkage of foods consumed to foods analysed and uncertainty due to the limited size of the dataset for both concentration and food consumption data.

Sampling uncertainty refers to possible targeted sampling of those commodities expected to contain the chemical at concentrations above a legal limit and occurs because monitoring is conducted for law enforcement reasons. It is not possible to quantify this type of uncertainty, but a qualitative statement can be made; exposure assessments using these data likely overestimate the true exposure.

The other sources of uncertainty mentioned above cannot be quantified and will either result in a possible overestimation and/or underestimation of exposure. An exception is formed by uncertainties due to the limited size of the dataset that can be quantified by using the bootstrap method (Efron, 1979; Efron and Tibshirani, 1993). With this method a bootstrap database is generated of the same size as the original database for both the food consumption and concentration database by sampling with replacement from the original datasets. These bootstrap databases are considered as databases that could have been obtained from the original population if another sample was randomly drawn. These two bootstrap databases are then used for the exposure calculations and derivation of the relevant percentiles. Repeating this process many times results in a bootstrap distribution for each percentile that allows for the derivation of confidence intervals around it. The bootstrap approach was used by generating 100 food consumption and 100 concentration bootstrap databases and calculating the chronic or acute (with at least 10,000 iterations each) dietary exposure. Of the resulting bootstrap distributions per percentile a 95 % uncertainty interval was calculated by computing the 2.5 % and 97.5 % points of the empirical distribution. Note that by bootstrapping both the consumption and concentration database in one analysis it is not possible to quantify which part of the uncertainty was due to a limited number of consumption or concentration data. For the acute cumulative dietary exposure an uncertainty analysis using the bootstrap approach was only applied using approach where the concentrations were summed per sample (Approach 1). With Approach 2, in which the exposure distributions per compound are summed, uncertainty analyses have not yet been implemented. For the results of this on the exposure percentiles, see Appendix H. It should be realised that this uncertainty also includes the uncertainty in the exposure due to the number of iterations used, the so-called ‘Monte Carlo’ uncertainty. The number of iterations was however selected in such a way that this uncertainty was negligible in the exposure calculations reported here.

2.7 Risk assessment

2.7.1 Decision tree of VWA panel

In 2007 a scientific panel of experts was established in the Netherlands to discuss how to perform a risk assessment for children aged 0.5 to 12 years in those cases where the dietary exposure exceeds the acceptable or tolerably daily intake (ADI/TDI) (VWA, 2008). This panel identified that children may be more sensitive to neurotoxic, endocrine and immunological toxic effects up to 4 years of age due to significant postnatal development of different organ systems during childhood. This is also true for

children aged 5 to 12 years, however to a lesser extent for immunological toxic effects (VWA, 2008). For compounds with these types of effects a 'temporary' exceedance of the health limit during childhood may be adverse, even though the exposures decrease below the limit when the children mature.

This panel proposed a decision tree, which includes a tiered approach, when assessing possible health risks when health limits are exceeded (VWA, 2008). This decision tree is applicable to evaluate chronic effects for which a health based limit value (HBLV; such as ADI, TDI) is established. The decision tree is not meant to evaluate compounds with acute toxic effects or genotoxic carcinogens. In this tiered approach, the risk assessment is refined via different steps including a refinement of the exposure assessment, examination of possibilities to lower the exposure (*e.g.,* by adjustment of processing techniques, removal of foods from the market or changes in dietary patterns) and a critical evaluation of the toxicity database (including reproduction toxicity data). Important in this is whether the HBLV was derived from a study in which young animals received a higher exposure than the full-grown animals due to their lower body weight and relative high food consumption. In those cases, an additional safety factor of two is present in the derivation of the HBLV, and consequently an exposure level that exceeds the HBLV with a factor two or less may not give reason for concern (VWA, 2008)[4]. Based on this evaluation different conclusions regarding the compound are possible according to the panel, namely:

- The exposure does not exceed the HBLV. There is therefore no additional health risk.
- The exposure exceeds the HBLV with a factor two or less. However, an additional safety factor of two is present in the derivation of the HBLV and therefore the extent and duration of the exceedance can be considered to pose a very limited health risk.
- The exposure exceeds the HBLV with a factor two or less and no additional safety factor of two can be applied (derivation of the HBLV is not based on a study in which young animals received a higher exposure than full-grown animals) or the exposure exceeds the HBLV with more than a factor two, irrespective of whether an additional safety factor of two can be applied:
    - Based on the available data there is a (very) limited health risk. However, additional research is needed to optimize the risk assessment.
    - There is insufficient data to establish the size of the health risk.
    - Based on the available data the exposure will result in a considerable health risk.

In these conclusions the exposure refers to a high percentile of exposure (VWA, 2008).

2.7.2 Risk assessment of chemicals addressed

In the present report the decision tree of the panel was used for the risk assessment of chemicals for which non-carcinogenic, chronic toxic effects were the most critical, although there were some deviations (Appendix I). In short, to assess the consequences of any exceedance of the ADI/TDI, the available toxicity data were reviewed with special attention to effects in children. In this respect, especially the potential adverse effects in reproductive toxicology studies and on the (developing) endocrine, immunological and neurological system were addressed. In addition, it was determined whether the critical effect of the HBLV was relevant for young children and whether there were indications that the sensitivity of children to the respective chemical may differ from the general population. In addition, when the ADI/TDI was exceeded with a factor two or less, it was examined whether the young animals (in the study on which the ADI/TDI was based) received a higher exposure than the full-grown animals due to their lower body weight and higher food consumption. In that case,

[4] The panel indicates that when, in the toxicitiy study on which the HBLV is based, the young animals were exposed to constant concentrations of the compound via food or drinking water, they were exposed to concentrations about two times higher than adult animals. As a consequence, an exposure level exceeding the HBLV with a factor two or less does not give reason for concern, unless other data in the toxicity database indicate otherwise.

exceeding the HBLV with a factor two or less may not give reason for concern[4]. In the final step of the risk assessment, a conclusion about the health risk (*i.e.*, probability that an adverse health effect occurs) was drawn.

For the compounds acrylamide and aflatoxin B_1 for which carcinogenic effects with a genotoxic mechanism are relevant, no HBLVs such as the ADI/TDI are available. In these cases, a margin of exposure (MOE) was calculated by dividing the (2.5 %) lower confidence limit of the benchmark dose for a 10 % increase in cancer incidence ($BMDL_{10}$) by the exposure. If no $BMDL_{10}$ was available, the no observed (adverse) effect level (NO(A)EL) was used. The MOE was compared to a value of 10,000 as proposed by EFSA (2005a). An MOE of 10,000 or higher ensures a reasonable margin of safety between a dose that causes with 95 % certainty no more than a 10 % cancer incidence in an animal experiment and the estimated exposure in humans and would therefore be of low health concern (EFSA, 2005a).

For the OPs, for which acute toxicity is relevant in relation to health risks, the exposure was compared to the acute reference dose (ARfD). Note that also for acrylamide, aflatoxin B_1 and OPs the whole toxicity database was screened for relevant effects in children.

When the exposure to a chemical is below the HBLV or the MOE is higher than 10,000, the dietary exposure can be regarded as safe (or to pose a negligible health risk) for that specific chemical. If the exposure however exceeds the HBLV or results in an MOE below 10,000, there is a probability that an adverse health effect occurs. Depending on the quality of both the exposure and the toxicity data used, the extent of the probability may be identified.

In this report we aimed at dividing the compounds addressed into those for which the diet poses a negligible health risk and those for which this may not be true. For the latter group, if sufficient exposure and toxicity data are available, the possible health risk was classified using the terminology of the VWA panel, namely very limited, limited or considerable health risk (Figure 2-1). This classification in three degrees of severity should be seen as a guideline to prioritize resources to refine the risk assessment.

The decision whether the probability to develop an adverse health effect is deemed very limited, limited or considerable was based on four factors:

1. The percentage of children exceeding the HBLV (or with a MOE < 10,000).
2. The extent to which the HBLV is exceeded (or the size of the MOE).
3. The severity of the health effect (*e.g.*, the occurrence of some light adverse liver effects will be considered less severe than a teratogenic effect).
4. The slope of the dose-response curve (a steep slope implies that the response to a slight increase of the dose is relatively high).

When all four factors are considered ‘low’, the probability to develop an adverse health effect is judged to be ‘very limited’. On the other hand, when one or more of the factors is ‘high’ a thorough toxicological evaluation should form the basis for the decision whether the probability should be considered ‘limited’ or ‘considerable’. From factor 3, the severity of the health effect, it may be clear that the final conclusion on the probability to develop an adverse health effect cannot be reached in a fully quantitative way. Rather, it is based on the evaluation of both the quantitative and qualitative toxicological information present. For this reason, a conclusion on the health risk of a compound should always be accompanied by a narrative in which the decision is explained.

As described in section 2.4, concentrations of mycotoxins and pesticides used to estimate the exposure may be biased to higher concentrations than children are exposed to in real life. The reported exposure

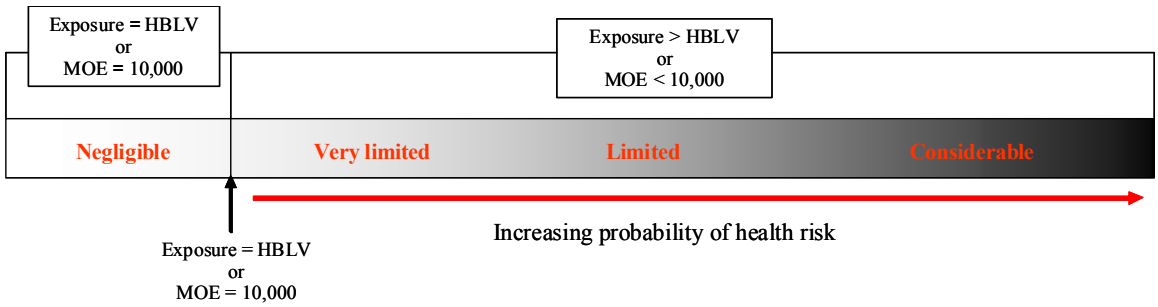

Figure 2-1. Different degrees of health risk
(HBLV = health based limit value; MOE = margin of exposure).

levels will therefore be very likely overestimations of the true exposures. When the exposure to these compounds does not exceed the HBLV or the MOE is 10,000 or higher, it is clear that there is a negligible health risk. However, when the exposure exceeds the HBLV or results in a low MOE, the conclusion is less clear. In those cases, the presented exposure levels indicate that the health risk may not be negligible, but due to possible overestimation of the exposure it is unclear whether this is true. The conclusion will then be that it is not feasible to determine whether there is a negligible health risk or not, and recommendations will be given how to refine the risk assessment. This conclusion can also be reached when other relevant data are lacking, such as certain toxicology data.

To summarise, the following conclusions can be drawn based on the risk assessments described in this report (Figure 2-1):

1. When the exposure does not exceed the HBLV, or exceeds the HBLV with a factor two or less in the case that an additional safety factor of two is present in the derivation of the HBLV or results in an MOE of 10,000 or higher, there is a negligible health risk (or negligible probability that an adverse health effect occurs). We did not use the same terminology as used by the panel (no additional health risk or limited health risk, section 2.7.1), because 'negligible' health risk is in our view a better description of the health risk related to such exposures.
2. When the exposure exceeds the HBLV, or exceeds the HBLV with a factor larger than two in the case that an additional safety factor of two is present in the derivation of the HBLV or results in an MOE below 10,000:
    a. There is a very limited health risk or probability that an adverse health effect occurs.
    b. There is a limited health risk or probability that an adverse health effect occurs.
    c. There is a considerable health risk or probability that an adverse health effect occurs.
    d. There is a possible health risk, but the extent cannot be identified
    e. Determination of the health risk is not feasible.

For an overview of the steps of the risk assessment procedure for all compounds addressed in this report and the possible conclusions, see Figure 2-2.

Selection reference value of exposure for risk assessment
To assess the health risk due to the dietary exposure to compounds, an exposure level needs to be selected for comparison to the HBLV or for the calculation of an MOE. Internationally there are no guidelines which dietary exposure percentile to select for this for both acute and chronic dietary exposure assessments. As discussed by the US EPA, which uses probabilistic modelling as part of the pesticide registration process in the USA, the choice of the percentile may, for example, depend on the (un)certainties related to the data used in assessment (EPA, 2000a). These are among others related to representativeness and size of the food consumption and concentration database (see also section 2.6).

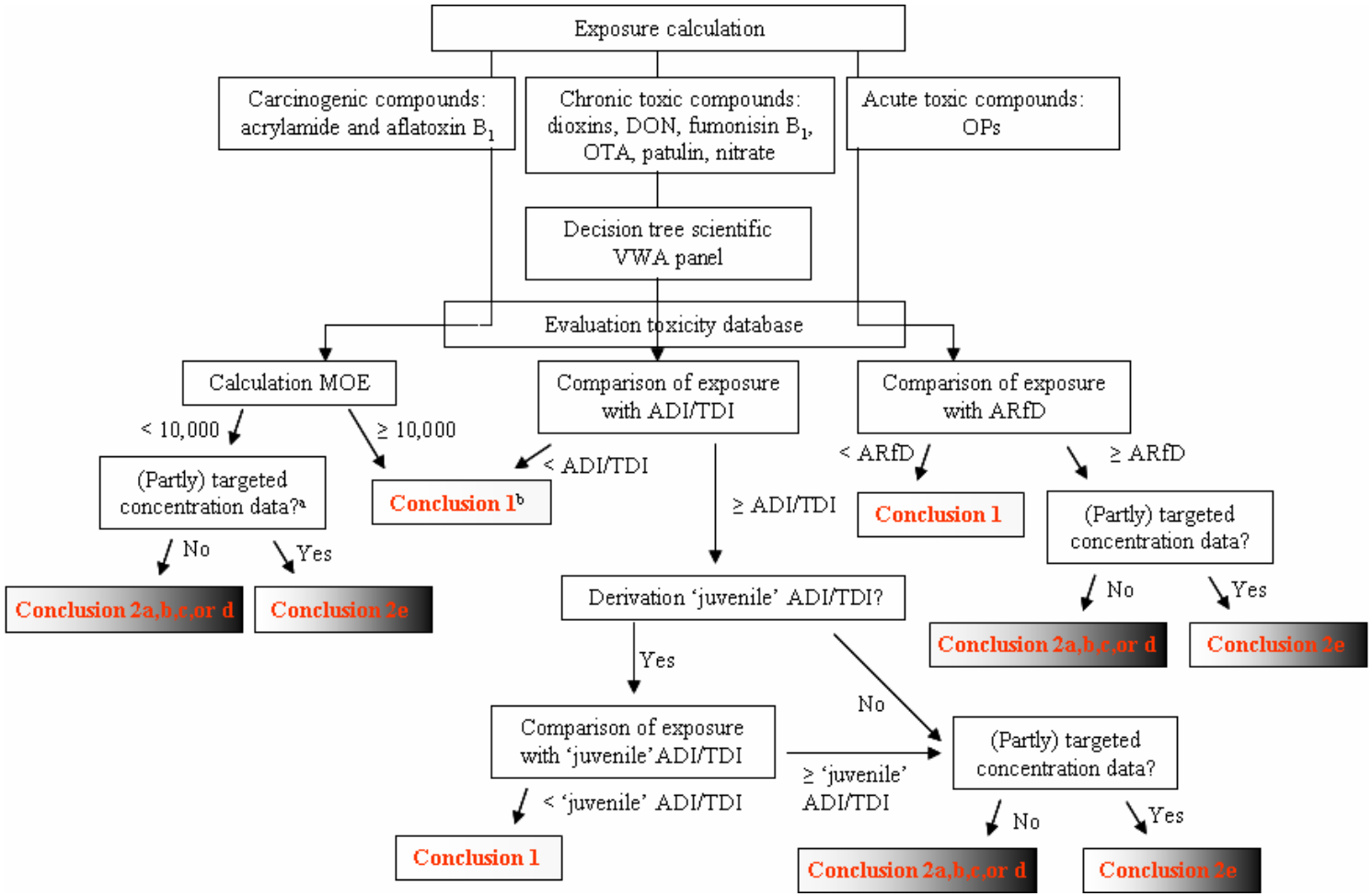

Figure 2-2. Flow diagram of the risk assessment. For more details, see section 2.7.2, and for an explanation of abbreviations used the Glossary.

[a] '(Partly) targeted concentration data' refers to the concentration data used to calculate the exposure to mycotoxins and pesticides and which may be (partly) biased to higher concentrations than those to which children are exposed in reality.

[b] Conclusion 1 = Negligible health risk; Conclusion 2a, b, c, or d = (Very) limited or considerable health risk, or there is a possible health risk, but the extent cannot be identified; Conclusion 2e = Determination of health risk is not feasible.

The less uncertainty there is in the data the higher the selected percentile can be.

For acute dietary exposure assessment (OPs), the US EPA (EPA, 2000a) advises the use of the P99.9 in probabilistic assessments of exposure to pesticides. The Board for the Authorisation of Pesticides in the Netherlands (CTB) followed this recommendation in their evaluation of pesticides using the probabilistic approach (CTB, 2001). Use of the P99.9 by both bodies is applicable to the regulation of pesticides, where consumption levels are combined with concentration data derived from experimental studies in which pesticides are applied to relevant crops. Which percentile to use when dealing with only monitoring concentration data, as in this study, has not been addressed anywhere so far. We therefore followed the recommendation of the US EPA and used the P99.9 to assess a possible health risk for OPs.

For long-term dietary exposure assessments (relevant for all chemicals addressed in this report except OPs) the VWA panel, via a footnote of the decision tree, observed that the P97.5 could be used as a reference point, stating that this level is applied in EFSA-opinions. However, they also stated that based on, for example, the compound another choice can be made.

Due to the extensive amount of consumption and concentration data available in the present study and because we are dealing here with young children, a vulnerable sub-group, a more conservative level of exposure, the P99, was chosen. When the P99 or P99.9 of exposure exceeded the corresponding HBLV the exact percentage of children exceeding this limit was calculated.

3 Dietary exposure and risk assessment of acrylamide

3.1 Introduction

When acrylamide was detected in foods heated at high temperatures in Sweden in 2002 (Tareke et al., 2002), this caused a worldwide concern, due to the classification of acrylamide as a group 2A carcinogen (probably carcinogenic to humans) (IARC, 1994). Subsequent research showed that acrylamide can be formed by heating of many starchy foods, such as French fries, biscuits and crisps. The compound is formed in the Maillard reaction (Mottram et al., 2002; Stadler et al., 2002). Precursors of acrylamide formation are asparagine and reducing sugars, such as fructose and glucose. The reaction only occurs at temperatures above 100°C (Friedman, 2003).

Since the detection of acrylamide, numerous actions have been, and are still, undertaken to reduce acrylamide concentrations in food. Reducing measures focus on several aspects, such as changing the baking agent, heating advices and selection of potato cultivar (Dybing et al., 2005; Stadler and Scholz, 2004). The CIAA (Confederation of the Food and Drink Industries of the EU) has developed a 'toolbox' approach, which provides a way to help food manufacturers to identify approaches to help control acrylamide in different types of foods. For example, several studies demonstrated that reducing temperatures during frying resulted in lower acrylamide concentrations in potato products (Gertz and Klostermann, 2002; Rydberg et al., 2003; Tareke et al., 2002; Taubert et al., 2004). Also the use of a different baking agent resulted in a reduction of acrylamide concentrations by more than 60 % in gingerbread (Amrein et al., 2004). However, apart from a positive effect on acrylamide concentration due to these (and other) changes in processing techniques, one should also be aware of possible negative side-effects of these changes, such as the increase in fat content at lower frying temperatures or possible loss of 'desired' Maillard products.

Due to the ongoing reduction measures, as well as the potential adverse health effects of acrylamide and high exposures, it is important to perform acrylamide exposure assessments at a regular basis and to use the most up-to-date acrylamide concentration data for this.

3.2 Exposure assessment

Exposure calculation
The median long-term exposure to acrylamide was 0.7 µg/kg bw/d (P95 = 1.2 µg/kg bw/d; Table 3-1). Of the children 1 % had an acrylamide exposure above 1.5 µg/kg bw/d. These results are based on the assumption that non-detects were assigned a concentration equal to ½LOR. Age dependency of the exposure was tested and no significant effect was observed. Chips (28 %), biscuits (18 %) and Dutch spiced cake (15 %) contributed most to the acrylamide intake in young Dutch children (Figure 3-1). The contribution of the food groups biscuits, cookies, spiced biscuits and children's biscuits together added up to 33 %.

Varying the concentration assigned to non-detect samples had a negligible effect on the exposure levels of acrylamide (Appendix E). This was mainly due to the limited number of samples with a concentration below LOR. Also the regrouping of the biscuits/cookies coded showed only a small decrease in exposure, 3-6 %, compared to the baseline calculations (Appendix J).

Table 3-1. Percentiles of long-term dietary exposure of children aged 2 to 6 years to acrylamide assuming samples with a concentration below LOR[a] to equal ½LOR.

Age (years)	Percentiles of exposure (µg/kg bw/d)		
	P50	P95	P99
2 -6	0.7	1.2	1.5

[a] LOR = Limit of reporting

Comparison with other studies

The exposure reported here is low compared to the most recent estimate of exposure to acrylamide in the Netherlands in small children (Boon et al., 2005). In that study, more than 10-year old food consumption data for 1- to 6-year olds derived from DNFCS-3 were combined with either European or only Dutch concentration data. The lower exposure is very likely not due to changes in dietary habits (Boon et al., 2009), but to decreased acrylamide concentrations in most food groups. A decrease in acrylamide concentration is brought about by different mitigation measures, such as replacement of the baking agent ammonium bicarbonate by sodium bicarbonate in ginger bread and frying French fries at 175°C (VWA, 2007a).

As in the 2005 estimate, chips contributed most to the intake of acrylamide. In the present estimate, crisps and bread were less important than in 2005, while presently biscuits and spiced cake have a larger share. To make a quantitative comparison between the present intake and the 2005 intake is however difficult since the dietary assessment methods are different. It can therefore not be excluded that observed differences are (partly) due to methodological differences in dietary assessment methods. Besides the European concentration data may not have been fully representative for the Dutch exposure in 2005.

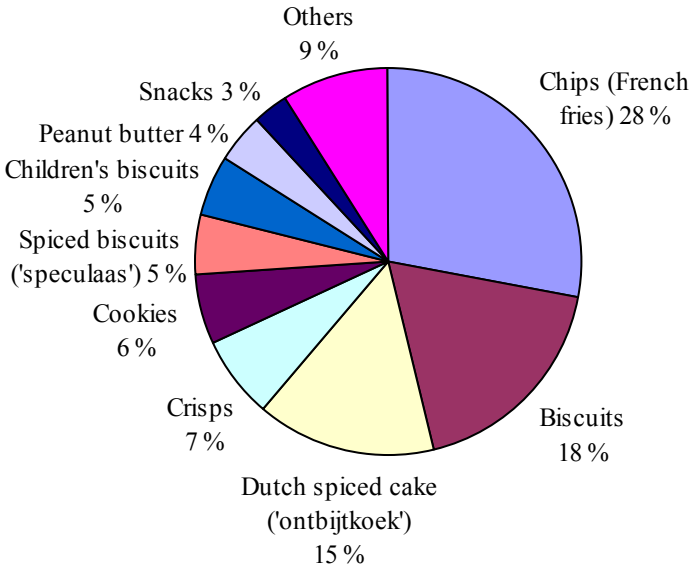

Figure 3-1. Contribution (%) of the most important food groups to the dietary exposure of children aged 2 to 6 years to acrylamide, assuming samples below the limit of reporting (LOR) to equal ½LOR.

Representativity of the concentration data
Acrylamide concentrations used in the assessment were the most up-to-date data available in the Netherlands. Due to mitigation measures it is important to have the most up-to-date data available for the assessment. In the analyses, all foods were included that had been shown to contain acrylamide in the analyses performed in 2002. It should be recognised that some other foods have incidentally been shown to contain acrylamide. Among these foods are olives, dried fruits (dried apricots and prunes) and popcorn (Dybing and Sanner, 2003). These foods were not included in the present calculations because no recent concentration data were available. As acrylamide concentrations in these foods are generally low (Dybing and Sanner, 2003) and these foods are not consumed regularly and/or in large amounts by young children, we do not expect that inclusion of the foods has affected the exposure significantly.

The level of acrylamide formation depends on the heating temperature and duration of heating, and the amount of asparagine and reducing sugars present in the food. The amount of asparagine and reducing sugars present in relevant ingredients of acrylamide containing foods (including potatoes, cereals and coffee beans), is in turn influenced by agronomical aspects like soil quality, cultivar, time of harvest and fertilizer use (Dybing et al., 2005). As a result of this, acrylamide concentrations can vary substantially between but also within food groups.

For some of the food groups the acrylamide concentration was based on less than five samples (Appendix A). Even though this number of samples is very likely too small to result in a reliable estimate of the acrylamide concentration, the concentrations were included in the analyses because the food groups concerned very likely contain acrylamide based on their characteristics or analyses performed in the past. Excluding them from the analyses resulted in a decrease in exposure of 10 % (results not shown).

In every food group different types and brands were analysed. It is assumed that these foods were representative for the foods coded in the food consumption survey belonging to the food group. Furthermore, of every food only one sample per brand was analysed, resulting in no information on the variability within a brand. This probably did not affect the intake estimate, because foods were aggregated in groups and it is likely that the variability within a brand or food type is smaller than the variability within a food group, as was shown for potato chips (Roach et al., 2003). Furthermore in long-term intake calculations the mean concentration per food group is used, because variability in concentrations level out in the long run.

3.3 Risk assessment

3.3.1 Toxicology

In experimental animals, acrylamide is rapidly and extensively absorbed from the gastrointestinal tract following oral administration. Acrylamide is metabolized to a chemically reactive epoxide, glycidamide. An alternate pathway for metabolism of acrylamide is conjugation with glutathione. The pivotal effects of acrylamide in humans are neurotoxicity and carcinogenity (EFSA, 2005d; FAO/WHO, 2006; SCF, 2002b). For a more elaborated toxicological profile of acrylamide, see Appendix K.

Neurotoxicity
Many studies conducted in a number of animal species and humans have shown that the nervous system is a principal site of acrylamide-toxicity. Repeated exposure to acrylamide causes degenerative peripheral nerve changes that result from an accumulation of damage at the sites of toxicity, as was

Table 3-2. Points of departure for the hazard assessment of acrylamide[a].

Study	Toxicological endpoint	Point of departure[b]	Reference
Subchronic study rats	Electron-microscopical morphological nerve changes	NOAEL: 0.2 mg/kg bw/d	(Burek et al., 1980)
Chronic study rats	Degenerative nerve changes	NOAEL: 0.5 mg/kg bw/d	(Friedman et al., 1995; Johnson et al., 1986)
Chronic study rats	Mammary tumours	$BMDL_{10}$: 0.30 mg/kg bw/d	(Johnson et al., 1986)
Chronic study rats	Thyroid follicular adenomas	$BMDL_{10}$: 0.63 mg/kg bw/d	(Friedman et al., 1995)

[a] Taken from JECFA (FAO/WHO, 2006).
[b] NOAEL = No-observed adverse effect level; $BMDL_{10}$ = 2.5 % lower confidence limit of the benchmark dose for a 10 % increase in cancer incidence.

shown in subacute and subchronic studies with rats. Continued dosing (subchronic and chronic studies) with acrylamide has been shown to induce nerve terminal degeneration in brain areas critical for learning, memory and other cognitive functions (*i.e.*, cerebral cortex, thalamus and hippocampus) and these lesions may precede the morphological changes in nerves. In rats exposed to acrylamide in drinking water for 90 days, the no-observed adverse effect level (NOAEL) for morphological changes in nerves detected using electron microscopy was 0.2 mg/kg bw/d.

Carcinogenicity
Although acrylamide did not show mutagenicity in the Ames Salmonella assay, glycidamide clearly did. Acrylamide is both clastogenic and mutagenic in mammalian cells *in vitro* and *in vivo*. In addition, dominant lethality studies have shown acrylamide to be a germ cell mutagen in male rodents. The mutational spectra produced by acrylamide and glycidamide in transgenic mouse cells are consistent with formation of pro-mutagenic purine DNA-adducts *in vivo*. Glycidamide is much more reactive than acrylamide with DNA and metabolism of acrylamide to glycidamide appears to be a prerequisite for the genotoxicity of acrylamide *in vitro* and in experimental animals.

Acrylamide was evaluated by the International Agency for Research on Cancer (IARC) in 1994 and classified as a group 2A carcinogen (probably carcinogenic to humans) (IARC group 2A), based on a positive cancer bioassay result and supported by evidence that acrylamide is efficiently biotransformed to a chemically reactive genotoxic metabolite, glycidamide, in both rodents and humans (IARC, 1994).

Health based limit values (HBLVs) for both neurotoxicity and carcinogenity were not derived due to the rather weak database. JECFA (Joint FAO/WHO Expert Committee on Food Additives) (FAO/WHO, 2006) analyzed the dose-response relationships in the studies by Johnson et al. (1986) and Friedman et al. (1995) to establish a point-of-departure for the calculation of margins of exposure (MOEs)[5]); this procedure was also accepted by EFSA (2005a). The NOAELs, BMDs and $BMDL_{10}$s (2.5 % lower confidence limit of the BMD for a 10 % increase in cancer incidence above the control) serving as these points of departure are summarized in Table 3-2.

[5] The MOE is defined as the point of comparison on the dose-response curve (usually based on animal experiments in the absence of human data) divided by the estimated exposure by humans.

Table 3-3. Percentiles of long-term dietary exposure to acrylamide and corresponding margins of exposure (MOE) for neurotoxicity and carcinogenicity.

	Percentiles of dietary exposure (μg/kg bw/d) and MOEs								
	P50			P95			P99 (L of CI)[a]		
(Sub)population	Exp[b]	MOEn[c]	MOEc[c]	Exp[b]	MOEn[c]	MOEc[c]	Exp[b]	MOEn[c]	MOEc[c]
Children (2 - 6 years)[d]	0.7	286	429	1.2	167	250	1.5 (1.7)	133 (118)	200 (176)
Total population (1 - 97 years)[e]	0.3	667	1,000	0.7	286	429	1.0	200	300

[a] L of CI = Limit of 95 % confidence interval. For exposure 97.5 % upper confidence limit, and for MOE 2.5 % lower confidence limit.
[b] Exp = Exposure
[c] MOEn = Margin of exposure for neurotoxicity (NOAEL = 0.2 mg/kg bw/d); MOEc = Margin of exposure for carcinogenicity ($BMDL_{10}$ = 0.30 mg/kg bw/d)
[d] This study
[e] Exposures from DNFCS-3 (Konings et al., 2003).

Observations in humans
Epidemiological studies of human industrial and accidental exposures suggest that the nervous system is a principal site for toxicity in humans. Epidemiological studies carried out in recent years on the general population have produced inconsistent results concerning carcinogenicity (Appendix K). Based on this the 11th EFSA colloquium on acrylamide in May 2008 concluded that acrylamide intake is not likely to be very strongly carcinogenic in humans (EFSA, 2008c).

Relevance for children
With respect to its neurotoxic effects and hence its effect on learning, memory and other cognitive functions (section 3.3.1), the toxicity of acrylamide is particularly relevant for children (and adolescents). With respect to the carcinogenic effects, in general long-term exposure is needed for cancer to develop. From this point of view the (possible) carcinogenicity of acrylamide is for children as relevant as for adults.

3.3.2 Overall risk assessment

To perform a risk assessment, the MOEs for children and adults for the P50, P95 and P99 (including its 97.5 % upper confidence limit; Appendix H) of exposure were calculated (Table 3-3). Based on the MOEs for neurotoxicity it can be concluded that neurotoxic effects are highly unlikely, even at the P99 of exposure: Although the database is not complete, the NOAELs for neurotoxicity in the two chronic studies were a factor 2.5 higher than the NOAEL for neurotoxicity from the subchronic study (Table 3-2). The risk assessment can thus be based on an MOE of 100 based on the latter NOAEL. Generally an MOE of 100 is considered high enough to preclude adverse toxic effects at any point in (human) life, including sensitive subpopulations (such as children) (EFSA, 2005a).

The MOEs for carcinogenicity, however, are below 10,000. In general, an MOE for carcinogenic effects should be that order of magnitude to ensure a reasonable margin of safety between a dose that causes a low but measurable response in an animal experiment and the dose representing an acceptable risk for humans (EFSA, 2005a). The MOE for children appears to be somewhat lower compared to the MOE for the total population (Table 3-3), but it should be noted that the MOEs for the total population include the childhood period and thus represent that for the average exposure during full lifetime. Obviously children have temporarily a lower MOE, which is increased at adulthood, although still low

compared to an MOE of 10,000. This is true for the whole group of children: the P1 of exposure (0.2 µg/kg bw/d) resulted in an MOE of 1,500.

Unfortunately the data do not allow any conclusion regarding the time it takes to develop cancer caused by acrylamide exposure. However, in the near future more toxicity/carcinogenicity data are to be expected from new animal studies. These data may be useful to establish the time needed to develop cancer, and thus to address the relevancy of a low MOE in relation to the cancer risk for children.

Overall it is concluded that due to the relatively small MOE for acrylamide there is a probability that an adverse health effect will occur related to carcinogenic effects. However, based on the current epidemiological studies (section 3.3.1) it is not possible to draw firm conclusions on the extent to which this could happen (conclusion 2d, section 2.7.2).

3.4 Conclusion and recommendations

Acrylamide can be formed by heating of many starchy foods, such as French fries, biscuits and crisps. The compound is formed in the Maillard reaction (Mottram et al., 2002; Stadler et al., 2002). Precursors of acrylamide formation are asparagine and reducing sugars, such as fructose and glucose. Since its detection, many studies have been performed to fathom the formation of acrylamide. Thanks to this knowledge, mitigation measures on acrylamide concentrations of foods have been developed resulting in a decrease in acrylamide levels since its discovery, and resulting exposure levels. Due to its genotoxic carcinogenity and the assumption that no threshold levels of these types of effect exist, exposure to the compound should be minimized as far as possible. It is therefore very important to keep on developing tools to decrease acrylamide concentrations further, such as the tool-box of CIAA. This was also concluded by JECFA. This committee arrived at an MOE of 300 for carcinogenity for an average intake level and at an MOE of 75 for a high percentile (P97.5) intake level (FAO/WHO, 2006). These two MOEs were judged to be low for a compound that is both genotoxic and carcinogenic. JECFA therefore indicated that there may be a human health concern, and that appropriate efforts to reduce concentrations of acrylamide in food and beverages should be continued. At the EFSA Colloquium on acrylamide, it was stated that additional mitigation measures earlier in the food chain may be possible (EFSA, 2008c). During this colloquium the conclusion of JECFA that acrylamide may be of health concern was felt to be still relevant. Results presented in this report are in line with these conclusions

However, as described by Seal et al. (2008), a decrease of acrylamide exposure due to lower acrylamide levels in foods may not only result in a decrease in risk, but may also be associated with a loss in benefit or an increase in another risk. In this paper, an example was given of using sodium bicarbonate as a baking agent instead of ammonium bicarbonate, resulting in a decrease in acrylamide exposure (from 0.41 to 0.31 µg/kg bw/d). However, also the intake of sodium was affected, namely slightly shifted up (from 42 to 44 mg/kg bw/d). Whether these changes, due to the complexity of translating changes in intake levels of sodium in terms of higher incidence of high blood pressure (sodium) and that of acrylamide in a lower number of cancer cases are also significant, is unclear and uncertain. However, this example shows very clearly that lowering acrylamide concentrations should be viewed in combination with possible, not-desired, side effects. Another example is the reduction of frying temperatures of French fries resulting in an increase in fat content, which is undesirable in view of increased incidence in obesity, both in the Netherlands and world wide. These undesirable side-effects (risk) should be considered when reducing acrylamide concentrations in foods (benefit). Models to perform such analyses (so-called risk-benefit analyses) are available (Seal et al., 2008; van der Voet et al., 2007).

A relatively low MOE for acrylamide was derived, showing that the health risk related to the exposure to acrylamide in young children living in the Netherlands may not be negligible. It was therefore concluded that there is a probability that an adverse health effect will occur given the calculated exposure levels of acrylamide in this age group. However, due to inconsistent results concerning carcinogenicity of acrylamide in epidemiological studies, it is not possible to draw firm conclusions on the extent to which this could happen. For this, data on the toxicity of acrylamide need to be generated.

Recommendations

To quantify the possible health risk related to the exposure to acrylamide in young Dutch children we recommend to obtain a better understanding of the toxicological effect of acrylamide, taking into account that epidemiological studies do not show that acrylamide is a strong carcinogenic in humans.

Furthermore we recommend:

- to improve the exposure assessment by increasing the number of analyses of all food groups, but especially of those with less than 10 samples and relatively high acrylamide concentrations, including children's cookies, crisps, peanut butter, breakfast cereals and chocolate (Appendix B);
- to perform risk-benefit analyses where possible adverse side-effects (risk) of reductions in acrylamide levels in foods (benefit) are addressed.

4 Dietary exposure and risk assessment of dioxins

4.1 Introduction

For this report the collective term dioxins encompasses the following groups of compounds: polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), mono-ortho polychlorinated biphenyls (mo-PCBs) and non-ortho polychlorinated biphenyls (no-PCBs). Dioxins are omnipresent in the environment, they degrade slowly, are lipophilic and bioaccumulate. PCBs were produced in large quantities until the late 1970s. Nowadays use of PCBs is prohibited in many countries, only in some countries their use is still allowed in closed systems like transformers. Environmental contamination with PCBs mainly occurred by waste disposal, resulting in regulations for safe PCB waste removal (EC, 2006a). PCDDs and PCDFs are formed as a result of combustion processes, such as waste incineration, and production of chemicals and power. Contamination of foods with dioxins occurs through deposition of contaminated emissions on farmland and uptake by farm animals, while fish accumulate dioxins through contamination of the aquatic environment (Baars et al., 2004; EC, 2006a; SCF, 2001).

4.2 Exposure assessment

Exposure calculation
The median dietary exposure of children to dioxins was estimated at 1.5 pg TEQ/kg bw/d for 2-year olds and 1.2 pg TEQ/kg bw/d for 6-year olds (Table 4-1). The exposure at the P99 was 2.8 and 2.3 pg TEQ/kg bw/d, respectively. The foods that contributed most to total dioxin exposure are presented in Figure 4-1. The main foods are cheese, milk, beef and vegetable oils, making up over 75 % of the exposure.

The dioxin exposure estimates assigning ½LOR were 20 % higher compared to the lower bound scenario estimate (Appendix E). Earlier calculations showed that exposure estimates, in which ½LOR was assigned to all congeners with a value below LOR, including vegetables, fruits and cereals, were about twice as high than the lower bound scenario estimates. Although it is likely that all foods are contaminated by dioxins during growth and/or production, due to the presence of dioxins in soil, air and surface water, it is uncertain which of the scenarios is closest to the real situation. US Food and

Table 4-1. Percentiles of long-term dietary exposure of children to dioxins as a function of age, assuming congener concentrations below LOR[a] to equal ½LOR, except for vegetables, fruits and cereals for which congener concentrations below LOR were assumed to be zero.

Age (years)	Percentiles of exposure (pg TEQ/kg bw/d)		
	P50	P95	P99
2	1.5	2.4	2.8
3	1.4	2.2	2.7
4	1.3	2.1	2.5
5	1.2	2.0	2.4
6	1.2	1.9	2.3

[a] LOR = Limit of reporting

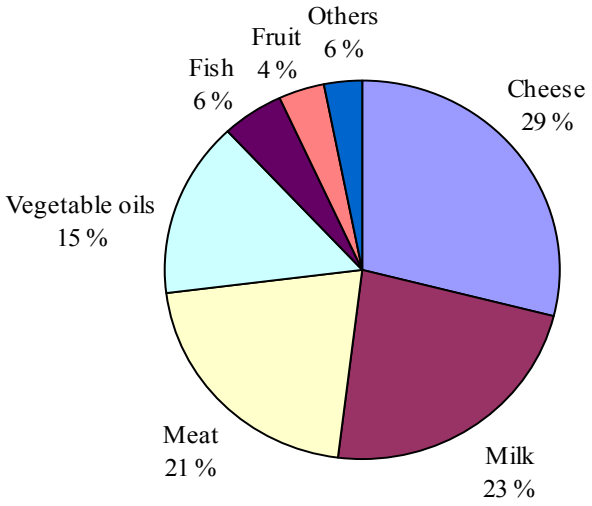

Figure 4-1. Contribution (%) of the most important food groups to the long-term dietary exposure of children aged 2 to 6 years to dioxins in which congener concentrations below limit of reporting (LOR) were equalled to ½LOR, except for vegetables, fruits and cereals for which congener concentrations below LOR were assumed to be zero.

Drugs Administration stated that assigning ½LOR to congeners with a concentration below LOR for vegetables and fruits results in overestimates of exposure because concentrations of dioxins are low in these foods (FDA, 2004). We therefore assumed congener concentrations of dioxins in vegetables, fruit and cereal samples below LOR to be zero. To circumvent this uncertainty in the future the limit of quantification of the analytical method should be decreased and sample size should be increased.

Comparison with other studies

The most recent published exposure calculations of dioxins in Dutch children reported a median intake for 2-year olds of 2.8 pg TEQ/kg bw/d (Baars et al., 2004). These exposure levels were estimated using dioxin concentrations analysed in 1999 combined with food consumption data of DNFCS-3. The reported median intake in the present report is almost a factor two lower. This is consistent with the known decrease in dioxin concentrations in the environment, resulting in lower exposure levels (Huwe, 2002). Also de Mul et al. (2008) showed a declining trend in median dioxin exposure was reported from around 9 pg TEQ/kg bw/d in the late seventies to 0.7 pg TEQ/kg bw/d in 2004 for a 40-year old adult. A confounding factor in comparing the present results with those reported by Baars et al. (2004) is the use of the recently re-evaluated TEFs in the present study. These TEFs differ primarily from the old values of 1998 in the reduction of the TEFs for mo-PCBs and in the adjustment of the TEF values to half-log values (before 0.5, now 0.3). An exposure assessment of dioxins using the old and new TEF values showed that the exposure calculated with the new TEFs was about 10 % lower than the estimate using the old TEFs. In the report on the re-evaluation of the TEFs (van den Berg et al., 2006), a decrease of 10 to 25 % was observed in exposure and concentrations. Taking the use of different TEFs into account we can still state that the current exposure to dioxins in 2-year olds has decreased since the last exposure estimate.

It was shown that in small children especially cheese and milk contributed largely to the total exposure to dioxins, together 52 %, followed by meat (21 %) and vegetable oils (15 %). Due to lack of information on contribution of foods to the exposure in small children, the present results were compared to those recently reported in the total Dutch population (de Mul et al., 2008). In this study, dioxin concentrations of 2004 were combined with food consumption data of the DNFCS-3. Also in

this study dairy products (including milk (products) and cheese) contributed most to the exposure, namely 31 %, followed by meat and vegetable oils (both 17 %). The higher contribution of dairy products in the present study compared to the study of de Mul et al. (2008), 52 vs. 31 %, is very likely due to differences in eating habits between young children and the general Dutch population (higher consumption levels of milk). In the Netherlands, exposure to dioxins in small children could be reduced most by (further) decreasing dioxin concentrations in milk and cheese. Another strategy could be a reduction in consumption levels of these products to bring levels closer to recommended levels. As reported by Ocké et al. (2008), Dutch children aged 2 to 3 years consume on average more milk products than the recommended level (438 g/d vs. 300 g/d), while children aged 4 to 6 years tend to consume a little more cheese than recommended for this age group (12 vs. 10 g/d).

Representativity of concentration data
Dioxin concentrations used in the assessment were the most up-to-date data available in the Netherlands. The selection of samples, the use of composite samples and the low number of composite samples (Appendix A) introduce uncertainty in the calculation. This choice of sampling is based on the available resources (measurements are expensive) and chronic toxicity of dioxins. For most food groups, more than one composite sample was analyzed. Due to this way of sampling quantification of the variability (and uncertainty) in the dioxin concentrations is not possible. However, we expect this variability (and uncertainty) to be substantial, and therefore a relevant point for future research could be the analysis of individual food items or, to reduce costs, to analyse more (composite) samples.

For a few food groups no samples were analysed, thus concentrations for these products had to be estimated based on concentrations in other food groups (Appendix D). This was the case for vegetables, fruits, vegetable oils and some fish types.

Effect of processing
The effect of cooking on dioxin concentrations was not integrated in the current exposure assessment, and to our knowledge also not in any other exposure assessment. Only limited research has so far been conducted to study the effect of the processing on dioxin concentrations. However the results vary and are inconsistent. Hori et al. (2005) compared dioxin concentrations in fish and meat before and after preparation and concluded that cooking practices decrease dioxin concentrations in animal products. Their experiment showed that the reduction varied from 14 to 40 % depending on the type of fish and meat and the cooking method used. Zabik and Zabik (1995) showed a decrease of TCDD by 30 to 50 % with several cooking techniques and a 100 % reduction with smoking. Another study showed an increase of dioxin concentrations with the smoking of eel (van Leeuwen et al., 2007). Rose et al. (2001) studied the effect of different cooking methods of meat dosed with five dioxin congeners, and concluded that the changes in dioxin concentrations were due to water loss and leakage of fat. The total amount of dioxins in meat plus fat did not, however, change. Because of these highly variable effects and information on only a limited number of foods, processing effects were not included in the present calculation. For more precise exposure estimates in the future, information on processing effects in more (cooked) consumed foods is necessary.

4.3 Risk assessment

4.3.1 Toxicology

Toxicokinetics
Dioxins have a very long half-life and thus accumulate in the human body. The biochemical and toxicological effects of these compounds are therefore directly related to their concentrations in tissues,

and not to the daily ingested dose. The most appropriate measure of dose would therefore be the concentration at the target tissue; however, this is seldom known. The body burden is strongly correlated with the concentrations in tissue and serum, and is therefore a more appropriate measure of dose than the daily dose.

The long half-life of dioxins has several implications for the period of intake that is relevant for a risk assessment. First, the concentration of toxic equivalents (TEQs) in the body will increase over time as more of the compounds is ingested. Second, after cessation of exposure, the body's concentration of stored TEQs will decline slowly (only half of the accumulated TEQs disappears over a period of about seven years), resulting in a pseudo-steady state only after decades. Third, because of this long-term storage in the body and the consequent daily exposure to the body's stored TEQs, intake on a particular day will have a small or even negligible effect on the overall body burden. Hence, the appropriate period for evaluating the average intake of these compounds is at least a week (SCF, 2000c, 2001) or a month (FAO/WHO, 2001a). For a more elaborated toxicological profile of dioxins, see Appendix L.

Most sensitive effects
The most sensitive adverse effects of dioxins are on rat offspring development and immunological deficits in the same species after prenatal exposure to TCDD. Animal studies provide evidence that adverse effects occur on the reproductive system in male offspring of pregnant rats given TCDD. The studies show reductions in daily sperm production and other effects on the reproductive system in male offspring associated with maternal steady-state body burdens of TCDD of $\geq$ 25 ng/kg bw. More detailed information on these studies can be found in Appendix L.

Health based limit value
On the basis of the available toxicity data, a tolerable intake can be derived for TCDD on the basis of the assumption that there is a threshold for all effects, including cancer (Appendix L). The lowest observed adverse effect level (LOAEL) and NOAEL were provided by studies of Faqi et al. (1998) and Ohsako et al. (2001), respectively. Applying appropriate toxicokinetic conversions and assessment factors (see Appendix L for more details), these levels result in a provisional tolerable daily intake (PTDI) of 2.5 to 3.1 pg/kg bw/d based on the NOAEL, and 1.5 to 2.1 pg/kg bw/d based on the LOAEL.

Scientific Committee on Food (SCF) chose the midpoint of these ranges expressed on a weekly basis, and arrived at a provisional tolerable weekly intake (PTWI) of 14 pg/kg bw (per week) (SCF, 2000c, 2001). JECFA (FAO/WHO, 2001a) chose the midpoint expressed on a monthly basis, resulting in a provisional tolerable monthly intake (PTMI) of 70 pg/kg bw (per month). Both organisations concluded that these tolerable intakes should be applied to intake of PCDDs, PCDFs and coplanar PCBs, expressed as TEQs (see section 2.4 for an explanation of the TEQ concept).

For pragmatic reasons a PTDI of 2 pg TEQ/kg bw/d was used in, for example, exposure calculations, if it is kept in mind that an incidental high intake on a particular day will have a small or even negligible effect on the overall body burden. This PTDI is sufficiently low to avoid body burdens to rise above the level at which the toxicity of dioxins can come to expression at any time in life.

Relevance for children
The toxicological effects of dioxins are directly related to their concentrations in tissues, and not to the daily ingested dose. Since a child's body burden is lower than the body burden of an adult (due to the accumulating properties of dioxins), children are not more sensitive to dioxin toxicity than adults. The most critical toxic effects of dioxins are on the offspring, which causes women in the reproductive age (*i.e.*, the unborn child) to be the most sensitive subpopulation.

Table 4-2. Percentage of children with a long-term dietary exposure above the provisional tolerable daily intake (pTDI; 2 pg TEQ/kg bw/d) as a function of age, assuming congener concentrations below LOR[a] to equal ½LOR, except for vegetables, fruits and cereals for which congener levels below LOR were assumed to be zero.

Age (years)	Percentage of children exceeding pTDI
2	14
3	10
4	7
5	5
6	3

[a] LOR = Limit of reporting

However, the accumulation of dioxins is particularly high during childhood, firstly because the intake of dioxins per kg bw via the food in childhood is higher compared to adults (due to higher energy intake), and secondly because the biotransformation and excretion of dioxins in children is not yet fully developed. As a result, the rate of body burden increase is high during the first years of life, and slows down during puberty, adolescence and adulthood. From this point of view any attempt to decrease the exposure to dioxins during the first years of life will contribute substantially to a lower body burden later in life, and thus to a decrease in the risk for dioxin toxicity at higher ages.

4.3.2 Overall risk assessment

To perform a risk assessment the percentage of children exceeding the PTDI (2 pg TEQ/kg bw/d) was calculated. The results (Table 4-2) show that 3 % of the 6-year olds up to 14 % of the 2-year olds exceeded this level: the P95 of exposure is 2.4, and the P99 2.8 pg TEQ/kg bw/d (with a 97.5 % upper confidence limit of the latter percentile of 3.4 pg TEQ/kg bw/d; Appendix H). The PTDI was exceeded with less than a factor two. Therefore, we studied whether exceeding the HBLV with a factor two or less could be accepted (section 2.7). However, the PTDI of dioxins is based on studies in which mother animals were considered and not young animals given a constant (*i.e.,* not corrected for bw) dose. An additional safety factor of two is therefore not present in the PTDI.

Since the PTDI is exceeded, a refinement of the exposure and/or hazard assessment is recommended. Refinement of the exposure assessment by using a more sophisticated approach for assigning concentrations to non-detects will not very likely reduce the exposure very much, since the lower bound scenario already resulted in an exceedance of the TDI for the P99 in 2-year olds (Appendix E). Obtaining reliable data on processing would be a better option to refine the exposure assessment. Refining the hazard assessment by performing a BMD analysis would be difficult for dioxins, as the relevant toxicity studies (Faqi et al., 1998; Ohsako et al., 2001) do not show a dose-response effect. (Note also that for many young children the dioxin body burden is driven by the exposure during the period of breast feeding).

The estimated exposure is not much higher than the PTDI and will not result in any toxicity during childhood. However, levels exposed to during this period will add to the total body burden accumulated during whole life.

4.4 Conclusion and recommendations

Dioxins are environmental contaminants present in the environment. Efforts to reduce the levels have been made and are still continuing, resulting in lower exposure and body burden levels (de Mul et al., 2008). Also this study showed lower exposure levels compared to a study performed in 2004 using dioxin concentrations analysed in 1999, which is in line with the expectations. Nevertheless, a certain percentage of the children still exceeded the PTDI of 2 pg TEQ/kg bw/d (3 % of the 6-year olds up to 14 % of the 2-year olds). It should however be realised that the PTDI is a long-term toxicological reference value. This value protects humans from accumulating dioxins to body burden levels that may result in adverse effects. As long as high levels encountered in early life are balanced with lower levels when growing into adulthood there may not be a health risk. This is generally thought to be the case as it is known for numerous compounds that the intake decreases with age. Overall we conclude that, although the exposure to dioxins exceeded the PTDI, based on the available information the probability that an adverse health effect occurs is limited (conclusion 2b, section 2.7.2).

Recommendations
We recommend refining the exposure assessment by generating more information on the effect of processing on dioxin concentrations in food.

5 Dietary exposure and risk assessment of selected mycotoxins

5.1 Introduction

Mycotoxins are low-molecular weight toxins made by moulds (microfungi), which are toxic to animals and humans[6] in low concentrations. Due to their diverse chemical structures mycotoxins have very different biological effects. These toxins can appear in the food chain as a result of fungal infection of crops, and greatly resist decomposition or being broken down in digestion. Even temperature treatments, such as cooking and freezing, do not destroy them. Methods for controlling mycotoxins are largely preventive. They include good agricultural practice and sufficient drying of crops after harvest. Since fungi are present on foods by nature, formation of mycotoxins is often unavoidable. Minimisation of the contamination of foods by mycotoxins is mostly by screening and subsequently removal of non-compliant crops from the market. The mycotoxins included in the present study are aflatoxin B_1, deoxynivalenol, fumonisin B_1, ochratoxin A and patulin.

Aflatoxin B_1
Aflatoxin B_1 is a mycotoxin that is produced by moulds of the *Aspergillus* species, which contaminate plants and plant products, especially in areas of the world with hot, humid climates. Though a wide range of agricultural products may be contaminated with aflatoxin B_1, it is most commonly associated with maize, groundnuts, dried fruit, tree nuts, spices, figs, crude vegetable oils, cocoa beans, rice and copra. The hydroxylated metabolite of aflatoxin B_1, aflatoxin M_1 may be found in milk or milk products obtained from livestock that has ingested contaminated feed. Various other aflatoxins have been described, especially as mammalian biotransformation products of the major metabolites. However, aflatoxin B_1 is the most frequent aflatoxin present in contaminated products (Bennett and Klich, 2003). The IARC has classified aflatoxin B_1 as a (group 1) carcinogen (carcinogenic to humans) (IARC, 1993, 2002). The carcinogenic potency of aflatoxin M_1 is probably one or even two orders of magnitude lower than that of aflatoxin B_1 (Bennett and Klich, 2003; EFSA, 2004b; FAO/WHO, 2001b) as a conservative estimate, considered the potency of aflatoxin M_1 to be 10 % that of aflatoxin B_1

Deoxynivalenol
Deoxynivalenol (DON) is a mycotoxin belonging to the trichothecenes, a family of closely related compounds. This mycotoxin is produced by several plant pathogenic fungi, of which the *Fusarium* family is the most important. The geographical distribution of the *Fusarium* species appears to be related to temperature. DON is one of the most common mycotoxins found in cereals and grains such as barley, maize, oats, rice, rye, wheat. When ingested in high doses by agricultural animals it causes nausea, vomiting and diarrhea. Therefore DON is sometimes called vomitoxin or food refusal factor (Bennett and Klich, 2003; FAO/WHO, 2001b).

In 1998 and 1999, wheat contained high concentrations of DON in the Netherlands, resulting in high exposure levels. After the detection of high DON concentrations the Dutch government enforced national action limits. Separate from this, the primary sector and the grain processing industry took several contamination-reducing measures such as checking plant material for fungal contamination, improving methods for cultivation, harvest and storage, and removing contaminated lots from the food

[6] Fungal products that are mainly toxic to bacteria are called antibiotics, while those that are toxic to plants are called phytotoxins.

production process. As a result, the DON contamination of wheat was reduced by 55 %. The reduction of the DON concentration in some other foods was even higher (up to 90 % for children's food) (Pieters et al., 2004).

Fumonisin B_1
Fumonisin B_1 is the most prevalent member of a family of toxins produced by several species *Fusarium* moulds. Maize is the most significant crop in which fumonisins can be found. Fumonisins are formed in maize before harvest or during the early stage of drying. Infrequently fumonisins also occur in other foods, such as wheat, sorghum, asparagus, rice, mung beans, tea and cowpea (Bakker et al., 2003; EFSA, 2005b). Fumonisin B_1 on foodstuffs is generally accompanied by fumonisins B_2 and B_3 in a ratio of about 8:3:1 (Sydenham et al., 1993). The intake of fumonisin B_1 should therefore be increased with 50 % to obtain the intake of all three fumonisins together (Bakker et al., 2003).

Ochratoxin A
Ochratoxin A (OTA) is a mycotoxin that is produced by moulds of the *Aspergillus* and *Penicillium* (EFSA, 2004a). Materials used as animal feed, such as the cereals rye, barley, maize and wheat, are regularly contaminated by OTA. The products peanuts and soybean are also frequently contaminated with the toxin, but in lesser degree. Contaminations of green coffee and grapes with OTA, is often related to *A. ochraceus* (FAO/WHO, 2001b). The above mentioned products are the major sources of human OTA exposure (FAO/WHO, 2001b; Murphy et al., 2006). OTA production usually occurs due to poor and inadequate drying of cereals prior storage. Poor storage conditions attribute to the contamination by creating 'hot-spots' (EFSA, 2004a). The toxin is rather stable and degrades only partially under normal, cooking, roasting and fermenting processes (EFSA, 2004a; Murphy et al., 2006; van der Stegen et al., 2001; Yiannikouris and Jouany, 2002).

Patulin
Patulin is a mycotoxin produced by fungi belonging to several genera, including *Penicillum* and *Aspergillus*. It has been mainly isolated from apples and apple products (apple juice, apple sauce). The intake of patulin occurs via the consumption of processed fruit. The consumption of raw fruit is not an important source, since fruit affected by moulds will be discarded by the consumer.

5.2 Exposure assessment

5.2.1 Aflatoxin B_1

Exposure calculation
The calculations showed that more than 99 % of the children in the survey had a positive intake of aflatoxin B_1 on both days. The exposure percentiles for the whole population of 2-to 6-year olds demonstrate that the P99 was about 30 % higher than the median (Table 5-1). The main contribution to the daily intake of aflatoxin B_1 was caused by the consumption of nuts, peanut butter, maize, sunflower seed and rice (Figure 5-1). The contribution of milk was 1 % (Note that it was assumed that the potency of aflatoxin M_1 was 0.1 of that of aflatoxin B_1, which is a conservative estimate (Bennett and Klich, 2003; EFSA, 2004b; FAO/WHO, 2001b).

Varying the concentrations of the non-detects had a considerable effect on the exposure to aflatoxin B_1: the intakes of the middle bound scenario were about 50 % higher than those of the lower bound scenario (Appendix E). This was due to a large fraction of non-detects for aflatoxin B_1 (Appendix A). For nuts and milk, about one out of eight samples had a level above LOR, while for peanut butter this was about one out of 20.

Table 5-1. Percentiles of long-term dietary exposure of children aged 2 to 6 years to different mycotoxins assuming samples below LOR[a] to equal ½LOR.

Age (years) and mycotoxin	Percentiles of exposure		
	P50	P95	P99
Aflatoxin B_1 (in ng/kg bw/d)			
2-6[b]	0.8	1.9	2.7
DON (in µg/kg bw/d)			
2	0.3	0.5	0.6
3	0.3	0.4	0.5
4	0.3	0.4	0.5
5	0.3	0.4	0.5
6	0.2	0.4	0.4
Fumonisin B_1 (in µg/kg bw/d)			
2-6[b]	0.3	0.7	1.0
OTA (in ng/kg bw/d)			
2-6[b]	11	25	32
Patulin (in µg/kg bw/d)			
2-6[b]	0.03	0.1	0.2

[a] LOR = Limit of reporting
[b] Due to multimodal distribution of daily exposure, the ISUF method was used to model long-term exposure (section 2.6). This approach does not provide information on the age dependency of exposure.

Comparison with other studies
The EFSA has published an opinion on aflatoxin B_1 (EFSA, 2007b). The EFSA used the GEMS/Food consumption cluster diets database to estimate the intake of aflatoxin B_1 via the consumption of nuts and other foods. Analytical results on the occurrence of aflatoxins were submitted from 22 EU Member States in response to a call for information issued by the European Commission. For children the EFSA used German consumption data (Banasiak et al., 2005) covering children aged 2 to 4 years. Using these data EFSA calculated a lower (< LOR = 0) and upper (< LOR = LOR) bound median exposure of total aflatoxins of 0.6 and 1.1 ng/kg bw/d, respectively. These values are similar to the values presented here (P50 of 0.5 and 1.0 ng/kg bw/d for the lower and upper bound scenario, respectively, see Appendix E).

In a duplicate diet study performed at the RIVM in 2006, aflatoxin B_1 was analysed in 123 duplicate diet samples of children aged 2 to 6 years (van Egmond, 2007a). In 54 % of the samples, aflatoxin B_1 was present at concentrations below the limit of quantification (LOQ; 5 ng/kg), and in 35 % the concentration was between the limit of detection (LOD; 1.5 ng/kg) and LOQ. The aflatoxin B_1 intakes that could be calculated based on the concentrations present in the samples with a level above the LOD ranged from 0.06 to 0.4 ng/kg bw/d. This is considerably lower than the estimated intakes in the present study in which the P50 of exposure was equal to 0.8 ng/kg bw/d (Table 5-1). The concentration of aflatoxin M_1 analysed in all duplicate diet samples was below the LOQ (23 ng/kg), and in only 10 % the concentration was between the LOD (5 ng/kg) and LOQ. Thus, the intake of aflatoxin M_1 by these children was very low.

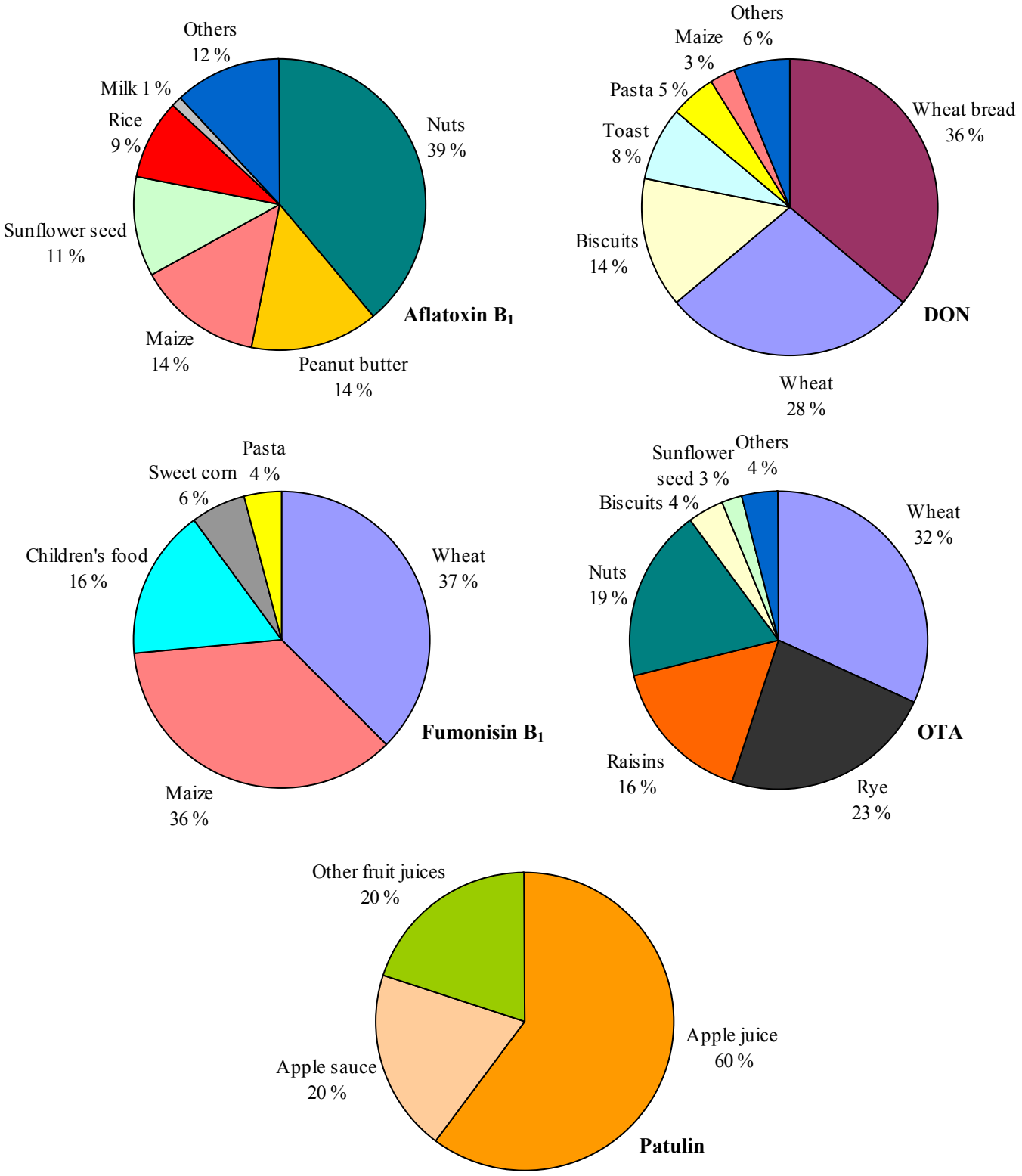

Figure 5-1. Contribution (%) of the most important food groups to the long-term dietary exposure of children aged 2 to 6 years to different mycotoxins. Samples with levels below the limit of reporting (LOR) were assigned ½LOR. For more details, see text. Food group 'children's food' refers to porridge and children's food in jars.

Table 5-2. Median long-term dietary exposure (µg/kg bw/d) of children to DON as a function of age by Pieters et al. (2001, 2004) assuming samples below LOR[a] to equal ½ LOR. Between brackets the 95th percentiles of exposure are reported.

Age (years)	Median exposure (µg/kg bw/d)	
	Survey 1[b] 1998-1999	Survey2[c] 2000-2002
2	1.3 (2.4)	0.4 (0.9)
3	1.1 (2.2)	0.4 (0.8)
4	1.0 (2.0)	0.3 (0.7)
5	1.0 (1.8)	0.3 (0.7)
6	0.9 (1.7)	0.3 (0.6)

[a] LOR = Limit of reporting
[b] Pieters et al. (2001)
[c] Pieters et al. (2004)

5.2.2 Deoxynivalenol (DON)

Exposure calculation
According to the calculations, more than 99 % of the children in the survey had an intake of DON on both days. The calculated long-term exposure percentiles varied around 0.3 µg/kg bw/d (P50) up to 0.5 µg/kg bw/d (P99), decreasing by about 20 % from the age of 2 to 6 years (Table 5-1). The main foods contributing to the intake of DON were wheat and wheat bread (Figure 5-1).

Assigning the value of ½LOR to the non-detects resulted in exposures which were about a factor two higher than those of the lower bound scenario (Appendix E). Also here this was due to a relatively high fraction of samples with a level below LOR for DON: one out of five samples (Appendix A).

Comparison with other studies
Two previous surveys on the exposure to DON in the Netherlands have been carried out, one with concentration data from 1998/1999 (Pieters et al., 2001) and one with concentration data from 2000-2002 (Pieters et al., 2004). In both studies, consumption data of DNFCS-3 were used. In the 2004 study, the exposure to DON had declined to about 30 % of the 2001 study due to a decrease in DON-concentrations in wheat. Unfortunately, a trend analysis with previous findings in the Netherlands cannot be made, as the method of dietary assessment differs and a calibration study has not (yet) been conducted. However a crude comparison shows that the exposure estimated in the present study is in the same order of magnitude as in the study of Pieters et al. (2004) using DON concentration data from 2000-2002 (Table 5-2).

In another study on the dietary exposure to DON, duplicate diet portions collected in a Dutch infant study were analysed for DON to estimate the true exposure in infants aged 8 to 12 months. To this end the diets of 74 infants with the highest cereal consumption levels were analysed for DON (Schothorst et al., 2005). The duplicate diet study resulted in a (short-term) median daily exposure to DON of 0.7 µg/kg bw/d, while intake calculations using the food diaries of the baby study and concentration data of 2000-2002 resulted in an exposure of 0.4 µg/kg bw/d (Schothorst et al., 2005). The results of the infant study do not differ much from the values of the present study.

Calculated average intakes of DON in the SCOOP report on *Fusarium* toxins show that the intake of young children in Germany (average body weight of 20 kg) was comparable to that of Dutch children (average body weight of 17 kg), namely 1.0 and 0.8 µg/kg bw/d respectively (SCOOP, 2003). The calculated intake of children from the UK was lower, namely 0.5 µg/kg bw/d. Intakes were calculated as mean consumption × mean concentration divided by mean body weight and are therefore different from the results presented here.

In the RIVM children's duplicate diet study of 2006, DON was detected in all 123 samples (van Egmond, 2007b). The resulting intakes were 0.26 ng/kg bw/d (P50) and 0.5 ng/kg bw/d (P90), while the maximum intake was 0.8 ng/kg bw/d. These values are similar to the intakes estimated in the present study (Table 5-1).

5.2.3 Fumonisin B_1

Exposure calculation
The calculated exposure to fumonisin B_1 of 2- to 6-year olds ranged from 0.3 µg/kg bw/ d (P50) to 1.0 µg/kg bw/d (P99; Table 5-1) The main food groups contributing to the exposure were maize, sweet corn, children's food (*i.e.,* porridge and children's food in jars) and pasta (Figure 5-1).

Assigning concentrations of 0, ½LOR or LOR to the non-detects had a considerable effect on the exposure (up to a factor four for the P50, Appendix E). This was mainly due to a high fraction of non-detects for analysed foods other than maize and sweet corn (Appendix A).

Comparison with other studies
In a previous study into the exposure to fumonisin B_1 in the Netherlands Dutch concentration data on maize (from 1997 and 2000) and data on maize and other cereals reported in the EU (SCOOP, 2003) were combined with consumption data of DNFCS-3 (Bakker et al. 2003). Bakker et al. (2003) calculated a median intake of 0.09 µg/kg bw/d for 1-year old children (P99: 0.26 µg/kg bw/d), using ½LOR for maize, wheat and rice. A true comparison of the study of Bakker et al. (2003) with the present study cannot be made, due to the differences in methodology in the two consumption surveys used in the different studies (see also section 5.2.2). A crude comparison indicates that the intakes in the previous study were somewhat lower than the calculated exposures reported here (P50 of 0.26 and P99 of 1.05 µg/kg bw/d). This difference in exposure is likely due to higher concentrations of fumonisin B_1 in maize in the present study (1350 µg/kg vs. 360 µg/kg).

In the RIVM children's duplicate diet study of 2006, fumonisin B_1 was only detected in 28 % of the 123 samples (van Egmond, 2008). The resulting estimated intakes of the positive samples varied between 8 and 288 ng/kg bw/d. The intakes estimated in the present study were higher: the maximum intake of the duplicate diet samples corresponds with the P50 of the present study.

5.2.4 Ochratoxin A (OTA)

Exposure calculation
The calculations showed that more than 99 % of the children in the survey had a positive intake of OTA on both days. The results of the intake calculations are listed in Table 5-1. The P99 of exposure (32 ng/kg bw/d) was a factor three higher than the median exposure level. The main food groups contributing to the daily intake of OTA were wheat, rye, raisins and nuts (Figure 5-1). Note that the concentrations of raisins originated from targeted survey samples (section 2.4).

Results of the middle bound scenario were 20 to 50 % higher than those of the lower bound scenario, and about 15 to 30 % lower than those of the upper bound scenario (Appendix E). This was mainly due

to the high fraction on non-detects for wheat and wheat products (60 to 90 %; Appendix A). The uncertainty in the calculated exposure of OTA was larger than for the other mycotoxins (the lower and upper limits of the confidence interval differed more than a factor two, see Appendix H). This is most likely due to the relatively large variation in OTA concentrations in food products. This variation is addressed in the uncertainty analysis (bootstrap) of MCRA (section 2.6).

Comparison with other studies
In a previous survey on the exposure to OTA in the Netherlands concentration data reported by different institutions (VWA, EC and JECFA) were combined with consumption data of DNFCS-3 (Bakker and Pieters, 2002). In that study, median intakes of 2- to 6-year olds decreased from 3 to 2 ng/kg bw/d. The intake of 2- to 6-year olds in the present study is a factor four higher. This large difference is likely mainly due to a difference in OTA concentrations in wheat (0.3 µg/kg (½LOR) vs. 1.4 µg/kg (present study)), and not to a difference in the methodology used to measure food consumption levels in both studies or a difference in wheat consumption between 1997/1998 and 2005/2006. A large part of this difference in OTA concentrations originates from differences in LORs. In the study of Bakker and Pieters (2002), LORs were used ranging from 0.25 to 1.0 µg/kg, while in the present study an LOR of 2 µg/kg was used. This resulted in a large impact of the level assigned to non-detects on the mean OTA concentration of wheat as used in the exposure assessment. Concentrations of OTA in raisins were lower in the 2003 study than in the present study (1.9 µg/kg vs. 5.3 µg/kg). However, the representativity of the concentration data used in the 2003 study is unknown.

OTA was also analysed in the RIVM children's duplicate diet study of 2006. In this study, OTA was detected in 123 samples (van Egmond, 2007a). The resulting intakes were 1.2 ng/kg bw/d (P50) and 3.4 ng/kg bw/d (P90) with a maximum intake of 10 ng/kg bw/d. These values are lower than the intakes estimated in the present study.

5.2.5 Patulin

Exposure calculation
The long-term exposure of children aged 2 to 6 years varied from 0.03 µg/kg bw/d (P50) to 0.2 µg/kg bw/d (P99; Table 5-1). Patulin was detected in apple juice and apple sauce. Apple juice contributed most to the intake in young children (Figure 5-1).

The influence of the concentrations assigned to the non-detects on the exposure was high: exposure results of the lower bound scenario were low compared to the middle and upper bound scenario (Appendix E). Up to about 80 % of the samples were non-detects (Appendix A).

Comparison with other studies
The intake of 2- to 6-year olds as reported here can be compared to the intake of children aged 3 to 6 years as reported in the 2002 SCOOP report by Germany, Austria and France (SCOOP, 2002b). While in the present study the median intake is 0.03 µg/kg bw/d and the P95 is 0.14 µg/kg bw/d, the SCOOP-report reports comparable exposures: 0.03 and 0.09 µg/kg bw/d as mean and high intakes, respectively. Beretta et al. (2000) calculated the intake via baby food for 1-year olds in Italy. Using the highest concentrations measured, the intake was 0.04 µg/kg bw/d, which was similar to the median intake reported in the present study.

5.2.6 Discussion exposure assessment

Representativity of concentration data
An important source of uncertainty in the dietary exposure calculations of mycotoxins is the representativity of the concentration data, which can be divided in sample size and bias. The sample

size should be high enough to give a reliable mean value. As the concentrations of mycotoxins vary from year to year, due to different weather conditions in different years, the concentration data from a range of five years were used.

With respect to bias, the sampling must be non-targeted. As set out in section 2.4, we removed the targeted samples from the available mycotoxin concentration dataset (except for OTA in raisins) and used only monitoring results. However, also these samples may not be completely at random, and focussed on those food products that are suspected to be contaminated. The resulting exposure calculations of mycotoxins are therefore very likely overestimates of the true exposure. However, the extent of this overestimation is unclear. In comparison, a duplicate diet study performed in 2006 in Dutch children of the same age range showed considerably lower intakes of aflatoxin B_1, fumonisin B_1 and OTA. Typically, the maximum intake of these compounds in the duplicate diets corresponded with the P50 estimated in the present study. Hence, the findings confirm the notion that the intake of these mycotoxins based on monitoring data is overestimated.

Multimodal distributions
Except for DON, the distributions of positive (daily) intakes of all mycotoxins were multimodal (*i.e.,* they consisted of more than one peak). The most likely explanation for this is that there are subgroups in the population which have different eating habits (*e.g.,* apple juice consumers versus non-consumers in the case of patulin). These multimodal intake distributions cannot be transformed to a normal distribution (which is needed to estimate long-term exposure) by the BBN model, unless the different subgroups are analysed separately (section 2.6). Since the separation of subgroups is difficult and time-consuming and will result in loss of statistical power, the ISUF model was used to calculate the long-term exposures of aflatoxin B_1, fumonisin B_1, OTA and patulin. The ISUF model is a very flexible model to transform daily intake distributions to normality. However, it has the disadvantage that it cannot calculate the intake as a function of age. Furthermore, the ISUF model may also not be optimal when handling multimodal intake distributions (section 2.6). Preliminary results from simple simulation studies seem to indicate that ISUF most likely overestimates the true exposure. However, whether this is always true in more complex realistic situations is yet unclear. We therefore recommend that more research is necessary to develop a method to perform long-term exposure calculations based on multimodal exposure distributions in combination with age-dependency of the exposure.

Reporting limits
Some of the mycotoxins addressed in this report were only detected in a low fraction of the analysed samples (*e.g.,* fumonisin B_1, OTA and patulin). In these cases, the value of the LOR assigned to non-detects will drive the exposure of the middle and upper bound scenarios, especially if these LORs are high relative to the analysed values. This was true for the mycotoxins addressed here[7]. The reason for this is that VWA, for the analysis of mycotoxins, uses a method to screen samples on all mycotoxins simultaneously, to test whether samples exceed the product limits. When a sample is positive for a mycotoxin with this multi-method, the actual concentration is quantified with a method specific for that substance. For a screening purpose, this method is very practical. However, for dietary exposure assessments in which also the presence of low concentrations in food products is important, this practice is far less suitable.

Due to this phenomenon the exposure levels calculated with the middle and upper bound scenario for all mycotoxins (except for fumonisin B_1) were considerably higher than those of the lower bound

[7] We used concentration values from 2002-2006. VWA has used the multi-method since 2004. Although in the years 2002 and 2003 the analysis methods had lower LORs, for practical reasons we used the LORs of the multi-method to calculate the middle and upper bound scenarios.

scenario (Appendix E). To reduce the uncertainty in the exposures of the middle and upper bound scenarios, the samples need to be analysed with a method with a lower LOR. Another, perhaps more practical solution would be the use of a new statistical method with which the concentrations in the non-detects can be estimated based on a concentration distribution containing all positive concentrations. Of course this method can only be applied if the fraction of non-detects is fairly low.

Effect of processing
Information on processing was available for all mycotoxins except for patulin. For aflatoxin B_1, processing factors were used for boiling of rice and baking of maize tortillas. Processing factors for the preparation of peanut butter and cooking of maize, which each contribute significantly to the total intake, were not available. For DON and OTA, the information on cooking of rice, noodles and pasta was used. For DON these three cover the factors needed for the exposure calculation, since concentrations of bread and bakery products were available. However, for OTA this was not the case and a processing factor for the baking of bread and bakery products with wheat was lacking and therefore not taken into account. For fumonisin B_1 the available processing factors cover most of the relevant processes: for maize boiling and baking of tortillas and cornflakes, and for wheat baking of cake and bread. Factors for cooking of pasta were not available, but pasta contributes only for 4 % to the total intake of fumonisin B_1. Overall the availability of processing factors for mycotoxins was incomplete.

Duplicate diet studies
An alternative way to estimate the intake of compounds present in food is to analyse these compounds in duplicate diets. Duplicate diets give a direct measure of the exposure, which is a large advantage compared to the exposure estimates based on combining food consumption surveys with concentrations from monitoring programmes. On the other hand, duplicate diet studies have three major drawbacks, namely 1) the compound may often not be detected in the duplicate diet samples, due to dilution with non-contaminated foods and drinks, 2) the sources of the exposure cannot be identified and 3) the number of duplicate diet samples is normally very low and is therefore not representative of the population addressed. Furthermore duplicate diet studies are used to assess the exposure during a short period of time, mostly one day. Intake results obtained during one day are not suitable to assess the long-term exposure.

Nevertheless, as indicated above, the intake estimations as performed in the present study are also subject to difficulties. For mycotoxins two problems were encountered, namely biased sampling and multimodal intake distributions. In these cases it is helpful to have the results of both types of study. Exposure estimates calculated with data from food consumption surveys and monitoring programmes have the advantages of a large (representative) population, identification of the sources of exposure and relatively low costs, while the benefit of the duplicate diet study is a direct measure of exposure.

5.3 Risk assessment

Below the most relevant adverse effects of the different compounds will be addressed. For a more elaborated toxicological profile of the different mycotoxins, see Appendix M.

5.3.1 Aflatoxin B_1

5.3.1.1 Toxicology

Toxicokinetics
Absorption of aflatoxins in the rat small intestine is a rapid. Aflatoxin B_1 is metabolised to different metabolites, including aflatoxin M_1 and the critical product aflatoxin B_1-*exo*-8,9-epoxide. Aflatoxin B_1-*exo*-8,9-epoxide may be detoxified by glutathione conjugation and hydrolysis. However, it is very reactive and may form DNA- and protein adducts. The metabolites are excreted in urine and bile. Aflatoxin M_1 appears as a metabolite in milk.

Most sensitive effects
The primary toxic effects of aflatoxin B_1 are its genotoxicity and carcinogenicity (see Appendix M for other toxic effects). Aflatoxin B_1 is mutagenic in bacterial systems and in eukaryotes, usually requiring an exogenous bioactivation system (IARC, 1993). Sufficient experimental evidence is available for the carcinogenicity of aflatoxin B_1. In rodents the principal tumours are hepatocellular carcinomas (HCC). Rats were found to be the most sensitive mammal with (male) Fisher rat as the most sensitive strain. Species differences may reflect the differences in the rates and extents of metabolic activation and detoxification.

Aflatoxin M_1 is a metabolic hydroxylation product of aflatoxin B_1 which is excreted in milk. Based on studies in rats, FAO/WHO (2001b) as a conservative estimate, considered the potency of aflatoxin M_1 to be 10 % of that of aflatoxin B_1.

Observations in humans
Reasonably consistent associations have been found between estimates of dietary exposure to aflatoxins and HCC rates (IARC, 1993). Overall, published studies show a positive correlation between population estimates of aflatoxin exposure and the proportion of HCC (Wild and Turner, 2002). The IARC classified aflatoxins as 'human carcinogens' (IARC, 1993, 2002), and concluded in 1993 and 2002 that there was sufficient experimental evidence for the carcinogenicity of both aflatoxin B_1 and aflatoxin M_1.

Health based limit value
In 2007, the EFSA panel on contaminants in the food chain (CONTAM) considered liver carcinogenicity of aflatoxins to be the pivotal effect for risk assessments (EFSA, 2007b), and used therefore the 2.5 % lower confidence limit of the BMD, calculated for a 10 % increase in cancer incidence ($BMDL_{10}$), as a reference point (EFSA, 2005a). The panel used the most sensitive strain (Fisher rat) and sex (male) in a precautionary approach for risk characterization. It considered a study in male Fisher rats performed by Wogan et al. (1974) as the most adequate study for this. The calculated $BMDL_{10}$ values ranged from 0.17 to 0.34 µg/kg bw/d. The lowest $BMDL_{10}$ of 0.17 µg/kg bw/d was used in the risk assessment.

Epidemiology data indicate a clear association between exposure to dietary aflatoxins and liver cancer. However the relationship is confounded by high incidences of hepatitis B (> 20 %), which is a recognised risk factor of aflatoxins. On the basis of data from human populations, including the most

Table 5-3. Points of departure for the risk assessment of aflatoxin B_1.

Study	Toxicological endpoint	Point of departure, $BMDL_{10}$[a]	Reference
Rats	Hepatocellular carcinoma	170 ng/kg bw/d	(EFSA, 2007b) (based on (Wogan et al., 1974))
Human data	Liver cancer	870 ng/kg bw/d	(EFSA, 2007b) (based on (Yeh et al., 1989))

[a] $BMDL_{10}$ = 2.5 % lower confidence limit of the benchmark dose for a 10 % increase in cancer incidence.

sensitive subgroups with high prevalence of chronic hepatitis B infection, the CONTAM derived $BMDL_{10}$ value of 870 ng/kg bw/d. For an overview of the derived BMDLs, see Table 5-3.

Relevance for children
With respect to the carcinogenic effects, in general long-term exposure is needed for cancer to develop. From this point of view the carcinogenicity of aflatoxin B_1 is as relevant for adults as for children.

5.3.1.2 Overall risk assessment

The intake of aflatoxin B_1 was calculated with the contribution of aflatoxin M_1 in milk. The contribution of milk to the total intake was (almost) negligible (Figure 5-1).To perform a risk assessment, the MOEs of children to aflatoxin B_1 for the P50, P95 and P99 (including its 97.5 % upper confidence limit; Appendix H) of exposure were calculated. The results are summarized in Table 5-4.

In general, an MOE for carcinogenic effects should be in the order of magnitude of at least 10,000 (EFSA, 2005a). In this respect, CONTAM noted that to date there have been no conclusions on the magnitude of an MOE based on human data that would be of low concern (EFSA, 2007b). The MOEs for carcinogenicity are all far below 10,000, even when the samples with aflatoxin B_1 levels below LOR are considered to contain no aflatoxin B_1 at all (results not shown). The use of human data as a basis for risk assessment is however limited. All the studies used sensitive populations with a high prevalence of chronic hepatitis B, but very limited information on aflatoxin B_1 exposure. The incidence of liver cancers in nonhepatitis B infected individuals in the available studies was low. These limitations increase the uncertainty in the accuracy of the $BMDL_{10}$ estimates (Table 5-4).

In addition, the exposure assessment of aflatoxin B_1 is very likely an overestimation of the true exposure, due to the high number of non-detects (were assigned ½LOR of the screening method),and non-random sampling. The extent of the overestimation is however unclear. We therefore recommend that a study should be performed to obtain representative concentration data of aflatoxin B_1 to determine whether or not there is a negligible health risk related to aflatoxin B_1.

Taken together the derivation of a health risk related to the presence of aflatoxin B_1 in food (including aflatoxin M_1 in milk) in young children in the Netherlands is presently not feasible, because the quality of the concentration data was considered too low (conclusion 2e, section 2.7.2). To ascertain whether there is a negligible health risk or not representative levels of aflatoxin B_1 in relevant foods need to be made available.

Table 5-4. Percentiles of long-term dietary exposure of children aged 2 to 6 years to aflatoxin B_1 (including aflatoxin M_1 in milk) and corresponding margins of exposure (MOE) for carcinogenicity.

Age (years)	Dietary exposure (ng/kg bw/d) and MOEs[a]								
	P50			P95			P99 (L of CI)[b]		
	Exp[c]	MOE_{10} animal	MOE_{10} human	Exp[c]	MOE_{10} animal	MOE_{10} human	Exp[c]	MOE_{10} animal	MOE_{10} human
2-6	0.8	221	1130	1.9	88	448	2.7 (3.6)	63 (47)	323 (242)

[a] MOE_{10} animal and MOE_{10} human refer to the margins of exposure calculated with the $BMDL_{10}$ based on rat data and the $BMDL_{10}$ based on human data, respectively (Table 5-3).
[b] L of CI = Limit of 95 % confidence interval. For exposure 97.5 % upper confidence limit and for MOE 2.5 % lower confidence limit.
[c] Exp = Exposure

5.3.2 Deoxynivalenol (DON)

5.3.2.1 Toxicology

Toxicokinetics
After oral dosing of deoxynivalenol (DON) a high bioavailability of approximately 55 % was reported in pigs (Goyarts and Danicke, 2006; FAO/WHO, 2001b), but no values are available for rats, mice or humans. DON is rapidly absorbed after oral dosing in pigs, suggesting that the absorption takes place in the upper parts of the gastrointestinal tract (Danicke et al., 2004; FAO/WHO, 2001b; Goyarts and Danicke, 2006). Due to the rapid excretion of DON, no accumulation is expected.

One study addressed possible differences in toxicokinetics of DON in weanling and adult mice. When given doses of 5 mg/kg bw/d by gavage the uptake of DON in plasma, spleen, liver, lung and kidney was approximately twice as high in weanling mice compared to adult mice. However, DON concentrations in tissue and plasma of weanling mice approached the concentrations in adult mice within two hours, suggesting that differences in the clearance are less pronounced than differences in the uptake of DON (Pestka and Amuzie, 2008).

Most sensitive effects
Oral exposure to DON can result in adverse effects on the gastrointestinal tract and the immune system. Acute toxic effects on the gastrointestinal tract result in nausea, vomiting, abdominal pain and diarrhea. (Sub)chronic low dose toxicity of DON in experimental animals is characterized by a reduced body weight gain, which can be ascribed to a decreased uptake of nutrients from the intestine and/or reduced apatite. Reduced body weight gain is generally viewed as the critical effect of DON exposure. However, evaluation of the toxicity of DON showed that adverse effect of DON on the immune system, most notably serum IgA levels, may also be important (FAO/WHO, 2001b).

Health based limit value
For DON an extensive toxicity database is available. Short- and long-term studies have been performed in a variety of animal species (such as mice, rats, dogs and pigs). These studies also included studies performed in young animals (weanlings). On the basis of this extensive database a TDI of 1 µg/kg bw/d was established by RIVM (Pieters et al., 1999), JECFA (FAO/WHO, 2001b) and SCF (1999, 2002a). This TDI was based on a NOAEL of 0.11 mg/kg bw/d in a 2-year feeding study in mice in which the critical effect was reduced weight gain. It is considered that this TDI is also adequately protective for effects on body weight in young children.

Table 5-5. Percentage of children that exceeded the tolerable daily intake (TDI) for DON as derived by the Dutch Health Council (0.5 µg/kg bw/d) (Health Council of the Netherlands, 2001) and the $BMDL_5$[a] as derived by Slob and Pieters (1998) (0.6 µg/kg bw.d) as a function of age, assuming samples below LOR[b] to equal ½ LOR. The other TDI (1 µg/kg bw/d) (FAO/WHO, 2001c; Pieters et al., 1999; SCF, 1999) was not exceeded.

Age (years)	Percentage of children exceeding	
	TDI (0.5 µg/kg bw/d)	$BMDL_5$ (0.6 µg/kg bw/d)
2	3	1
3	2	0
4	1	0
5	1	0
6	0	0

[a] $BMDL_5$ = 2.5 % lower confidence limit of the benchmark dose for a 5 % reduction in body weight gain.
[b] LOR = Limit of reporting

The Health Council of the Netherlands used the same study to derive a TDI of 0.5 µg/kg bw/d by applying an uncertainty factor of 210, composed of uncertainty factors of 10 for intra- and 3 for interspecies differences, and a scaling factor of 7 for differences in energy use, as an indicator for metabolism, between man and mice (Health Council of the Netherlands, 2001). The latter was considered appropriate by the Health Council of the Netherlands because exposure to DON may influence body weight gain which is related to the level of metabolism (Health Council of the Netherlands, 2001).

Relevance for children
All adverse effects of DON, and especially reduced body weight gain and effects of serum IgA levels, are relevant for children.

5.3.2.2 Overall risk assessment

To perform a risk assessment the percentages of children exceeding the TDIs of DON were calculated. The results (Table 5-5) show that the TDI of the Health Council of the Netherlands (0.5 µg/kg bw/d) was slightly exceeded by 2-year olds (also when the uncertainty was taken into account, see Appendix E), when it was assumed that samples < LOR contain DON at a concentration of ½LOR. The TDI of 1 µg/kg bw/d was not exceeded.

Since the TDI of 0.5 µg/kg bw/d was exceeded with less than a factor two, it was studied whether exceeding the HBLV with a factor two or less could be accepted (section 2.7). The underlying study for the derivation of the TDI was a 2-year feeding study with mice, in which 0, 1, 5 or 10 ppm DON was added to the feed. Because of this constant concentration in the feed, an additional safety factor of two is present in the derivation of the HBLV. We can thus conclude that there is a negligible health risk related to the slight exceedance of the TDI by the 2-year olds.

Due to the availability of a BMD-analysis for DON, also these data were used to examine the possible health risk of DON. As stated above, the most sensitive adverse health effect of DON is reduced body weight gain. A previous probabilistic derivation of a BMD for humans from above mentioned 2-year feeding study in mice based on a 5 % reduction in body weight yielded a value of 8.6 µg/kg bw/d, with

a lower confidence limit of 0.6 μg/kg bw/d (Pieters et al., 2001; Slob and Pieters, 1998). The value of 0.6 μg/kg bw/d equals the P99 of dietary exposure in 2-year olds (Table 5-1), who had the highest exposure. So, 1 % of the 2-year olds exceeded this value. When the quantified uncertainty in the calculation was taken into account 4 % of the 2-year olds and 1 % of the 3- and 4-year olds could potentially exceed this value (Appendix H). So, based on the BMD approach there is a very limited risk for reduced body weight gain in a small percentage of these age groups.

Apart from affecting body weight gain, DON has also been shown to have an adverse effect on serum IgA levels. The lowest dose resulting in increased IgA levels in mice was 0.03 mg/kg bw/d, which is three-fold lower than the NOAEL for reduced body weight in the above mentioned 2-year feeding study in mice. To assess the relevance of this observation in relation to exposure of children to DON three aspects were considered. First, in two short-term oral toxicity studies in piglets, which resemble humans physiologically more closely than mice, no effects on serum IgA were observed in piglets fed 0.01 to 0.1 mg/kg bw/d for four weeks (Accensi et al., 2006; Goyarts et al., 2006). Second, in mice the effects of DON on serum IgA levels were less pronounced in the 2-year oral toxicity study (NOAEL 0.1 mg/kg bw/d (FAO/WHO, 2001b)). Third, the effects of DON on the immune system as well as on body weight gain in experimental animals appear to be reversible, based on two feeding studies in mice, one of which used intermittent exposure (Banotai, 1999; Dong and Pestka, 1993). Based on these observations in experimental studies in rats and pigs we conclude that 1) exceeding the TDI may result in adverse effects on body weight and serum IgA levels that are likely reversible and 2) (long-term) exposure to low doses of DON equal to or below the TDI does not likely result in adverse effects on human health. Given these conclusions, it should also be taken into account that DON intake by children decreases with increasing age. Whereas the estimated intake of DON by 2-year olds slightly exceeded the TDI derived by the Health Council of the Netherlands, the estimated intake of 6-year olds did not (P99, middle bound scenario).

A study in mice suggests that children may be more sensitive to DON induced toxicity due to possible toxicokinetic differences. Two-fold higher plasma and tissue levels of DON were observed in weanling mice (supposedly comparable to humans aged 1 to 2 years) compared to adult mice fed an equivalent (single) dose of 5 mg/kg bw (Pestka and Amuzie, 2008). At the moment it is not clear how these results relate to the most sensitive effects of DON in mice, namely reduced body weight gain and increased IgA levels. Further research is necessary to determine whether the increased plasma and tissue DON levels indeed augment the toxic effects of DON at a given dose and, importantly, how this relates to the current TDIs. With respect to the latter, it should be taken into account that a two-fold difference in sensitivity may be accounted for by the 10-fold safety factor for human variability.

Taken together, the TDI for DON was exceeded with a factor less than two (which was acceptable given the design of the study on which the TDI was based), while 1 % of the 2-year olds exceeded the BMD. Since the sampling for DON is also very likely biased (section 5.2.6) and exceeding the TDI with a factor two or less was acceptable, it is concluded that the health risk of DON for children aged 2 to 6 years is negligible (conclusion 1, section 2.7.2). However, additional information on toxicokinetics and immunotoxicity of DON is desirable to further support this conclusion. Furthermore, the exposure assessment of DON may be refined by using alternative methods for handling non-detects and by obtaining concentrations representative of the levels children are exposed to in real life.

5.3.3 Fumonisin B_1

5.3.3.1 Toxicology

Toxicokinetics

There are no kinetic data available for fumonisin B_1 in humans. In many animal species, including swine, rat, mouse and non-human primates, fumonisin B_1 is poorly absorbed (< 6 %) after oral intake. Distribution and elimination occurs rapidly and it is recovered mainly unmetabolized in the faeces. Small amounts are excreted in the urine. Studies have shown that in several animal species it is not transferred into milk and does not cross the placenta. A small amount of fumonisin B_1 was shown to be retained in the liver and the kidney (SCF, 2000b; WHO-IPCS, 2000).

Most sensitive effect

The major target organs of FB_1 are the kidney and the liver. Kidney and liver toxicity induced by fumonisin B_1 can result in both kidney and liver cancer (SCF, 2000b). In 1993 and 2002, IARC evaluated fumonisin B_1 and classified it as a group 2B carcinogen (possibly carcinogenic to humans), based on sufficient evidence in experimental animals but no adequate evidence in humans (IARC, 1993, 2002). There is no evidence that fumonisin B_1 is carcinogenic via a genotoxic mechanism (SCF, 2000b). In experimental animals, kidney or liver toxicity are a prerequisite for the development of cancer in the respective organ, indicating that there is likely a threshold for carcinogenesis induced by fumonisin B_1.

Health based limit value

Two identical TDI values for fumonisin B_1 have been derived. In 2000, SCF derived a TDI of 2 µg/kg bw/d based on the overall NOAELs for kidney toxicity of 0.2 and 0.25 mg/kg bw/d respectively in a subchronic (90 days) and a chronic (2-year) oral toxicity study in rats (SCF, 2000b). In 2001, JECFA derived the same TDI based on the same study for fumonisin B_1, B_2 and B_3, alone or in combination (FAO/WHO, 2001b). This approach was also adopted by SCF in 2003 (SCF, 2003).

Relevance for children

The adverse effects of fumonisin B_1 on kidney and liver are both relevant for children and adults.

5.3.3.2 Overall risk assessment

The fumonisin B_1 intakes listed in Table 5-1 were compared to the health limit of 2 µg/kg bw/d. For neither of the scenarios the TDI was exceeded. This was also true when the contributions of the intakes of fumonisin B_2 and fumonisin B_3, estimated as 50 % of the intake of fumonisin B_1, were taken into account. When the quantified uncertainty in the exposure calculation was considered, the TDI was slightly exceeded by 1 % of the children when the contributions of fumonisin B_2 and fumonisin B_3 were also considered (1.4 ng/kg bw/d fumonisin B_1 + 0.5 x 1.4 ng/kg bw/d fumonisin B_2 and fumonisin B_3 = 2.1 ng/kg bw/d; see Appendix H). Because the TDI is in this worst case situation only slightly exceeded by a small percentage of the children, refinement of the exposure or hazard assessment was considered not necessary.

There were no indications that the critical effect for adults is different from the critical effect for young children or to assume that children are more sensitive to toxic effects of fumonisin B_1. Therefore it is concluded that the health risk for children aged 2 to 6 years at current intake levels of fumonisin B_1 is negligible (conclusion 1, section 2.7.2).

5.3.4 Ochratoxin A (OTA)

5.3.4.1 Toxicology

Toxicokinetics

Following oral administration, OTA is relatively rapidly, although incompletely (40 to 66 %) absorbed. OTA is extensively bound to plasma proteins and distributed mainly to the kidneys, and to a lesser extent to liver, muscle and fat. OTA can cross the placenta and is metabolized only to a minor extent in the liver. In many species, including monkeys and humans, the major route of excretion is renal elimination. In rodents biliary excretion seems to prevail. Reported elimination half-lives were five to six days in rats and pigs, and 19 to 21 days in vervet monkeys. A human volunteer study indicated a fast elimination and distribution phase (half-life about 20 hours) followed by a slow elimination phase (plasma half-life of 35 days). In addition, OTA has been detected in human, rat and rabbit milk.

Most sensitive effects

The most sensitive and pivotal effects of OTA are its effects on kidneys in rats and pigs. Pigs appear to be the most sensitive animal species. In 5-weeks and 3-months feeding studies in female pigs, a LOAEL of 8 µg /kg bw/d was derived based on effects on renal enzymes and renal function tests. In a 2-year study in female pigs, progressive nephropathy but no renal failure was seen at a dose of 40 µg/kg bw/d. No nephropathy was observed at 8 µg/kg bw/d.

OTA is genotoxic both *in vitro* and *in vivo*, but the mechanism of genotoxicity is unclear and there is no evidence that it is mediated by direct interaction with DNA. Recent scientific evidence indicates that the site-specific renal toxicity as well as the DNA damage and genotoxic effects of OTA, measured in various *in vivo* and *in vitro* studies, are most likely attributable to cellular oxidative damage. Other toxic effects of OTA are described in Appendix M.

Observations in humans

No cases of acute intoxication in humans have been reported. OTA is found more frequently and at higher average concentrations in blood from people living in regions where a fatal human kidney disease (known as Balkan endemic nephropathy) occurs and is associated with an increased incidence of tumours of the upper urinary tract. However, similar average concentrations have been reported in several other European countries where this disease is not observed.

Health based limit value

Considering the lack of evidence for the existence of OTA-DNA adducts, the CONTAM used a threshold-based approach to derive a tolerable intake of OTA (EFSA, 2004a). For this, the LOAEL of 8 µg/kg bw/d was used, representing an early marker of renal toxicity in pig. To account for interspecies differences the default factor 2.5 was used for toxicodynamic differences (WHO-IPCS, 1999) and a factor six for kinetic differences (half-life) in consideration of the kinetic data mentioned above. For the intraspecies factor the default factor 10 was used. In addition, a factor three was applied in order to take into account the use of a LOAEL instead of a NOAEL. This resulted in a composite uncertainty factor of 450. Given the relatively long half-life of OTA in humans, the CONTAM concluded that a tolerable weekly intake (TWI) should be derived. This resulted in a TWI of up to 120 ng/kg bw/week, equal to a TDI of 17.1 ng/kg bw/d.

Relevance for children

Repeated dose studies have shown that OTA induces kidney damage in young as well as adult animals, although there are indications that adult animals may be more sensitive than young animals. Thus the

Table 5-6. Percentiles of long-term dietary exposure of children aged 2 to 6 years to OTA and percentage of the tolerable daily intake (TDI) (17.1 ng/kg bw/d) assuming samples with concentrations below LOR[a] to equal ½LOR.

Percentiles of exposure	Exposure (ng/kg bw/d)	%TDI
P50	11	64
P95	25	146
P99	32	187

[a] LOR = Limit of reporting

TWI for OTA is relevant for the general population, including children. Accordingly, the risk assessment of dietary exposure of children to OTA will be based on the TDI set by CONTAM in 2005.

5.3.4.2 Overall risk assessment

The intakes of OTA were compared to the TDI of 17.1 ng/kg bw/d (Table 5-6). The P80 of the intake distribution was equal to this level (not shown), so 20 % of the children had an exposure to OTA above the TDI. Taking the quantified uncertainty into account, a much higher percentage of the children exceeded the TDI (the 97.5 % upper confidence limit of the P50 equalled 15.8 ng/kg bw/d). At the highest percentiles children had an intake of about twice the TDI (considering the quantified uncertainty the intake may even be up to four times the TDI). By calculating the intake of only the 2-year olds, only the 3-year olds, etc., it was estimated that the percentage of children exceeding the TDI decreased from about 40 % for 2-year olds to 11 % for 4-year olds and older children (data not shown)[8]. So, the present exposure results show that more than 20 % of the 2- to 6-year olds exceeded the TDI with a maximum of about four times the TDI for a relatively long period of time.

Since the TDI of 17.1 µg/kg bw/d was exceeded with about a factor two, we also here studied whether exceeding the TDI with a factor two or less could be accepted (section 2.7). However this was not possible. In the underlying feeding study with pigs, the young pigs were exposed to doses of OTA corrected for their body weight.

It should be noted that the TDI is considered to present the TDI for a life-long exposure. Due to a lower energy and nutrient requirements per kg bw in older children and adults, in these groups the food consumption, and accordingly the intake of OTA, will be lower as compared to young children. Indeed, previous intake data indicate that the OTA intake in young children (< 6 years) was approximately a factor two higher than in older children (> 10 years) and adults. Thus it is likely that with increasing age the fraction of the population exceeding the TDI for OTA will decrease considerably. Furthermore, for OTA the exposure was very likely overestimated due to (partly) targeted sampling. In view of this, the following actions could be considered:

- Refinement of the exposure assessment, by generating OTA concentration data that are representative of the concentrations in food children are exposed to in real life. In addition, also the generation of data on processing would make the exposure estimation more reliable.
- Refinement of the risk assessment by performing a BMD analysis for the nephrotoxicity endpoint. No relevant studies on renal toxicity in pigs were available to perform such an analysis as part of this study.

[8] Note that these age groups consist of about 250 children each, which is rather low for this type of calculation. Therefore the given percentages are only an indication.

Overall, it is concluded that it is presently not feasible to determine whether there is a negligible health risk or not related to the presence of OTA in food in young children in the Netherlands, due to the use of likely (partly) targeted concentration data that resulted in an overestimation of the exposure (conclusion 2e, section 2.7.2).

5.3.5 Patulin

5.3.5.1 Toxicology

Toxicokinetics

The information on (toxico)kinetics of patulin is limited. *In vitro* studies showed that patulin is rapidly degraded in whole blood, with only 6.1 % of patulin detected after two minutes of incubation. From this study it was concluded that patulin from food is likely degraded and will not reach other tissues than the gastrointestinal tract. A similar conclusion was drawn in a study on the absorption of patulin from perfused rat stomachs (Rychlik, 2003; Rychlik et al., 2004).

Most sensitive effect

The signs of acute oral toxicity of patulin at high doses (> 16 mg/kg bw/d) in experimental animals include toxicity to the gastrointestinal tract, agitation, dyspnea and lung toxicity. In the only available 2-year toxicity study, the most sensitive toxic effect of patulin was a decreased body weight observed in male rats at oral doses above 0.1 mg/kg bw by oral gavage three times a week. Oral dosing of 1.5 mg/kg bw/d resulted in increased mortality in both sexes. It is indicated that patulin induced mortality in (sub)chronic toxicity studies in experimental animals due to dilatation of the gut and/or pneumonia. The latter is thought to be secondary to the antibiotic effects of patulin on gut bacteria, which could give a selective advantage to pathogenic bacteria. This hypothesis is supported by a 13-week study in pathogen free rats in which no such mortality was observed at similar dose levels (FAO/WHO, 1996).

Health based limit value

JECFA estimated a provisional maximum TDI (PMTDI) for patulin of 0.4 µg/kg bw/d based on a NOAEL of 0.1 mg/kg bw/d for decreased body weight in the above mentioned 2-year toxicity study in rats. Body weights of male but not female rats were reduced when dosed 0.5 mg/kg bw/d. The highest dose of patulin (1.5 mg/kg bw/d) resulted in increased mortality in rats of both sexes. As patulin was administered only three times a week the NOAEL of 0.1 mg/kg bw/week was recalculated to a daily intake of 43 µg/kg bw/d. A safety factor of 100 was applied (FAO/WHO, 1996). In 2000, SCF endorsed the PMTDI of 0.4 µg/kg bw/d (SCF, 2000a).

Relevance for children

The toxic effect of patulin on the gastro-intestinal tract is as relevant for children as for adults.

5.3.5.2 Overall risk assessment

All exposure levels, including the 97.5 % upper confidence limit of the P99 (Appendix H), were below the PMTDI (Table 5-1). It can be concluded that the health risk for children aged 2 to 6 years at current exposure levels of patulin is negligible (conclusion 1, section 2.7.2).

5.4 Conclusion and recommendations

Table 5-7 summarizes the exceedances of the health based limit values and the margin of exposure of the studied mycotoxins for children aged 2 to 6 years. The health risk is considered negligible for three (fumonisin B_1, patulin and DON) out of five mycotoxins (Table 5-7). However, additional information

Table 5-7. Margin of exposure (MOE) and percentage children exceeding the (provisional maximum) tolerable daily intake ((PM)TDI) of children aged 2 to 6 years at calculated dietary exposures. For DON also the percentage of children exceeding the $BMDL_5$[a] is listed.

Mycotoxin	MOE or percentage > (PM)TDI or $BMDL_5$	Conclusion
Aflatoxin B_1[b]	MOE P50 100-1100; P99 30-300	Health risk cannot be determined (= conclusion 2e, section 2.7.2)
DON	1 to 3 % of the 2- to 5-year olds exceeded the TDI of the Health Council of the Netherlands[c]. When applying an extra safety factor of two (section 2.7) the TDI was not exceeded. The $BMDL_5$ was exceeded by 1 % of the 2-year olds.	Taking the (partly) targeted sampling into account: negligible health risk (= conclusion 1, section 2.7.2)
Fumonisin B_1[d]	TDI was not exceeded.	Negligible health risk (= conclusion 1, section 2.7.2)
OTA	20 % of the 2- to 6-year olds exceeded the TDI with a factor of about two.	Health risk cannot be determined (= conclusion 2e, section 2.7.2)
Patulin	The PMTDI was not exceeded.	Negligible health risk (= conclusion 1, section 2.7.2)

[a] $BMDL_5$ = 2.5 % lower confidence limit of the benchmark dose for a 5 % reduction in body weight gain.
[b] Including aflatoxin M_1 in milk, assuming a potency factor of 0.1.
[c] The TDI of the SCF, JECFA and RIVM was not exceeded.
[d] Including fumonisin B_2 and B_3, assuming that these added 50 % to the exposure to fumonisin B_1.

on the toxicity of DON and patulin (the former on toxicokinetics and immunotoxicity, the latter on neurotoxicity and endocrine toxicity) is desirable to support these conclusions. For two mycotoxins, aflatoxin B_1 and OTA, it was not feasible to determine whether or not there is a negligible health risk due to the likely overestimation of the exposure.

Recommendations
To establish whether or not the health risk related to the exposure to aflatoxin B_1 and OTA in young children is negligible we recommend:

- to refine the exposure assessment:
  - by conducting a study into aflatoxin B_1 and OTA concentrations present in foods representative of those consumed (*e.g.,* as bought at the supermarket);
  - by generating processing factors for OTA;
  - by developing a method to perform long-term exposure assessments based on multimodal short-term intake distributions, including age-dependency of the exposure;
  - by reducing the uncertainty in the calculated mycotoxin exposures by analysing mycotoxins with a method with a lower detection limit. As a practical solution, the use of an alternative statistical method to estimate concentrations assigned to non-detect samples is recommended.
- to refine the risk assessment:
  - by generating for OTA suitable data for the performance of a BMD analysis for the nephrotoxicity endpoint.

6 Dietary exposure and risk assessment of nitrate

6.1 Introduction

Nitrate is naturally present in the environment as part of the nitrogen cycle. Nitrate occurs in most plants. Its concentration varies between species and variety and is influenced by various growth conditions such as sun light and fertilizer use. A regulation of the European Commission (194/97) requires member states to monitor the concentrations of nitrate in lettuce and spinach to ensure that they remain acceptable for human consumption. For these vegetables, concentrations might be higher in the winter compared to the summer due to different lighting conditions. In addition, nitrate occurs naturally in ground water, which is used as a source of tap water. Farming and effluent can increase nitrate concentrations. The WHO guideline for drinking water is 50 mg/L (WHO, 2003).

The toxicity of nitrate is usually the result of the *in vivo* conversion of nitrate into the more toxic nitrite (Hartman, 1983). Recently, the CONTAM compared the risk and benefit of exposure to nitrate from vegetables (EFSA, 2008a). They concluded that overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks.

6.2 Exposure assessment

Exposure calculation
The median intake of nitrate was highest in 2-year olds in the summer period, namely 1.9 mg/kg bw/d (Table 6-1). In the same period, the P99 ranged from 3.5 mg/kg bw/d in 6-year olds up to 4.7 mg/kg bw/d in 2-year olds. Corresponding numbers in the winter period were 2.4 and 3.6 mg/kg bw/d, respectively. The most important sources contributing to the average intake of nitrate were foods with relatively low nitrate concentration but high consumption levels, such as potatoes, tap water and apples (Figure 6-1). Differences between the summer and winter period appeared because of differences in consumption patterns (in summer higher consumption levels of tap water, beetroot, cucumber and lettuce) and due to differences in concentration levels (higher concentrations in potato and apple in summer). Differences between summer and winter were only significant at the age of 5 at P50 and P95.

It was shown that the exposure to nitrate decreased with age, mainly because of a decrease in food consumption levels per kg bw. There were no significant changes in absolute vegetable consumption levels with age visible in the research group (Ocké et al., 2008). The most important sources contributing to the average intakes were foods with relatively low nitrate concentrations but relative high consumption levels like potatoes (25 to 26 %), tap water (9 %) and apples (6 %).

Varying the concentrations assigned to non-detect samples had a negligible effect on the exposure levels of nitrate (Appendix E). This was mainly due to the limited number of samples with a concentration below LOR (Appendix A).

Comparison with other studies
The long-term nitrate intake of Dutch children was evaluated before with consumption data of the DNFCS-3 linked with concentration data from 1994 to 2002 (KAP database; (Westenbrink et al., 2005)). The nitrate intake was calculated with the Nusser method. Results reported are listed in Table 6-2. At P50 and P95, nitrate intakes in the current study are slightly lower compared to the

Table 6-1. Percentiles of long-term dietary exposure of children to nitrate as a function of age and season, assuming samples belowLOR[a] to equal ½LOR.

Age (years)	Percentiles of exposure (mg/kg bw/d)		
	P50	P95	P99
Summer			
2	1.9	3.6	4.7
3	1.8	3.4	4.4
4	1.6	3.1	4.1
5	1.5	2.9	3.8
6	1.4	2.7	3.5
Winter			
2	1.8	2.9	3.6
3	1.6	2.6	3.3
4	1.4	2.4	3.0
5	1.3	2.2	2.7
6	1.2	2.0	2.4

[a] LOR = Limit of reporting

previous calculations (Westenbrink et al., 2005). Main contributors to nitrate intake were similar for both studies, except for a higher contribution of cereal foods in the study of Westenbrink et al. (2005). Another reason for the slightly lower intake in the current study might be the tendency for lower nitrate

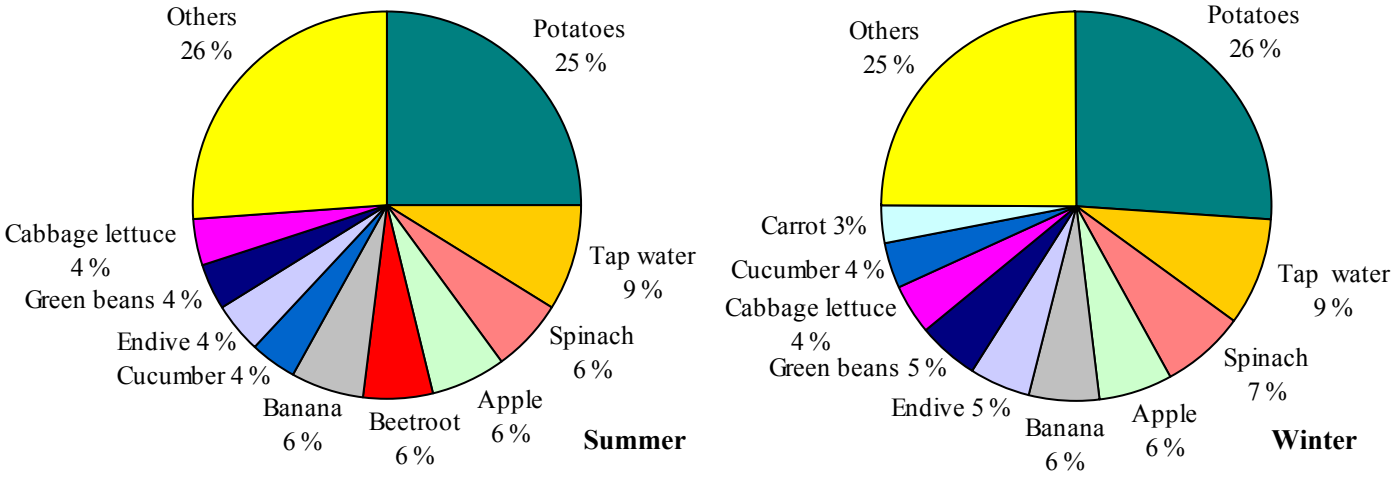

Figure 6-1. Contribution (%) of the most important food groups to the long-term dietary exposure to nitrate in summer and winter in children aged 2 to 6 years, assuming samples below the limit of reporting (LOR) to equal ½ LOR. The food group 'spinach' includes both frozen and canned spinach.

Table 6-2. Long-term dietary exposure to nitrate in mg/kg bw/d during all seasons based on food consumption data of DNFCS-3 (Westenbrink et al., 2005).

Age class (years)	Number of children	Percentiles of exposure (mg/kg bw/d)		
		P50	P95	P97.5
1-3	156	2.3	4.3	5.1
4-6	289	2.0	4.0	4.4

concentrations in lettuce as has been described by VWA (2005). In addition, the studies applied different dietary assessment methods, which may cause differences in intake.

Representativity of concentrations and processing factors
All consumed vegetables were covered in the sampling for nitrate (Appendix A). Leafy vegetables and lettuces were sampled extensively and included also samples of the frequently consumed frozen spinach. The sampling of other foods than vegetables was more limited. In this study, only old data were available for fruits and the number of samples was low. Not all fruits consumed were covered by the nitrate samples taken for fruit. Because it is expected that nitrate concentration in fruits are low, there is very likely no perceived need for authorities to sample these foods even when they are sometimes consumed in higher amounts than leafy vegetables. Nitrate concentrations of tap water were analysed frequently.

For nitrate, the effect of boiling vegetables was estimated by the results of the publication of Meah et al. (1994). This publication provides information for a number of vegetables (Appendix F). For other vegetables not included in this publication an average loss due to cooking of 51 % was assumed. Loss due to cooking, however, varies because of differences in cooking methods. For example, adding more or less water and/or draining more or less water may affect nitrate concentrations. Therefore, this aspect can either increase or decrease nitrate exposure. We also included the effect of peeling a banana on nitrate levels in the assessments as derived from Dejonckheere et al. (1994) (Appendix F).

Table 6-3. Percentage of children that exceeded the acceptable daily intake (ADI) of 3.7 mg/kg bw/d for nitrate as a function of age and season, assuming different levels of nitrate in tap water. The level of 5.1 mg/L was used in the basic analyses. Levels below LOR[a] were equalled to ½LOR.

Age (years)	Percentage of children exceeding ADI					
	Summer			Winter		
	5.1 mg/L	15 mg/L	30 mg/L	5.1 mg/L	15 mg/L	30 mg/L
2	5	9	19	1	2	7
3	3	5	13	0	1	4
4	2	3	8	0	0	2
5	1	2	5	0	0	1
6	1	1	3	0	0	0

[a] LOR = Limit of reporting

Nitrate concentrations in tap water
Concentrations of nitrate differ in tap water in the Netherlands, depending on the region. In the present assessment, nitrate concentrations were assumed to be equal to 5.12 mg/L tap water, the mean concentration analysed in 2006 (Appendix A). To also assess the exposure in children living in areas with higher nitrate concentrations in tap water than the average value, nitrate concentrations were assumed to be three to six times higher than the mean value, 15 and 30 mg/L. It is shown that a substantial proportion of the children living in these areas with these high levels will exceed the ADI (Table 6-3). Even though the nitrate levels are still well below WHO standards for drinking water (50 mg/L), this shows that children in such areas may be at risk, especially if they live there for longer periods. Further research is needed to establish the factors contributing to high nitrate concentrations in tap water. It should be noted that it was assumed for different food groups (soft drinks, juices reconstituted or not) that they consisted for 100 % of tap water, which might overestimate the intake of nitrate via tap water.

6.3 Risk assessment

6.3.1 Toxicology

In experimental animals prolonged oral exposure to nitrate was shown to result in growth depression and inanition. Due to the endogenous conversion of nitrate to nitrite, also methemoglobin (metHb) formation has to be considered as a toxic effect of nitrate. This holds particularly for children, because their endogenous formation of nitrite following nitrate intake is higher than in adults (EFSA, 2008a; SCF, 1997). A NOAEL of 370 mg/kg bw/d for the nitrate ion was derived from long-term studies in rats and a subchronic toxicity study in dogs, both with growth depression as the critical toxic endpoint. Applying an uncertainty factor of 100 resulted in an ADI of 3.7 mg/kg bw/d for nitrate (ion) (EFSA, 2008a; SCF, 1997). Since this ADI is based on growth depression, it encompasses children in particular. For a more elaborated toxicological profile of nitrate (including nitrite and N-nitrosodimethyl-amine), see Appendix N.

Based on the toxic effect of nitrite on heart and lung (Appendix N), a 'transposed' NOAEL for nitrate was established based on the NOAEL of nitrite (6.7 mg/kg bw/d), which also covers the toxicity due to metHb formation. This derivation resulted in a 'transposed' ADI of 3.2 mg/kg bw/d. Because this was in the same range as the originally derived ADI for nitrate (3.7 mg/kg bw/d), there was no justification to amend this (EFSA, 2008a; SCF, 1997).

When nitrate is consumed as part of a normal diet containing vegetables, the endogenous reduction of nitrate to nitrite, together with amines in concomitantly consumed food (particular fish), contribute to the formation of nitrosamines. This was shown in an *in vitro* model in which the formation of N-nitrosodimethylamine (NDMA) was observed after gradually adding nitrite to food samples (cod fish) (EFSA, 2008a; Zeilmaker et al., In preparation). NDMA is carcinogenic in all animal species tested, inducing benign and malignant tumours. Human HBLVs were estimated using dose-response modelling to derive a BMD associated with a 10 % increase in tumour incidence (BMD_{10}) in a large chronic toxicity/carcinogenicity study. The 2.5 % lower confidence limit ($BMDL_{10}$) of the BMD_{10} was taken as the starting point for extrapolation to the human virtual safe dose (VSD)[9], resulting in a VSD of 0.4 ng/kg bw/d (Zeilmaker et al., In preparation). Since NDMA was also proven to be carcinogenic in rats after one single dose, in a similar way a VSD for acute exposure was derived at 110 ng/kg bw/d

[9] The amount of a chemical that, if ingested daily over a lifetime, will result in one additional case of cancer in one million exposed individuals.

Table 6-4. Summary of the health based limit values (HBLVs) for nitrate, nitrite and N-nitrosodimethylamine (NDMA).

Study	Toxicological endpoint	NOAEL / $BMDL_{10}$[a]	HBLV[b]
Nitrate			
Subchronic study dogs (Lehman, 1958)	Growth depression	NOAEL: 370 mg/kg bw/d	ADI: 3.7 mg/kg bw/d[c]
Chronic study rats (Walker, 1990)	Growth depression	NOAEL: 370 mg/kg bw/d	
Nitrite			
Chronic study rats (Gruener and Shuval, 1973)	Heart and lung toxicity	NOAEL: 6.7 mg/kg bw/d	ADI: 0.07 mg/kg bw/d[c]
NDMA			
Chronic study rats (Peto et al., 1991a, b)	Liver tumors	$BMDL_{10}$: 29 µg/kg bw/d	VSD (chronic exposure): 0.4 ng/kg bw/d[d]
Single exposure study rats (Driver et al., 1987)	Kidney tumors	$BMDL_{10}$: 11 mg/kg bw/d	VSD (short-term exposure): 110 ng/kg bw/d[d]

[a] NOAEL = No-observed adverse effect level; $BMDL_{10}$ = 2.5 % lower confidence limit of the benchmark dose for a 10 % increase in tumor incidence.
[b] ADI = Acceptable daily intake; VSD = Virtual safe dose (see section 6.3.1)
[c] (EFSA, 2008a)
[d] (Zeilmaker et al., In preparation)

(Zeilmaker et al., In preparation). For more details, see Appendix N. An overview of all health based limit values is presented in Table 6-4.

Relevance for children
The adverse effects of nitrate on heart and lung, as well as its carcinogenic effect via the formation of NDMA are relevant for both children and adults.

6.3.2 Overall risk assessment

To perform a risk assessment the percentage of children exceeding the ADI of nitrate (3.7 mg/kg bw/d was calculated. The results (Table 6-5) show that the ADI for nitrate is exceeded in the summer period by 1 % of the 6-year olds up to 5 % of the 2-year olds. In the winter period, however, only 1 % of the 2-year olds exceeded the ADI. The 97.5 % upper confidence limit of the P99 exceeded the ADI also in 3-year olds in the winter (Appendix H).

Since in all cases the ADI was exceeded with less than a factor two, we also studied whether exceeding the TDI with a factor two or less could be accepted for nitrate (section 2.7). This was indeed possible, since the doses administered in the relevant studies were not corrected for body weight. Thus, exceeding the TDI of nitrate with a factor less than two poses a negligible health risk.

Although metHb formation is an important aspect of nitrate/nitrite toxicology, it is only relevant for infants up to the age of 3 months (Appendix N). In this respect, the nitrate exposure of infants aged up to 3 months is needed to address this risk with more confidence.

Regarding the endogenous formation of NDMA, Zeilmaker et al. (In preparation) concluded on the basis of model experiments that at the current exposure levels 4 % of the adult population (> 25 years

Table 6-5. Percentage of children that exceeded the acceptable daily intake (ADI) of 3.7 mg/kg bw/d as a function of age and season, assuming samples below LOR[a] to equal ½LOR.

Age (years)	Percentage of children exceeding ADI	
	Summer	Winter
2	5	1
3	3	0
4	2	0
5	1	0
6	1	0

[a] LOR = Limit of reporting

of age) exceeds the chronic VSD, while virtually nobody exceeds the $1{:}10^5$ lifelong additional cancer risk level. Conservatively estimated, the fraction of the population of young children (*i.e.*, children aged 5 years) exceeding the VSD appeared to be 47 %. Of these, 3.6 % exceeded the $1{:}10^5$ lifelong additional cancer risk level. (Note that they will exceed this limit for a shorter time-period than life-long). The fraction exceeding the $1{:}10^4$ lifelong additional cancer risk level was virtually 0 (Zeilmaker et al., In preparation). Since NDMA was also shown to be carcinogenic after one single dose, the same authors also calculated the risk for acute exposure. Conservatively estimated they reported that the acute VSD is exceeded by 1 % of the children aged 5 years on at least 3 % of the days, and by 3.3 % of the children (aged 5 years) on at least 2 % of the days (Zeilmaker et al., In preparation). It must be noted that the authors emphasized the large uncertainties in these estimations, mainly due to 1) the extrapolations from the model experiments to the actual *in vivo* situation, 2) the uncertainties of the exposure levels, and 3) the quite conservative extrapolations from cancer incidences in experimental animals to very low doses in humans (Zeilmaker et al., In preparation). Therefore it is difficult to assess the relevance of these exceedances for the cancer risk of children. Obviously one important aspect is the time it takes to develop a tumour following exposure associated with a certain risk. Results of estimations by Zeilmaker et al. (In preparation) indicate that the cancer risk of exposures to NDMA above the VSD might result in a shortening of human life of at most a few days. This would suggest a rather minimal risk of children exceeding the VSD of NDMA for a short period of time.

Overall we conclude that the health risk of nitrate exposure in children aged 2 to 6 years at the present exposure levels is negligible (conclusion 1, section 2.7.2).

6.4 Conclusion

Nitrate is a naturally occurring compound that is part of the nitrogen cycle and is an important component of vegetables due to its potential for accumulation. In the past, the attention to lower the exposure to nitrate via food has been focussed on lowering the concentration of nitrate in lettuce and spinach, the main source of exposure, by setting maximum levels for these vegetables. Despite developments in good agricultural practice and ALARA (as low as reasonably achievable) principle, concentrations can still be high and result in exceedances of the ADI. This is mainly because certain factors that stimulate the formation of nitrate in crops, such as sun light and temperature (Dich et al., 1996), are difficult, if not, to influence for crops grown in open air. This dependency has resulted, for example, in different maximum permitted nitrate levels for the summer and winter period, with higher

concentrations allowed in the winter period. Based on the risk assessment the probability that an adverse health effect occurs due to the exposure to nitrate was estimated to be negligible in young children.

Nitrate is present in vegetables, foods that are known to be beneficial to human health (reduced risk of cancer) and of which national and international governments stimulate the consumption. Encouraging the consumption of vegetables on the one hand, but also restricting it due to the presence of nitrate, can be confusing and therefore counterproductive. In 2008 the CONTAM performed a scientific risk assessment into nitrate exposure taking into account all relevant considerations on risks and benefits by weighing the possible negative impact of nitrate versus the possible positive effects of eating vegetables, such as antioxidant activities or other properties that might in some way counteract or provide a balance to the risks from nitrate (EFSA, 2008a). In this opinion, it was concluded that the beneficial effects of consumption of vegetables prevail above a possible health risk due to the exposure to nitrate. However, this conclusion was not quantified.

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7 Dietary cumulative exposure and risk assessment of organophosphorus pesticides

7.1 Introduction

Organophosphorus pesticides (OPs) are a group of pesticides which have a common mode of action: inhibition of acetylcholinesterase (AChE) by phosphorylation, resulting in a spectrum of acute cholinergic effects (ILSI, 1999; Mileson et al., 1998; Pope, 1999). To address the risk of exposure to this group of compounds, the individual exposures should be addressed simultaneously.

In this chapter the RPF method (section 2.4) was applied, in combination with the probabilistic approach, to estimate the acute cumulative dietary exposure of children aged 2 to 6 years to OPs using concentration data of OPs of 2005 and 2006. Acephate was used as index compound due to the availability of an extensive toxicity database. In a recently published opinion of the EFSA panel on Plant Protection Products and their Residues (PPR) on cumulative exposure modelling in relation to MRL setting (EFSA, 2008b), a tiered approach was proposed. The RPF method combined with the probabilistic approach belonged to one of the highest tiers, only followed by physiologically-based toxicokinetic (PBTK) modelling as an even more refined approach. However, it was also recognised that PBTK modelling is presently very resource intensive and demands specialised expertise, and is therefore unlikely to be routinely used in the near future (EFSA, 2008b). In the RPF method, it is assumed that the individual effects of the compounds are dose additive and that there are no synergistic or antagonistic effects (Seed et al., 1995). The PPR concluded that based on the low levels in which individual pesticides are present in the diet the assumption of dose additivity is feasible (EFSA, 2008b).

As described in section 2.6, the RPF method is still a new methodology to assess the cumulative exposure to pesticides. In this report, the acute cumulative dietary exposure was calculated following two different implementations of the RPF method in practice, Approach 1 and 2. For more details, see section 2.6.

7.2 Exposure assessment

Samples with multiple pesticide residues
In total 9734 composite samples were analysed for OPs in Dutch monitoring programmes in 2005-2006 (excluding commodities not consumed by Dutch young children). This included in total 91 different raw agricultural commodities (RACs). Of all samples, 1464 (15 %) contained a positive concentration for at least one OP and 379 (4 %) contained a combination of OPs (excluding the omethoate-dimethoate combination since omethoate is a metabolite of dimethoate). Combinations ranged from two up to seven pesticides per sample with a combination of two pesticides occurring most frequently (75 %). RACs containing the largest number of samples with two or more OPs each were the citrus fruits mandarin and orange (26 % and 18 %, respectively).

Exposure calculation
Table 7-1 lists the percentiles of the distribution of acute cumulative dietary exposure of young children to OPs for both approaches of calculation. Very briefly, in Approach 1 the acute cumulative exposure to OPs is estimated by linking daily consumption levels of RACs with summed OP concentrations per relevant RAC sample. In Approach 2, first the acute dietary exposure distribution per OP is calculated,

Table 7-1. Percentiles of acute cumulative dietary exposure (µg/kg bw/d) of children aged 2 to 6 years to OPs[a] calculated via two approaches[b].

	Exposure (µg/kg bw/d)	
Percentiles	Approach 1	Approach 2
P50	0.2	0.6
P90	1.1	1.7
P95	1.6	2.5
P99	4.1	6.7
P99.9	19	27

[a] OPs = Organophosphorus pesticides
[b] Approach 1: OP levels are summed per sample; Approach 2: Exposure distributions per OP are summed (section 2.6)

which are subsequently summed to generate an acute cumulative exposure distribution. For more details, see section 2.6.

The P99.9 of exposure was lower for Approach 1 compared to 2: 19 vs. 27 µg/kg bw/d. Figure 7-1 shows the top five RACs contributing to the total acute cumulative exposure distributions of OPs in young Dutch children for both approaches. Wheat contributed most to the exposure in both approaches, followed by rye, citrus fruits (mandarin and orange) and apple. The ranking of the RACs differed between the approaches. In Approach 1, rye was second after wheat with a contribution of 20 %, while

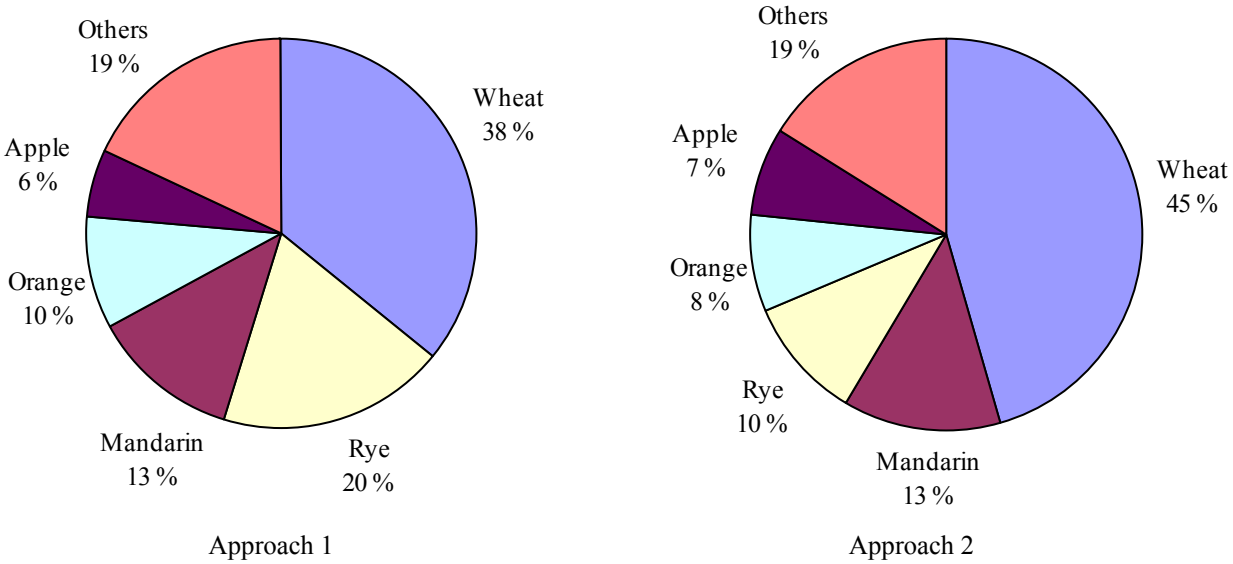

Figure 7-1. Contribution (%) of the five most important raw agricultural commodities to the acute cumulative dietary exposure of children aged 2 to 6 years to OPs according to two approaches of calculation; Approach 1: OP levels are summed per sample, and Approach 2: exposure distributions per OP are summed (section 2.6).

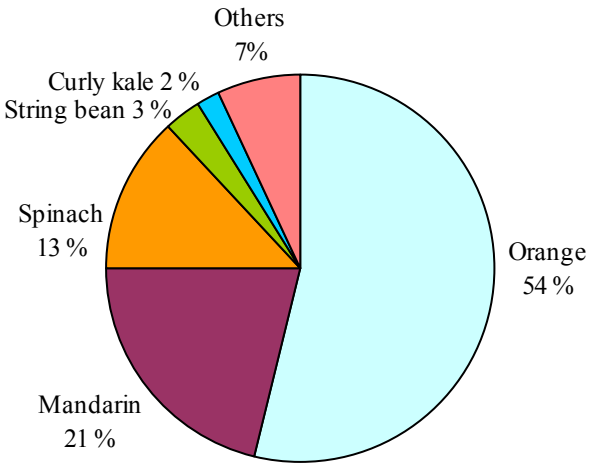

Figure 7-2. Contribution of the five most important raw agricultural commodities to the upper part of the acute cumulative dietary exposure distribution of children aged 2 to 6 years to OPs, calculated with Approach 1 (OP levels are summed per sample) (section 2.6).

in Approach 2 rye was third, after wheat and mandarin, with a contribution of 10 %. We also calculated the contribution of RACs to the upper 0.1 % of the exposure distribution, that part of the distribution with an exposure level above the reference value (P99.9). Result is plotted in Figure 7-2. This outcome can only be calculated with Approach 1. In the upper part, citrus fruits contributed most to the exposure.

In Approach 2 the acute cumulative exposure is calculated by summing up the exposure distributions of the individual OPs. Because of this, the contribution of the individual compounds to the total acute cumulative exposure to OPs can be calculated. This was done by multiplying the mean exposure level per OP with the corresponding RPF and dividing this by the overall mean exposure to all OPs (1 µg/kg bw/d). Figure 7-3 shows the percentage contribution of eight OPs contributing for more than 90 % to the total mean exposure level to OPs. The main drivers for the exposure to OPs were pirimiphos-methyl and dichlorvos. These compounds contributed each for 27 % to the acute cumulative dietary exposure, followed by fenthion (14 %) and omethoate (7 %).

Methodology of assessing the cumulative dietary exposure to OPs
The calculation of the cumulative exposure to a group of compounds with a common mechanism of action like OPs is still in its infancy. In November 2006, the EFSA organised a colloquium on this subject (EFSA, 2007a), and in 2008 an opinion was released by the PPR addressing cumulative exposure modelling in relation to MRL setting (EFSA, 2008b). In this opinion, the different methods available to calculate the cumulative exposure to compounds with a common mechanism of action were discussed, including the Hazard Index (HI), the Reference Point Index (RfPI), the Relative Potency Factor method (RPF) and physiologically-based toxicokinetic (PBTK) modelling. All these methods have their limitations. The RPF method for the calculation of cumulative exposure can only be used if the effects of the individual substances are dose-additive and in combination with probabilistic modelling. The PPR concluded that at the low levels at which pesticides are present in food interaction is not likely to occur and dose-additivity is to be expected. Given the complexity of PBTK modelling and expert knowledge needed to perform this type of modelling, the RPF method is presently the most advanced and refined method available to calculate cumulative exposure.

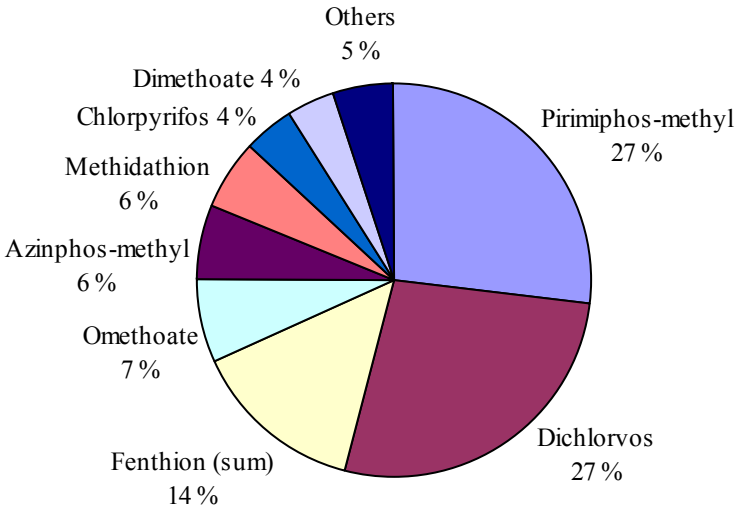

Figure 7-3. Contribution (%) of the individual OPs to the overall mean acute cumulative dietary exposure of children aged 2 to 6 years to OPs, calculated with Approach 2 (exposure distributions per OP are summed) (section 2.6).

However, how to apply this method has not yet been discussed. In this study, the acute cumulative dietary exposure to OPs was calculated using the RPF method via two different approaches (section 2.6). In one approach, OP concentrations were summed per sample using RPFs and calculated the acute cumulative exposure in equivalents of the index compound, while in another approach we first calculated the dietary exposure per OP and then summed the exposure distributions using the RPF per OP. The first approach results in realistic estimates of exposure if all compounds addressed are always analysed on each sample, because in this approach no distinction is made in assigning a concentration of zero to compounds that are analysed at a concentration below LOR or compounds that are not analysed at all. An analysis of the samples present in both the KAP and EWRS database showed however that not always all OPs were analysed in each sample. Assuming a zero when a compound is not analysed may therefore not always be correct and may via an increases in the number of zero levels in the concentration database result in an underestimation of the overall acute cumulative exposure. How large this underestimation will be depends on the percentage of RAC-compound combinations that are not analysed while a compound is likely to be present, based on, for example, other analyses that show positive concentrations or legislation.

To determine which RAC-compound combinations that were not analysed may very likely contain a pesticide if they had been analysed and subsequently to ascertain the distribution of concentrations that could have been present, is a huge undertaking and was outside the scope of this project. A pragmatic approach was therefore taken in which the exposure to all compounds was modelled separately, and subsequently summed the individual compound specific acute exposure distributions using the RPFs per compound (Approach 2). In this way, no zeros were assigned to samples that were not analysed for a certain compound. However, in this approach the chemical profile of the sample is not addressed. For example, the use of a certain pesticide on orange may preclude the presence of another compound, because they are not used in combination. By modelling the dietary exposure independently per compound this aspect is not addressed, resulting possibly in an overestimation of the exposure. For example, in the monitoring programme levels above LOR were analysed in mandarin for 11 different OPs. By addressing these compounds independently combinations may be generated that are not likely to occur in reality. Because only a very small part of the samples contained a combination of OPs (only

3 % of all samples analysed for OPs in 2005 and 2006), a possible realistic third approach could be to model the exposure in such a way that an unit fruit or vegetable consumed by an individual on a certain day can only be contaminated by one OP.

Apart from the above considerations, both approaches have some additional advantages and disadvantages that may plead for using one model above the other, or applying them both in an assessment. An advantage of Approach 1, apart from including the chemical profile of a sample, is that uncertainty intervals can be estimated around the exposure percentiles, as well as the contribution of the RACs to the upper part of the exposure distribution (*e.g.*, highest 1 %). Both are not possible in the way in which Approach 2 is implemented in MCRA. Approach 2 has currently however the advantage that processing can be applied per RAC-compound combination, as well as variability. Also, as mentioned above, the contribution of the different compounds to the cumulative exposure distribution can be calculated using this approach. This is presently not possible in the way that Approach 1 is implemented in MCRA. Due to the summing up of OP levels per sample, in Approach 1, information at compound level is lost at the very beginning of the analysis. Which approach gives the best estimate of exposure is difficult to establish, due to absence of information about correlations in pesticide application, and likelihood of presence of pesticides that are not analysed in the respective sample. Additionally it should be considered that in Approach 2 processing can be included at compound level and not at 'group' level (Approach 1). When processing effects are available and differ largely between compounds this may be a reason to prefer this approach above Approach 1.

It should be noted that both approaches used a combined OP intake of all foods for a whole day, and that thus the time-course of the exposure was not addressed. This implies, for example, that the effect of a compound ingested during breakfast and still being present in the body when a compound of the same mechanism group is ingested during dinner is not taken into account. Also not whether compounds ingested yesterday are still present the next day. Examination of this was outside the scope of this project, but can play a further role in refining exposure scenarios if information on toxicokinetics and/or -dynamics might become available.

Uncertainties
Also the present calculations were subject to uncertainties related to the input data of the assessment and the approach used to cumulate the exposure to OPs. Three of these uncertainties were quantified, namely the model uncertainty (Table 7-1), the uncertainty related to the consumption and concentration data (Appendix H) and approach taken to include variability in the assessment (see Appendix G). Especially assigning variability factors equal to 3, 5 or 7 depending on unit weight instead of 3 resulted in an increase of exposure with a factor 1.5. This shows that the exposure assessment is susceptible to which variability factors are used. However, the exposure levels remained below the relevant toxicological reference value (section 7.4).

Comparison with other studies
Only a few studies have so far reported on the cumulative exposure to OPs. A summary of these studies is reported in Boon et al. (2008) and reproduced here. Studies reported include cumulative exposure estimates for Brazil (Caldas et al., 2006), the Netherlands (Boon et al., 2008; Boon and van Klaveren, 2003; van Klaveren et al., 2006) and the US (EPA, 2006). Results of these studies are summarised in Table 7-2. These results show differences in exposure, at the P99.9 level, between the studies. One explanation for the differences is that both the Dutch study of 2003 (Boon and van Klaveren, 2003) and the one of Brazil (Caldas et al., 2006) studied the exposure to OPs and carbamates simultaneously, because both groups of pesticides are known to inhibit AChE. The carbamates were not included in the present calculations because of a difference in mechanism of action between both groups of chemicals.

Table 7-2. Overview of acute cumulative dietary exposure estimates reported in literature to organophosphorus pesticides (OPs) and carbamates in young children.

Country	Compounds	Exposure (P99.9)	%ARfD[a] index compound	Age range (years)	Index compound (ARfD[b] in ug/kg bw/d))	Reference
The Netherlands	OPs/carbamates	32	64	1-6	Acephate (50)[c]	(Boon and van Klaveren, 2003)
	OPs/carbamates	60	134	1-6	Phosmet (45)	(Boon and van Klaveren, 2003)
	OPs	25	50	1-6	Acephate (50)	(van Klaveren et al., 2006)
	OPs	57	114	1-6	Acephate (50)	(Boon et al., 2008)
	OPs	27	53	2-6	Acephate (50)[c]	Present study
Brazil	OPs/carbamates	8.0	80	0-6	Methamidophos (10)	(Caldas et al., 2006)
	OPs/carbamates	85	169	0-6	Acephate (50)	(Caldas et al., 2006)
US	OPs	2.3	23	3-5	Methamidophos (10)	(EPA, 2006)

[a] ARfD = Acute reference dose (see 6th column for the level of the ARfD in brackets).
[b] The dose reported in brackets is the ARfD reported in the respective studies or established by the JMPR and compiled by the International Programme on Chemical Safety (IPCS) (WHO-IPCS, 2006).
[c] In the present study we used an ARfD of 100 µg/kg bw/d for comparison with the P99.9 (see section 7.3). However, for reasons of comparison with other studies we used in this table an ARfD of 50 µg/kg bw/d for acephate.

The most important difference is that OPs inhibit AChE mostly irreversibly, while carbamates inhibit the enzyme reversibly (minutes to hours). Because of this difference, van Raaij et al. (2005) concluded that cumulating the daily exposure to both chemical groups will probably result in an overestimation of the risk.

The results presented in this study can thus only be compared to those of the US (EPA, 2006) and the 2006 (van Klaveren et al., 2006) and 2008 Dutch studies (Boon et al., 2008). The exposure to OPs calculated in the present study is comparable to the one reported in the 2006 Dutch study (27 vs. 25 µg/kg bw/d), but much lower compared to the 2008 Dutch study (27 vs. 57 µg/kg bw/d). Compared to the US exposure levels, exposure to OPs is very low in the US compared to the Netherlands (Table 7-2). Differences in exposure between the studies are due to differences in contamination levels of relevant foods and/or differences in eating habits between countries or in time, and/or differences in modelling of cumulative exposure. Also differences in toxicity between the index compounds used in the different studies may play a role. For example, in the 2006, 2008 and present Dutch study acephate was used as index compound, while the US study used methamidophos as index compound, a more toxic compound with a lower ARfD (Table 7-2). To address this, the exposure estimates were normalised by expressing them as percentage of the ARfD. The difference in exposure between the present study and the US study was reduced by a factor six, but the exposure remained higher in the Netherlands.

Contribution of RACs and individual pesticides to the exposure
The top five RACs contributing most to the total cumulative exposure was similar in both approaches, with the largest contribution for wheat. However, the ranking of the RACs differed per approach. In Approach 1, wheat was followed by rye (20 %), while in Approach 2 wheat was followed by mandarin (13 %), and then by rye (10 %; Figure 7-1). The reason for this difference is very likely the difference in methodology used to assess the cumulative exposure. Rye is a RAC with positive concentrations for only one OP, pirimiphos-methyl. OP levels assigned to rye differ therefore not between Approach 1 and 2. Mandarin, orange, wheat and apple, the other RACs in the top 5, were however all analysed for more than one OP, namely 11, 14, 3 and 4 respectively. If not all these OPs were analysed in each of the samples present in the database per RAC (*e.g.*, in some mandarin samples maybe only eight OPs were analysed), the concentrations in Approach 1 will be diluted with zeros assigned to these 'non-analysed OPs'. This being not true for rye, the contribution of rye to the total cumulative exposure distribution will be higher relative to the other RACs compared to Approach 2. In the upper part of the exposure distribution, the citrus fruits contributed most to the exposure. This result was consistent with that reported in the Dutch study of 2008 (Boon et al., 2008).

The results showed that pirimiphos-methyl and dichlorvos contributed most to the total cumulative exposure to OPs, followed by fenthion and omethoate. The high contribution of pirimiphos-methyl and dichlorvos, both with an RPF below 1, was due to their presence in wheat, a RAC frequently consumed by young Dutch children as, for example, bread and pancakes. Wheat determined 100 % of the exposure to dichlorvos and 59 % for pirimiphos-methyl. For malathion, another OP present in wheat, 89 % of the exposure was determined via wheat. However, due to the very low RPF of this compound (0.004) this compound did not contribute largely to the overall cumulative exposure. For fenthion and omethoate the contribution to the cumulative exposure distribution was determined by a high RPF (4.167 and 11.1, respectively). For these two compounds citrus fruits (orange and mandarin) were the most important source of exposure.

Processing factors
The information on processing effects per compound-RAC-processing type combination was very limited, necessitating the assumption that factors could be applied to a whole group of fruits or

vegetables (Appendix F). The variability in the actual processing level was covered by assuming that the processing factors follow a distribution. Apart from assuming that processing is applicable to a whole group of fruits or vegetables, it was also assumed that the processing factors were applicable to all OPs addressed. This is a simplification, because processing factors will differ per compound (EPA, 2006). Due to these two assumptions processing factors were set at relatively high levels with an upper bound level close to 1 for all processing type-RAC combinations, except for drying. A processing factor equal to 1 means that the concentration is not affected by processing (section 2.6). The calculated cumulative exposure levels for this group of compounds are therefore probably higher than those encountered in real life.

7.3 Risk assessment

OPs form a group of compounds that share a similar mode of action, namely inhibition of brain AChE (ILSI, 1999; Mileson et al., 1998; Pope, 1999), and should therefore be addressed simultaneously. The combined toxicity of two or more compounds can take three possible forms: dose-addition, response-addition or interaction. The following descriptions are taken from a publication on cumulative risk assessment by the PPR in 2008 (EFSA, 2008b).

- Dose-addition, also referred to as simple similar action, similar joint action or relative dose addition, occurs when chemicals in a mixture act in the same way, by the same mechanism/mode of action, and differs only in their potencies. Such compounds are said to belong to a 'common mechanism group' (CMG) and dose-addition implies that the effects of exposure to a mixture of such compounds are equivalent to the effects of the sum of the potency-corrected doses of each component compound.
- Response-addition, also referred to as simple dissimilar action, simple independent action or independent joint action occurs where the modes of action and possibly, but not necessarily, the nature and sites of toxic effects differ between the chemicals in a mixture and one chemical does not influence the toxicity of another. The effects of exposure to such a mixture are the combination of the effects of each individual compound.
- The term, interaction, embraces all forms of joint action that deviate from the two classes of combined toxicity described above. It implies that the combined effect of two or more chemicals is stronger (synergistic, potentiating, supra-additive) or weaker (antagonistic, inhibitive, sub-additive, infra-additive) than would be expected on the basis of dose-addition (if the chemicals belong to a CMG) or response-addition (if they do not belong to a CMG).

The mechanism of action for the neurotoxicity of OPs, *i.e.,* inhibition of brain AChE by phosphorylation, is well known. A careful evaluation of a study in which mixtures of five OPs were administered together to rats the evidence was in favour of dose addition for three of the four mixtures examined. The PPR concluded that there is empirical and mechanistic evidence that dose-addition can occur when OPs are administered at relatively low doses (EFSA, 2008b). Response-addition would be relevant only where exposures to individual OPs are at toxic levels, and this is not to be expected as a result of exposure to OPs in food. The PPR concluded that although toxic interactions from OPs in food cannot be ruled out, there is no empirical evidence for their occurrence, and they are much less likely to occur when exposures are below the effect levels for the individual OPs.

7.3.1 Toxicology

The toxicology of acephate has recently been evaluated by JMPR (FAO/WHO, 2002, 2005). An EFSA opinion on acephate is not yet available. The following summarizes the main conclusions of the JMPR 2002 and 2005 evaluations. Since in the present report only the risk of acute cumulative exposure of children to OPs is assessed, the toxicological profile presented below will mainly deal with the acute aspects.

Rat studies
After oral administration acephate is rapidly absorbed (peak plasma concentration half to one hour after dosing in rats, one to four hours in humans) and uniformly distributed. No relevant differences with respect to dose administered and C_{max}[10] between humans and rats were observed. In rats, a part of acephate is converted to methamidophos, which is also used as a pesticide. Excretion of acephate is rapid (half life ± 1.4 hours in rat, 3.5 to 6.6 hours in human) and mainly through urine. Less than 1 % was found as a residue in tissues and organs 72 hours after dosing. The main urinary metabolite is unchanged acephate (73 to 77 %) with methamidophos (0 to 5 %) as another significant compound in urine. Acephate and methamidophos do not accumulate. In pregnant and lactating rats treated with ^{14}C-acephate, radiolabel was recovered from the placenta, foetuses and suckling pups.

The most prominent and sensitive effect of acephate (and other OPs) following acute as well as chronic administration is inhibition of AChE activity. Inhibition of erythrocyte or brain AChE ≥ 20 % is considered toxicologically relevant. The overall acute NOAEL for acephate was 2.5 mg/kg bw/d, on the basis of a 30 to 34 % reduction in brain AChE activity in female rats observed in an acute (gavage) study. In a dietary repeated dose study in rats, a NOAEL (based on more than 20 % reduction in brain and erythrocyte AChE inhibition) of 10 ppm was derived, equal to 0.58 mg/kg bw/d.

Human studies
Volunteers receiving single oral doses of acephate showed no inhibition of erythrocyte AChE or any other adverse effects at doses up to and including 1.2 mg/kg bw/d (men) or 1.0 mg/kg bw/d (women). In a repeated dose study in male and female volunteers, erythrocyte AChE activity was not inhibited at 0.3 mg/kg bw/d of a mixture containing acephate and methamidophos at a 9:1 ratio throughout a 21-day test period. In a 28-day repeated dose study in human volunteers, daily oral doses of 0.25 mg/kg bw/d did not inhibit plasma or erythrocyte AChE activities at any time during the study, nor were there any treatment-related changes in hematology, clinical chemistry, electrocardiogram or urine analysis parameters, vital signs or physical examination.

JMPR noted that it is likely that the inhibitory effect of acephate is due to its conversion to methamidophos. No significant sex or species difference in cholinesterase inhibition was observed *in vivo*.

Health based limit value
JMPR 2005 established an ARfD of 100 µg/kg bw/d on the basis of the NOAEL of 1.2 mg/kg bw/d from the study of single doses in humans and an overall safety factor of 10. The overall safety factor of 10 was derived by dividing the default value of 10 by two (because inhibition of AChE activity depends on the C_{max}) and by multiplying by two (because some uncertainty remains with respect to the *in vivo* sensitivity to inhibition of human brain AChE activity relative to that of erythrocyte AChE activity, since brain AChE activity may be more sensitive than erythrocyte AChE activity). This ARfD is considered protective for the general population, including children. A detailed description of the argumentation for the setting of the ARfD is presented in Appendix O.

Relevance for children
The neurotoxic effects of acephate and other OPs are relevant for both children and adults.

[10] C_{max} = maximum plasma concentration achieved

7.3.2 Overall risk assessment

To assess whether there is a negligible health risk or not in children related to the exposure to OPs, the P99.9 of exposure was compared to the ARfD of acephate. It is clear that this level of exposure (Table 7-1) was well below the ARfD of 100 µg/kg bw/d. OPs pose therefore a negligible health risk for children aged 2 to 6 years (conclusion 1; section 2.7.2).

7.4 Conclusion and recommendations

Based on the risk assessment the health risk of exposure to OPs as a group appears negligible and no additional policy measures to reduce exposures further are necessary. It should however be noted that the calculation of the exposure to OPs, and other groups of chemicals with a common mechanism of action, is still in its infancy and that further model development is imperative.

Recommendations

We have shown that the RPF method can be used to assess the cumulative exposure to OPs. However, further method development, also in view of the EFSA opinion (EFSA, 2008b), is needed in the future to improve the estimation of the exposure to groups of compounds with a common mechanism of action. This includes OPs, but also other compound groups with a common mechanism of action, both known (like carbamates, anti-androgenic pesticides, pyrethroid pesticides, triazines and chloroacetanilides) but also unknown. Model improvement includes foremost the optimization of statistical methods to handle data sets in which not all compounds are analysed in each sample, and inclusion of the uncertainty in the derivation of the RPFs. For this the integration of exposure modelling with BMD modelling will be necessary. Also models that can address toxicokinetic (*e.g.*, half life of compounds) and toxicodynamic (*e.g.*, rates of spontaneous reactivation and aging of phosphorylated AChE) characteristics of compounds need to be developed to refine cumulative exposure modelling.

8 Discussion and conclusions

8.1 Main findings

The aim of the study described in this report was to assess the dietary exposure and the related possible health risk to a selected number of compounds in young Dutch children. For this, food consumption data of a recent DNFCS-Young Children 2005/2006 and recent concentration data derived from Dutch monitoring programmes were combined using advanced statistical models to assess both acute and chronic exposure. Based on the results we conclude that a compound is either safe (or poses a negligible health risk) or may form a very limited, limited or considerable health risk (Figure 2-1). This latter classification in three degrees of severity should be seen as a guideline to prioritize resources to refine the risk assessment. For compounds for which the data on exposure or toxicity were not sufficient to quantify the health risk (ranging from negligible up to considerable), it was concluded that the determination of a health risk was not feasible. For these compounds further research is needed to determine the health risk. Health risk is defined as the probability that an adverse health effect occurs.

For an overview of the results, see Table 8-1. For fumonisin B_1, patulin and OPs the exposure did not exceed the relevant health based limit values (HBLVs), indicating that these compounds pose a negligible health risk for young children. For dioxins, deoxynivalenol (DON), ochratoxin A (OTA) and nitrate HBLVs were however exceeded by part of the children. Based on a further examination of the toxicity database and/or refinement of the risk assessment, the health risk for DON and nitrate was judged negligible, while for dioxins there is a limited probability that an adverse health effect will occur. For OTA, a health risk was not determined due to the use of likely (partly) targeted concentration data. For the carcinogenic compound acrylamide a margin of exposure (MOE) was calculated far below 10,000, the value proposed by EFSA to indicate a possible health concern when dealing with genotoxic and/or carcinogenic compounds (EFSA, 2005a). It was therefore concluded that there is a probability that an adverse health effect will occur at the levels of acrylamide present in food in young children. However, as epidemiological studies show inconsistent results on the carcinogenic effects of acrylamide in humans, it was not possible to draw firm conclusions on the extent to which this could happen. Also for aflatoxin B_1 MOEs lower than 10,000 were calculated. However, as with OTA, a health risk was not determined for this compound due to the use of likely (partly) targeted concentration data.

Below these results will discussed further in relation to the methods and input data used to assess the exposure, as well as the meaning of exceedances of HBLVs or relatively low MOEs.

8.2 Methodological issues related to the exposure assessment

Information on food consumption
In the exposure assessments data of the DNFCS-Young Children 2005/2006 were used. In this survey, food consumption data were collected using the dietary record method. In this approach, caretakers of the children completed a detailed diary about all the foods and drinks consumed by the children. This methodology used to determine the food consumption habits of young children is regarded as the best method to assess individual dietary eating habits, and is best suitable to assess the acute and chronic dietary exposure to contaminants and pesticides. However, this method may underestimate

Table 8-1. Overview of dietary exposure levels to different compounds in Dutch children aged 2 to 6 years.

Compound (group)	P99 level of exposure[a]	Unit of exposure	%children > ADI, TDI or ARfD[b]	Margin of exposure[c]	Conclusion health risk[d]	
Acrylamide	1.5	µg/kg bw/d		200	Possible[e]	Conclusion 2d
Dioxins	2.3-2.8[f]	pg/kg bw/d	3-14		Limited	Conclusion 2b
Mycotoxins						
Aflatoxin B_1	2.7	ng/kg bw/d		63	Was not determined[g]	Conclusion 2e
DON	0.4-0.6[f]	µg/kg bw/d	1-3[h]		Negligible	Conclusion 1
Fumonisin B_1	1.0	µg/kg bw/d	-[i]		Negligible	Conclusion 1
OTA	32	ng/kg bw/d	20		Was not determined[g]	Conclusion 2e
Patulin	0.23	µg/kg bw/d	-[i]		Negligible	Conclusion 1
Nitrate						
summer	3.5-4.7[f]	mg/kg bw/d	1-5[j]		Negligible	Conclusion 1
winter	2.4-3.6[f]	mg/kg bw/d	0-1[j]		Negligible	Conclusion 1
OPs	27[k]	µg/kg bw/d	-[i]		Negligible	Conclusion 1

[a] All percentiles of exposure refer to chronic exposure, except for the OPs where the exposure refers to acute exposure.
[b] ADI = Acceptable daily intake; TDI = Tolerable daily intake; ARfD = Acute reference dose
[c] Margin of exposure is calculated by dividing the $BMDL_{10}$ (2.5 % lower confidence limit of the benchmark dose for a 10 % increase in cancer incidence) by the P99 of exposure.
[d] Health risk is defined as the probability that an adverse health effect occurs., see for more details section 2.7, and Figures 2-1 and 2-2.
[e] Due to inconsistent results on carcinogenicity of epidemiological studies, further identification of the possible health risk was not possible.
[f] Exposure range for 2- up to 6-year olds
[g] Due to the use of likely (partly) targeted concentration data which resulted in an overestimation of the exposure, determination of the health risk was not feasible.
[h] The estimated exposure to DON of 1-3 % for the 2- to 6-year olds exceeded the TDI of the Health Council of the Netherlands with less than a factor two. The TDI established by RIVM, JECFA and SCF was not exceeded. By refining the hazard assessment it was estimated that the exposure of 0-1 % of the children exceeded the $BMDL_5$ (2.5 % lower confidence limit of the benchmark dose for a 5 % reduction in body weight gain). Furthermore, the TDI was based on a study in which young animals received doses that were not corrected for body weight (section 2.7). Because of this, exceeding the TDI with a factor less than two was judged to pose a negligible health risk.
[i] Estimated P99 of exposure to fumonisin B_1 and patulin, and P99.9 of exposure to OPs did not exceed the relevant health based limit values. In those cases the exact percentage of children exceeding these limits was less than 1 % or 0.1 %, respectively, and therefore not further quantified.
[j] The estimated exposure to nitrate of 0-5 % for the 2- to 6-year olds exceeded the ADI with less than a factor two. Because the ADI was based on a study in which young animals received doses that were not corrected for body weight, exceeding the TDI with a factor less than two was judged to pose a negligible health risk (section 2.7).
[k] Refers to the P99.9 of exposure

consumption levels due to high participation burden (Biró et al., 2002). In the current food consumption survey, the data were checked by calculating the energy needed for basic metabolism. This showed that there was no discrepancy between energy demand and reported intake in the children's survey (Ocké et al., 2008).

Another disadvantage of food consumption surveys is that habitual eating patterns may be influenced or changed due to the recording process. The effect of this on the exposure calculations may differ. For example, due to the unhealthy image of crisps and French fries the consumption of these foods may be underestimated resulting in an underestimation of acrylamide intake. On the other hand, foods that are known to be healthy like fruits and vegetables may be eaten more on recording days than in practice, which may result in an overestimation of the intake of pesticides. The extent in which eating habits are changed due to the recording process and consequently the effect on calculated exposure levels is unknown.

As reported by Ocké et al. (2008) the food consumption data were not completely representative of that of all children aged 2 to 6 years living in the Netherlands. Densely populated areas were slightly underrepresented and the distribution by season was not completely homogeneous, with higher representation in 'Winter' and 'Autumn' than in 'Spring' and 'Summer' (Ocké et al., 2008). The statistical tools available to correct for these (small) deviations when addressing food safety are not available. How these deviations have affected the representativeness of the reported exposure estimates is unclear and will differ per compound. We recommend that factors to weigh the results for deviations in representativity are incorporated in the exposure models used in food safety.

Concentration data

We used monitoring concentration data to assess the exposure. The advantage of monitoring data is that it is available and generated as part of legislation obligations. The use of these data for risk assessment purposes is an efficient way of using data that are available. The disadvantage however is that the data are not generated for risk assessment purposes, but to ensure compliance with legal limits. Some of the monitoring regulations prescribe representative sampling procedures so that the generated data are comparable between countries or over time. In most cases, however, monitoring data may not always be representative of the concentrations people are exposed to. Sampling may be partly focussed on those samples suspected to contain concentrations above the legal limit (*e.g.,* due to previous problems, weather conditions). Use of this non-random sampled data in exposure assessment may thus lead to overestimates of the exposure. Non-random sampling is predominantly an issue for mycotoxins and pesticides. For mycotoxins, this is illustrated by the Dutch children's duplicate diet study (van Egmond, 2007a, b, 2008). The exposure estimated with the duplicate diets was considerably lower than the exposure estimated in the present study. Hence, this confirms the notion that the exposure of mycotoxins estimated with monitoring data is overestimated. For the other compounds addressed in this report, namely acrylamide, dioxins and nitrate, the presence in relevant food groups is rather constant (although maybe decreasing over time) and not dependent on the origin of the sample due, for example, to weather conditions or pesticide use. Besides non-random sampling, also sample size plays a role in the uncertainty of the concentration data, *i.e.,* a larger sampling size will result in an improved estimate of the concentration data. This sampling uncertainty is quantified in the bootstrap analysis.

In monitoring programmes, there is a tendency to use screening methods instead of compound specific methods because of their cost-effectiveness. Results generated with screening methods, and especially those with a high LOR, are less suitable for risk assessment purposes. An example in this report is the group of mycotoxins, which are analysed with a screening method with a high LOR. Negative screening samples are regarded as zeros, while positive screening samples (level above the LOR) are further analysed with a more precise compound specific analytical method. Because of the nature of

screening methods in general, and screening methods with a high LOR in particular, uncertainties are introduced when using these data in a risk assessment. This uncertainty can be addressed by sensitivity analyses where non-detects are replaced by ½LOR or LOR. This uncertainty can only be reduced by using more refined analytical methods. This is however not a realistic option because of the high costs involved. An alternative approach is the use of statistical methods in which non-detects are replaced with values drawn from a constructed concentration distribution using the positive values analysed. We therefore recommend the implementation of such a model into the present MCRA programme. Note that this will only be a feasible approach when there are enough samples with a level above the LOR.

Linking of food consumption data to concentration data
For most compounds addressed in this report (except acrylamide) concentration data are available mainly at the level of raw agricultural commodities (RACs; *e.g.,* vegetable, fruits, cereals, nuts, etc.) which can mostly not be linked directly to the foods as consumed. The food conversion model (section 2.5) was therefore used, which translates foods as eaten in RACs resulting in consumption patterns at RAC level. These consumptions can subsequently be linked to concentrations analysed at this level. The conversion model is therefore an essential tool to use monitoring data for risk assessment purposes. Presently foods as eaten are converted to a fixed (weight) fraction of a certain RAC per food. It should however be realized that food conversion percentages very likely vary. For example, the percentage of apple in apple pie will vary between different recipes. Apart from this variability, there is and always will be uncertainty regarding food conversion. Currently neither the variability nor the uncertainty of the food conversion is quantitatively addressed in exposure assessments. Furthermore, the food conversion model was set up in 1995. In the mean time, the recording of food consumption data has improved, resulting in the storage of more detailed information on the foods consumed in the DNFCS-Young Children 2005/2006 survey. This detailed information has not been used when converting foods recorded in this survey to their RAC ingredients. A recent study into the linkage of food consumption data to concentration data therefore advised to improve the linkage by updating the food conversion model using all the available detail information presently stored in food consumption data (Boon et al., 2008).

Another source of uncertainty is that by linking foods as consumed to concentrations in foods analysed. Acrylamide was analysed at the level of foods as they are consumed. These sampled foods were directly linked to consumed foods. Due to the diversity in foods consumed and the limited number of foods analysed, coded foods were grouped in such a way that they resemble best the foods analysed. The grouping was mainly based on similar characteristics and ingredients of foods as those analysed, including expert judgement. The choices made may affect the exposure assessment. The same applies to mycotoxins, for which grouping of consumed foods was also performed.

Linking of consumption and concentration data is an important step in the dietary exposure assessment and current knowledge concerning the uncertainty is limited. Hence characterization and quantification of the uncertainty associated with this step is important to examine in future research.

Processing factors
Almost all compounds are influenced by processing. If sampling is not performed at the level of foods as consumed, processing factors can be applied to make the exposure assessment more realistic. In the assessments presented in this report, processing factors were used if available. It is clear that there is a lack of processing factors for almost all compounds (except for DON and fumonisin B_1) and that generation of more processing factors for all different types of processing-food combinations would be important to generate a more refined estimate of exposure. This is above all important for those compounds that pose a possible human health risk. For dioxins, part of the children exceeded the TDI. This compound is very likely also susceptible to processing effects. These effects were not included in

the present calculations due to limited and highly variable (and thus unreliable) information (for more details, see section 4.2). Generation of processing data for dioxins is therefore very important to assess whether also after correcting for processing effects the TDI is still exceeded.

Modelling of exposure
Different models were used to calculate both the acute and chronic dietary exposure of compounds. Due to the expected effect of age on the intake, the BBN model was the preferred method to estimate the chronic exposure to compounds. In a study comparing BBN and ISUF, it was concluded that in situations where a logarithmic or power transformation results in approximately normal exposure data, the BBN model is preferred over the more complex ISUF model (de Boer et al., 2009). With covariates such as age or sex, it is even the only alternative. In our case, this was true for acrylamide, dioxins, DON and nitrate. However, for some compounds the BBN model was not applicable due to the multimodal character of the exposure distribution, where a logarithmic or power transformation does not result in approximately normal exposure data. In those cases, the ISUF model was used, although preliminary results of simulation studies indicated that ISUF possibly overestimates the true exposure. However, whether this also applies in more complex realistic situations than addressed in these simulation studies is yet unclear and should be investigated. If this is indeed the case, there is a need for an improved methodology that can handle multimodal exposure distributions, including, if feasible, covariate analysis.

Another issue is the methodology of calculating the acute cumulative dietary exposure to OPs. Two approaches were used as discussed in section 7.2. Approach 2 resulted in higher exposure levels compared to Approach 1, because in Approach 1 zero concentrations levels are assumed for pesticides that were not analyzed. This may have resulted in an underestimation of the exposure. In Approach 2 on the other hand, possible correlations between the uses of pesticides were ignored, which may have resulted in an overestimation of the exposure. To address these differences models should be developed that can address unbalanced concentration datasets.

In Table 8-2 the different sources that contribute to the total uncertainty of the exposure assessment to the compounds presented in this report are summarized, including the direction and magnitude of the uncertainty, using the format as proposed by EFSA (2006a). This table is certainly not exhaustive but addresses the most important sources contributing to the exposure assessment. Overall, we judge that the exposure estimates reported in this report will be slightly to fairly conservative due to the limited information on processing (dioxins and OTA), the substitution of non-detects with ½LOR (relevant for compounds with a high fraction of non-detects such as the mycotoxins) and the use of concentrations derived from non-random monitoring programmes (pesticides and mycotoxins) (Table 8-2). However, the exposure results presented in this report are the best estimates possible given the data and models currently available.

Note that also HBLVs are uncertain to some extent, depending on the underlying toxicity data and assessment factors. In the present study, the uncertainties in the hazard assessment are not transparent. A possible approach to improve the understanding of the uncertainty present in hazard assessments is proposed by van der Voet and Slob (2007). They present an Integrated Probabilistic Risk Assessment approach, which enables the inclusion and analysis of various sources of (quantitative) uncertainty in the exposure and hazard assessment.

Limitations of this study
In this report, we examined the intake of known chemicals present in food that may be relevant for children's health based on past estimates of exposure, for example, due to their eating habits or levels present in foods consumed by children. The substances were also selected based on the availability of

Table 8-2. Sources, direction and magnitude of uncertainty in the dietary exposure assessment to compounds in food.

Source and relevant substances	Source of uncertainty	Direction & magnitude[a]
Food consumption data		
All	Under / overreporting	-/+
All	Sampling uncertainty (bootstrap)[b]	--/++
Concentration levels		
Pesticides and mycotoxins	(Partly) targeted sampling	++
All (except pesticides and mycotoxins)	Representativity sampled RACs / foods	-/+
All	Non-sampled RACs / foods	-
All	Analytical precision	--/++
All	Sampling uncertainty (bootstrap)[2]	--/++
All (except pesticides)	Non-detect samples: ½LOR	+
Pesticides	Non-detect samples: 0 mg/kg	-
Linking food consumption and concentration data		
All (except acrylamide)	Calculation via RACs	--/++
Acrylamide	Calculation via food groups	--/++
Processing factors		
All (except acrylamide and dioxins)	Processing effects: limited data, conservative assumptions	++
Dioxins	No processing	+
Pesticides (cumulative)	Grouping of processing effects	++
Model uncertainty		
Pesticides (cumulative)	Summation of exposure distributions per pesticide	+
Pesticides (cumulative)	Summation of concentrations per composite sample	-
Pesticides (cumulative)	Summation of cumulative exposure during the day	++
Long-term	BBN for acrylamide, dioxins, DON and nitrate	-/+
Long-term	ISUF for aflatoxin B_1, fumonisin, OTA and patulin	+
Overall assessment: Based on this qualitative evaluation of different uncertainty sources we conclude that for OPs the exposure will be most likely conservative (*i.e.,* overestimating exposure levels) due to the use of monitoring data to assess the exposure. This is also true for all mycotoxins, including also a high LOR, and the use of the ISUF model to assess the long-term exposure for aflatoxin B_1, fumonisin, OTA and patulin. For dioxins the exposure may be slightly conservative due to limited information available on processing effects. For acrylamide the exposure may have been slightly underestimated due to incomplete coverage of foods and possible underreporting of unhealthy foods containing acrylamide (*e.g.*, crisps and chips) or adjusted eating habits during the recording days (like crisps and French fries).		+/++

[a] Key to direction and magnitude
+, ++, +++ = uncertainty likely to cause small, medium or large overestimation of exposure
-, --, --- = uncertainty likely to cause small, medium or large underestimation of exposure
[b] In the analyses sampling uncertainty of food consumption and concentration data was quantified simultaneously via a bootstrap analysis. Therefore it is not possible to quantify which part of the sample uncertainty was due to food consumption data and which due to concentration data. For more details, see section 2.6.

concentration data. In this report, the possible risks from emerging toxins or from other chemicals which have been identified as possible health risks, including, for example, furans, environmental contaminants like perfluorinated organic compounds and other processing contaminants than

acrylamide such as 3-MCPD (3-monochloorpropaandiol-1,2). This was mainly due to a lack of concentration data of those chemicals.

The exposure estimates were restricted to exposure via food consumption. Other routes, such as inhalation or dermal absorption, were not considered. However, for most of the substances addressed in this report, dietary intake is by far the most important exposure route. Only for acrylamide and nitrosamines, which may be present in consumer products like cosmetics, ignoring other exposure routes than dietary intake may have led to an underestimation of the total exposure. This needs to be examined further.

8.3 Risk assessment

The risk assessment of all compounds included a thorough literature research of adverse effects of the relevant compounds and comparison of the exposure with health based limit values (HBLVs; like ADI, TDI, ARfD) based on the most critical effect or the calculation of an MOE. Based on this risk assessment, four compounds exceeded the relevant HBLV, namely dioxins, DON, OTA and nitrate (Table 8-1). For two compounds, acrylamide and aflatoxin B_1, an MOE was estimated below the value of 10,000.

Exceeding the health based limit value
For compounds with an exposure above a HBLV it should be realized that such exposures are not directly associated with clinical effects in humans, especially if the exceedance is (very) limited. The larger the exceedance the higher the probability that a health risk may occur. Furthermore, HBLVs are set such to take into account potential inter- and intraspecies differences. Because of the variability and uncertainty in inter- and intraspecies differences, these limits are in most cases a factor 100 (10 for inter- and 10 for intraspecies) lower than the dose (*e.g.,* NOAEL or BMD) with no adverse effects in animals. When other uncertainties are present, this so-called uncertainty factor (or assessment factor) can be set to higher values than 100. For example, for OTA this factor was equal to 450, as a LOAEL was the point of departure instead of a NOAEL (section 5.3.4.1). Furthermore, HBLVs for chronic toxic compounds are set for lifelong exposure, while in this report the exposure of compounds was examined over a relatively short period of time, 2 to 6 years of age. It is well-known that children have higher exposures due to higher food consumption levels per kg bw and that exposures decrease with age, resulting in overall exposures during a lifetime that may be below the HBLV. Whether a short period of exceedance during early childhood may be critical will partly depend on the toxic effect of the compound, as well as on the size and duration of the exceedance.

For four compounds the P99 of exposure exceeded the HBLV, namely for dioxins, DON, OTA and nitrate. For dioxins the HBLV was based on toxic effects on the reproductive system in male offspring. However, for these compounds body burden levels are important when assessing the risk, which equals to the cumulative dioxin level people have been exposed to up to a certain point in their life. In childhood, due to the short period of exposure, body burdens are (still) low, and no toxic effects are expected. High levels of exposure during childhood however will contribute to higher body burden levels in adulthood. So in that sense a decrease in intake is recommendable, especially when a high intake of dioxins is associated with a certain life style which is continued in adulthood. Efforts to reduce the dioxins levels in the environment, and consequently foods, have been made resulting in lower exposures and thus body burden levels (de Mul et al., 2008). It was concluded that the exposure to dioxins in young children poses a limited health risk.

For DON the P99 of exposure exceeded the TDI of the Health Council of the Netherlands. This HBLV was however based on an animal study where the doses administrated were not corrected for body weight, and therefore exceeding the TDI with a factor two or less could be accepted (section 2.7). As young animals eat about twice as much as full-grown animals, the dose in young animals is twice as high as in the full-grown animals. This is comparable to the situation in humans. This resulted in a negligible health risk related to the slight exceedance of the TDI by 2-year olds. Due to the availability of a BMD-analysis for DON, also these data were used to examine the possible health risk of DON. This analysis demonstrated that the exposure of 1 % of the 2-year olds exceeded the $BMDL_5$. Based on all results, including the possible higher concentration of DON present in the foods analysed than those actually consumed, it was concluded that DON exposure results in a negligible health risk.

For OTA the HBLV is based on renal toxicity which is relevant for both children and adults. It was estimated that the exposure of 20 % of the children exceeded this HBLV, resulting in the conclusion that the exposure OTA resulted in a limited health risk. Exceeding the TDI with a factor two or less could not be accepted. The exposure assessment of OTA can however be seen as a worst case assessment because of non-random sampling (and even targeted sampling for raisins), and therefore we did not derive the health risk related to the intake of OTA through the diet in young children. Representative concentration data on the presence of OTA in food consumed by children are necessary to determine a reliable exposure estimate.

Finally, also the P99 of exposure to nitrate exceeded the HBLV. This HBLV is based on growth depression and therefore especially relevant for children. However, as for DON the HBLV was based based on an animal study where the doses administrated were not corrected for body weight, and therefore exceeding the TDI with a factor two or less could be accepted (section 2.7). Consequently, the health risk related to nitrate intake was judged negligible.

Margin of exposure
For genotoxic (DNA-damaging) and carcinogenic (leading to cancer) compounds like acrylamide and aflatoxin B_1, no safe intake levels are derived because it is assumed that even a very small dose can have an adverse effect. In 2005, EFSA released an opinion related to these types of compounds proposing a harmonized concept using the MOE approach, a methodology that enables the comparison of the risk posed by different genotoxic and carcinogenic substances (EFSA, 2005a). This approach is mainly seen as a risk management tool to prioritize risk reduction measures. When calculating the MOE for different compounds, those with the lowest MOE should be tackled first. However, apart from a tool to prioritize risk reduction measures, the EFSA Scientific Committee also gave guidance on how to interpret the MOE as such. It was stated that in general an MOE of 10,000 or higher, if it is based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management actions. Nevertheless, it was said that such a judgement is ultimately a matter for the risk managers.

We estimated that the MOE for acrylamide en aflatoxin B_1 based on $BMDL_{10}$ derived from an animal study equalled 200 and 63 respectively (Table 8-1). Compared to the value of 10,000 as proposed by EFSA these MOEs are very low. For acrylamide efforts have been and are being made to reduce acrylamide levels in foods and thus the exposure, including the evaluation of possible negative side effects (section 3.4). Acrylamide is a known carcinogenic compound (IARC, 1994). However, epidemiological studies carried out in recent years on the general population have produced inconsistent results concerning carcinogenicity (Appendix K). Based on this the 11th EFSA colloquium on acrylamide in May 2008 concluded that acrylamide intake is not likely to be very strongly carcinogenic in humans (EFSA, 2008c). Based on the results, we concluded that there is a probability that an adverse health effect occurs due to the exposure to acrylamide in young Dutch children, but that

it is not possible to draw firm conclusions on the extent of the health risk involved. Data on the toxicity of acrylamide need to be generated for this.

For aflatoxin B_1, the exposure levels reported here can be seen as worst case estimations (see section 2.4). It is therefore unclear if there is a negligible health risk or not related to the presence of aflatoxin B_1 in the diet of young children. Generation of realistic concentration data for aflatoxin B_1 is necessary for the derivation of a reliable health risk. Additionally, a refinement of the toxicity data available for the risk assessment could help to determine the true risk. Data from human studies were available resulting in an MOE of 323. However, the human data were derived from studies using sensitive populations with a high prevalence of chronic hepatitis B. Knowledge about liver cancers in nonhepatitis B infected individuals may make it possible to refine the assessment., although it is not expected that this will result in MOEs of more than 1,000 (factor 10 lower than 10,000 due to absence of interspecies factor).

Decision tree VWA panel
To assess the health risk related to the compounds studied in this report the decision tree as developed by the VWA panel (VWA, 2008) (section 2.7.1) was applied. This tree was only used for the compounds for which the exposure could be compared to a chronic HBLV. The steps as described in the decision tree were followed, although with some deviations (Appendix I). The most important ones were 1) only exposure from food was considered and not the exposure from other sources, 2) when the exposure was lower than the HBLV the toxicology database was nevertheless studied, although the decision tree considers this unnecessary, and 3) step 2 (determine if reduction of exposure is possible) was performed after step 5 (evaluation of the health risk), because we judged it only useful to lower the exposure when a health risk is present.

This is the first use of the decision tree in practice. It is appreciated by the researchers that the stepwise approach of the decision tree gives a clear structure to follow. Nevertheless, it is felt that, especially in step 3 (evaluation of the toxicology database), the proposed strategy was already the way of working beforehand. Therefore step 3 was not always followed in as much detail or in the same order as indicated by the VWA panel, especially by the more experienced toxicologists. On the other hand, the approach to allow the HBLV to be exceeded by a factor two when this limit value already takes into account a two-fold higher intake in children brings a new aspect to the risk assessment of chemicals in food. However, as indicated in the decision tree, it should be noted that this does not always apply, as for example shown in this report for ochratoxin A and dioxins. Thus, whether or not an exposure of children up to two times higher than the HBLV is acceptable should be assessed on a case-by-case basis, and should always be justified.

8.4 Recommendations

Based on the results presented in this report, two types of recommendations were formulated, those directed specifically at the compounds addressed aiming at refining the risk assessment and those of a more general nature covering, for example, input data, model development, etc. Below an overview is given of these recommendations. At compound level only the recommendations are summarised for those compounds for which no negligible health risk could be determined (Table 8-1). For more detailed information on these compounds we refer to the compound-specific sections. Note that the results presented in this report are not suitable (too premature) for the formulation of recommendations in the direction of policy makers regarding possible measures to reduce the exposure (see section 2.7).

8.4.1 Recommendations per compound

Acrylamide

To quantify the possible health risk related to the exposure to acrylamide in young Dutch children we recommend to obtain a better understanding of the toxicological effect of acrylamide, taking into account that epidemiological studies do not show that acrylamide is a strong carcinogenic in humans. Furthermore we recommend:

- to refine the exposure assessment by increasing the number of analyses of all food groups, but especially of those with less than 10 samples and relatively high acrylamide concentrations, including children's cookies, crisps, peanut butter, breakfast cereals and chocolate (Appendix B);
- to perform risk-benefit analyses where possible adverse side-effects (risk) of reductions in acrylamide levels in foods (benefit) are addressed.

Dioxins

We recommend refining the exposure assessment of dioxins by generating more information on the effect of processing on dioxin concentrations in food.

Aflatoxin B_1 and OTA

To establish whether or not there is a negligible health risk related to the exposure to aflatoxin B_1 and OTA in young children we recommend:

- to refine the exposure assessment:
    - by conducting a study into aflatoxin B_1 and OTA concentration data present in foods representative of those consumed (*e.g.*, as bought at the supermarket);
    - by generating processing factors for OTA.
- to refine the risk assessment by generating for OTA suitable data for the performance of a BMD analysis for the nephrotoxicity endpoint.

To give a ranking to the recommendations listed above, we advise to prioritise the generation of representative concentration data of aflatoxin B_1 and OTA, followed by the recommendations for acrylamide and dioxins in a decreasing level of importance.

8.4.2 General recommendations

Link concentration and food consumption data

We recommend updating the food conversion model used to link levels analysed at RAC level to food level. The examination of variability and uncertainty in the conversion model is advised, including the development of a method to address these quantitatively in an exposure assessment.

Model development

We recommend to (further) develop models that can:

- handle multimodal short-term exposure distributions, including age-dependency of the exposure;
- estimate concentrations to be assigned to non-detect samples, based on the concentration distribution of the detect samples and, for pesticides, including percentage crop treated;
- cumulate concentrations of compounds with a common mechanism of action;
- include weighing factors, for, for example, socio-demographic factors and season, to extrapolate the exposures to the general population.

Decision tree of VWA panel

Based on the experience of this study with the use of the decision tree, we recommend changing the order of the steps: perform step 2 (determine if reduction of exposure is possible) after step 5

(evaluation of the health risk). The reason for this is that it is only useful to lower the exposure when a health risk is present.

8.5 Conclusion

This report describes the dietary exposure and the related possible health risk to a selected number of compounds in Dutch children aged 2 to 6 years. For this food consumption data collected in a recent food consumption survey were combined with concentration values obtained from Dutch monitoring programmes to assess the exposure. By comparing the exposure with a health based limit value or by calculating a margin of exposure we assessed the possible health risk connected to the exposure to these compounds. For this we made use of the decision tree recently developed by the VWA panel.

It was demonstrated that based on the data available we were able to establish for which compounds the exposure through the diet is safe (or the health risk is negligible) for children, and for which compounds there may be a health risk. For compounds belonging to this latter group, recommendations were given for further research to refine the risk assessment.

Furthermore in this study, exposure and risk assessors worked closely together to establish the safety of compounds present in food in young Dutch children. This close cooperation has contributed to an increase in understanding of how risks related to compounds should be evaluated, interpreted and communicated. This knowledge will be useful in further research in food safety issues regarding the presence of adverse compounds in food.

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Appendix A List of concentrations used in the dietary exposure assessments (samples with concentration below LOR were assigned ½LOR)

Acrylamide

Acrylamide concentrations were obtained from (VWA, 2007a). All foods analysed were collected in 2006 from supermarkets, except for (prepared) French fries, which were sampled from snack bars in 2007. Organic crisps were omitted from the data since no consumption data were available. The LOR is 8 µg/kg. For an overview of the concentrations used in the exposure calculations, see Table A-1.

Table A-1. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) for acrylamide.

Food (group)	Total n samples	n samples > LOR	Mean concentration (µg/kg)
Children's cookie	1	1	1,630
Chips (French fries)	12	12	373
Dutch spiced cake	15	15	353
Crisps	7	7	343
Biscuits	10	10	325
Crisp bread	12	12	304
Spiced biscuits	15	15	284
Snacks	15	15	155
Children's biscuits	10	10	143
Cookies	14	14	115
Peanut butter[b]	2	2	113
Breakfast cereals	6	6	98
Chocolate (plain)[b]	2	2	96
Chocolate containing products (plain)[b]	2	2	96
Cornflakes	3	3	81
Crackers/toast	13	13	65
Peanuts[b]	4	4	46
Rye bread	4	4	45
Chocolate (milk)[b]	2	2	26
Chocolate containing products (milk)[b]	2	2	26
Coffee product[b]	2	2	21
Rusk	3	3	21
Coffee	18	18	15
Mixed nuts[b]	4	1	19.8

Food (group)	Total n samples	n samples > LOR	Mean concentration (µg/kg)
Chocolate drink and pudding[b]	4	1	6.8
Bread[b]	36	0	4

[a] LOR = Limit of reporting
[b] Not reported in (VWA, 2007a). Foods were however sampled in the same period and analysed by the same method (Erik Konings (VWA), personal communication).

Dioxins
Data on dioxin concentrations were obtained from the Dutch monitoring programme on dioxins of 2005-2006. Additional data on vegetables and fruits sampled in 2005 were extracted from (Traag and Hoogenboom, 2006). The LOR varies per congener and RAC or food analysed (W. Traag (RIKILT-Institute of Food Safety), personal communication)[11]. For an overview of the concentrations used in the exposure calculations, see Table A-2.

Table A-2. Total number of samples analyseda and the mean concentration (with samples with a concentration below LORb assigned ½ LOR) per food (group) for dioxins.

Food (group)	n samples	Mean concentration (in pg WHO-TEQ/g product or fat)[c]
Vegetable oils (pg/g product)		
Coconut fat	1	0.179
Olive oil	1	0.179
Sunflower oil	1	0.138
Maize/corn oil	1	0.179
Maize germ oil	1	0.179
Soya bean oil	1	0.179
Palm kernel oil	1	0.179
Peanut oil	1	0.179
Vegetable oils and fats	1	0.179
Vegetable & animal oils and fat	1	0.179
Potatoes (pg/g product)	1	0.0001
Cereals (pg/g product)		
Wheat	1	0.0001

[11] **Animal products** (incl. cheese and butter, no fish): LOR dioxins (PCDDs, PCDFs): 0.05-0.50 pg/g fat; LOR no-PCBs: 0.05 pg/g fat; LOR mo-PCBs: 10 pg/g fat
Fish: LOR dioxins (PCDDs, PCDFs): 0.05-0.50 pg/g product; LOR no-PCBs: 0.05 pg/g product; LOR mo PCBs: 10 pg/g product
Vegetables, fruits: LOR dioxins (PCDDs, PCDFs): 1-5 pg/g product; LOR no-PCBs: 5-10 pg/kg product; LOR mo-PCBs: 50-200 pg/kg product
Cereals: LOR dioxins (PCDDs, PCDFs): 0.05-0.50 pg/g product; LOR no-PCBs: 0.05 pg/g product; LOR mo-PCBs: 10 pg/g product

Food (group)	n samples	Mean concentration (in pg WHO-TEQ/g product or fat)[c]
Meat (pg/g fat)		
Meat of cow	17	1.135
Liver of cow	6	1.261
Fat of cow	1	1.501
Meat of pig	23	0.142
Liver of pig	2	0.492
Meat of sheep	7	1.639
Meat of horse	1	12.269
Meat of calf	15	0.493
Meat of chicken	9	0.186
Meat of turkey	9	0.186
Cow's milk (pg/g fat)	12	0.794
Cheese (pg/g fat)	5	0.649
Butter (pg/g fat)	1	0.533
Egg (pg/g fat)		
Whole egg, chicken	8	0.306
Egg yolk	8	1.019
Fish (pg/g product)		
Eel	6	7.569
Herring	4	1.253
Anchovy	4	1.253
Sardines	4	1.253
Mackerel	2	1.401
Tuna	3	0.066
Cod	2	0.341
Pollack, lithe	1	0.032
Plaice	2	0.247
Sole	1	0.217
Salmon	2	0.593
Fish medium fat	1	1.319
Shell fish (pg/g product)		
Crab	5	0.938
Shrimps	5	0.938
Mussel	4	1.057
Fruits (pg/g product)[d]	1	0.005

Food (group)	n samples	Mean concentration (in pg WHO-TEQ/g product or fat)[c]
Vegetables (pg/g product)		
Root/tuber vegetables	1	0.0002
Other vegetables (incl. mushrooms)[e]	1	0.001

[a] This number is also the total number of samples with a concentration above LOR. In every food group, at least one of the congeners was detected above LOR.
[b] LOR = Limit of reporting
[c] Congener concentrations below LOR in fruits, vegetables and cereals were assigned a zero concentration.
[d] Grapefruit, mandarin/clementine, pear
[e] Endive, lettuce, little gem, eggplant, French beans, sweet pepper, tomato

Mycotoxins

Aflatoxin B_1:
Aflatoxin B_1 concentrations were derived from monitoring programmes performed in the Netherlands in 2002-2006. The LOR of aflatoxin B_1 is 1 µg/kg, except for children's food for which the LOR is 0.01 g/kg (T. van der Horst (VWA), personal communication). For an overview of the concentrations used in the exposure calculations, see Table A-3.

Table A-3. Total number of samples analysed, number of samples with concentration above LORa and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) for aflatoxin B_1.

Food (group)	Total n samples	n samples > LOR	Mean concentration (µg/kg)
Sunflower seed	60	1	3.658
Maize	94	7	1.258
Rice	163	10	0.906
Sweet pepper	7	3	1.086
Biscuits	88	7	0.026
Peanut butter	639	29	0.576
Children's food	92	12	0.104
Popcorn	15	1	0.503
Vegetable oil	1	0	0.5
Goat's milk	179	34	0.001
Sheep's milk	179	34	0.001
Milk	179	34	0.001
Sesame seed	36	7	0.568
Nuts[b]	811	107	4.211
Ginger	57	30	2.097
Sweet corn	95	7	0.787
Date	216	3	0.499

Food (group)	Total n samples	n samples > LOR	Mean concentration (µg/kg)
Apricot-dried	71	1	0.010
Honey	7	1	0.1

[a] LOR = Limit of reporting

[b] Including hazelnut, peanut, walnut, coconut, Brazil nut, cashew nut, almond, pistachio, chestnut.

Table A-4. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) for DON.

Food (group)	Total n samples	n samples > LOR	Mean concentration (µg/kg)
Rusk/toast/crackers	11	4	91.4
Wheat bread	71	2	26.8
Biscuits and cookies	23	5	39.1
Semolina	31	14	171.8
Tortilla crisps	6	6	205.8
Popcorn	13	1	35.4
Wheat	487	227	110.8
Pasta	425	89	59.6
Rye	39	2	29.4
Barley	35	9	168.8
Oat	8	0	25.0
Maize	108	35	76.2
Rice	165	0	25.0
Nuts[b]	300	3	0.6
Sweet corn	108	35	59.3

[a] LOR = Limit of reporting

[b] Including hazelnut, peanut, walnut, coconut, Brazil nut, cashew nut, almond, pistachio, chestnut.

DON

The concentration data for DON were derived from monitoring programmes performed in the Netherlands in 2002-2006. The LOR of DON is 50 µg/kg (T. van der Horst (VWA), personal communication). For an overview of the concentrations used in the exposure calculations, see Table A-4.

Fumonisin B_1

The concentration data for fumonisin B_1 were derived from monitoring programmes performed in the Netherlands in 2002-2006. The LOR of fumonisin B_1 is 50 µg/kg (T. van der Horst (VWA) personal communication). For an overview of the concentrations used in the exposure calculations, see Table A-5.

Table A-5. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) for fumonisin B_1.

Food (group)	Total n samples	n samples .> LOR	Mean concentration (ug/kg)
Maize	97	71	1,354.3
Sweet corn	97	71	1,347.6
Children's food	29	5	349.8
Popcorn	14	2	44.6
Pasta	321	1	27.4
Barley	30	1	1.1
Wheat	340	2	25.9
Semolina	22	1	24.9
Rice	138	1	25.1

[a] LOR = Limit of reporting

OTA

The concentration data for OTA were derived from monitoring programmes performed in the Netherlands in 2002-2006, except for the concentration in raisins, which originated from targeted samples (section 2.4). Concentrations of OTA in meat originated from Germany (SCOOP, 2002a) and for poultry from Denmark (Jorgensen, 1998). The LOR of OTA is 2 µg/kg, with the exception of children's food for which LOR equals 0.02 µg/kg (T. van der Horst (VWA), personal communication). For an overview of the concentrations used in the exposure calculations, see Table A-6.

Table A-6. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) for OTA.

Food (group)	Total n samples	n samples >LOR	Mean concentration (ug/kg)
Wheat	91	12	1.4
Biscuits	57	33	0.5
Pork	58	8	0.0
Chicken	65	36	0.0
Raisins	7	7	5.3
Luncheon meat[b]	125	45	0.0
Cocoa beans	20	8	0.2
Children's food	56	20	0.1
Rice	19	3	0.9
Rye	6	2	22.7
Liver of pig	120	37	0.8
Nuts[c]	11	10	28.1
Grape juice	7	5	0.5
Liquorice salt	1	1	1.0

Food (group)	Total n samples	n samples >LOR	Mean concentration (ug/kg)
Plum	6	1	0.1
Sunflower seed	7	7	7.4
Tea	89	6	6.6
Meat of turkey	17	10	0.0
Buckwheat	1	1	20.7
Coffee beans	408	0	1.3
Ginger	5	4	4.4
Wine red	41	24	0.7
Dried apricot	6	3	10.7
Date	6	2	0.7
Brazil nut	11	10	28.2

[a] LOR = Limit of reporting
[b] Pork based
[c] Including hazelnut, peanut, walnut, coconut, Brazil nut, cashew nut, almond, pistachio, chestnut.

Patulin
The concentration data for patulin were derived from monitoring programmes performed in the Netherlands in 2002-2006. The LOR of patulin is 50 µg/kg (T. van der Horst (VWA), personal communication). For an overview of the concentrations used in the exposure calculations, see Table A-7.

Table A-7. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) for patulin.

Food (group)	Total n samples	n samples > LOR	Mean concentration (µg/kg)
Apple juice	40	12	20.4
Apple sauce	5	1	20.3
Other fruit juices[b]	76	0	25.0

[a] LOR = Limit of reporting
[b] In the calculations these samples were not used (section 5.2.5).

Nitrate
For potatoes and vegetables, the most recent analyses of 2002-2006 were used. Because spinach is mainly consumed as frozen spinach, nitrate concentrations of frozen spinach (VWA, 2005) analyzed in 2004 were added to the database. The LOR for vegetables was 50 mg/kg, both in the raw commodities as in frozen spinach (VWA, 2005). Very limited information was available on nitrate concentrations in wheat (1978-1988; 3 samples with an average concentration of 5 mg/kg). For fruits (only strawberry, apple, banana, bramble, grape, pear and orange were taken into account), VWA data of 1990-2001 were used, since more recent data were not available. For fruits the lowest level reported (LOR = 1 mg/kg) was assumed to be the LOR for fruit. Nitrate concentrations for vegetables and fruits were

calculated separately for the summer and the winter period. The summer period lasted from 1 April till 31 October and the winter period from 1 November till 31 March. This is according to the seasonal maximum limits set for spinach. These levels were linked to food consumption levels reported in the relevant period to assess the exposure to nitrate.

For tap water and drinks based on tap water (like. tea), the average nitrate concentration was used as reported by the tap water companies in 2006 (1118 samples; average level of 5.13 mg/L (range of 0.5 to 37 mg/L). The concentrations were kindly provided by the Centre of 'Inspectieonderzoek, Milieucalamiteiten en Drinkwater'. The average concentration was also applied to the water part of drinks (lemonades, soft drinks and fruit juices) and soup. Mineral and source water were assumed to contain 4 mg nitrate/L (information derived from Nestlé).

For an overview of the concentrations used in the exposure calculations, see Table A-8.

Table A-8. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned ½ LOR) per food (group) and season for nitrate.

	Summer			Winter		
Food (group)	Total n samples	n samples > LOR	Mean concentration (mg/kg)	Total n samples	n samples > LOR	Mean concentration (mg/kg)
Spinach frozen or canned	8	8	898.0	8	8	898.0
Tap water	1118	1118	5.1[b]	1118	1118	5.1[b]
Water mineral	1	1	4.0[1]	1	1	4.0[1]
Wheat	1	1	5.0	1	1	5.0
Maize	1	1	100.0	1	1	100.0
Buckwheat	123	123	45.3	123	123	45.3
Green/(garden) peas	2	2	100.0	2	2	100.0
Legume	10	11	93.2	3	3	100.0
Bean (scarlet/string/French)	7	7	550.0	7	7	731.4
Green beans	16	16	556.9	16	16	594.4
Lentils	1	1	100.0	1	1	100.0
Chicory	3	3	543.3	1	1	380.0
Endive	506	489	1680.2	227	223	1,645.7
Iceberg lettuce	212	216	893.5	94	94	1,060.7
Cabbage lettuce	433	428	2439.3	435	427	2,885.7
Celery leaves	1	1	2080.0	2	2	2,725.0
Spinach	309	290	1637.1	137	127	2,220.8
Parsley	2	2	725.0	1	1	1,000.0
Lambs lettuce	16	16	2881.3	5	5	2,424.0
Turnip tops/greens	1	1	4920.0	3	3	6,761.0
Asparagus	2	2	100.0	2	2	100.0

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Food (group)	Summer			Winter		
	Total n samples	n samples > LOR	Mean concentration (mg/kg)	Total n samples	n samples > LOR	Mean concentration (mg/kg)
Blanched celery	1	1	110.0	4	4	1,125.0
Broccoli	6	6	506.7	7	7	468.6
Cauliflower	21	21	231.0	7	6	282.9
Red cabbage	9	9	287.8	4	4	545.0
White cabbage	6	6	183.3	6	6	301.7
Brussels sprouts	2	2	100.0	7	7	100.0
Kohlrabi	1	1	2140.0	NA[c]	NA	NA
Curly kale	1	1	100.0	1	1	100.0
Chinese cabbage	4	4	1294.0	4	4	1,294.0
Savoy cabbage	2	2	570.0	4	4	247.5
Oxheart/conical cabbage	3	3	890.0	2	2	890.0
Onion, including pearl/cocktail	5	5	400.0	24	23	105.4
Leek	9	9	682.2	14	13	597.9
Garlic	1	1	100.0	3	3	100.7
Fennel	1	1	220.0	1	1	220.0
Potatoes	14	14	277.1	13	13	236.2
Carrot	14	15	164.3	18	16	241.2
Beetroot	83	83	2098.1	20	20	1,310.1
Radish	15	15	1663.3	5	5	2,720.0
Celeriac	1	1	1420.0	1	1	1,420.0
Cucumber	6	6	251.7	25	25	327.8
Tomato	12	11	93.8	20	17	89.1
Egg plant	1	1	430.0	8	8	452.5
Courgette	1	1	810.0	5	5	682.0
Sweet corn	1	1	100.0	1	1	100.0
Sweet pepper	6	6	103.3	31	19	90.5
Pumpkin	2	2	100.0	1	1	130.0
Gherkin/pickle	1	1	100.0	1	1	100.0
Mushroom	1	1	100.0	3	3	100.0
String bean	4	4	147.5	13	12	545.4
Banana	9	7	141.8	2	2	143.0
Papaya	3	3	400.0	3	3	400.0
Apple	31	25	33.4	60	49	24.6
Pear	8	12	35.3	12	6	9.3

Food (group)	Summer			Winter		
	Total n samples	n samples > LOR	Mean concentration (mg/kg)	Total n samples	n samples > LOR	Mean concentration (mg/kg)
Avocado	6	8	75.5	NA	NA	NA
Grape	55	36	46.8	28	22	47.1
Strawberry	25	22	133.0	5	3	161.2
Orange	1	1	0.2	NA	NA	NA

[a] LOR = Limit of reporting
[b] Unit: mg/L
[c] NA = Not available

OPs

The different OPs addressed in the cumulative exposure calculations are listed in Appendix C. These are the compounds found at levels above LOR in 2005-2006 in the Netherlands. LORs were in the range of 0.01 to 0.05 mg/kg product. Below we listed the cumulative levels per food analysed, treating levels below LOR as zero's, and using RPFs as listed in Appendix C.

For an overview of the concentrations used in the exposure calculations, see Table A-9.

Table A-9. Total number of samples analysed, number of samples with concentration above LOR[a] and the mean concentration (with samples with a concentration below LOR assigned zero) per food (group) for OPs.

Food (group)	Total n samples	n samples > LOR	Mean concentration (mg/kg)
Apple	439	81	0.0149
Apricot	32	4	0.0061
Egg plant	74	4	0.0885
Banana	112	3	0.0004
Barley	24	1	0.0079
Bean, (scarlet/string/French)	106	7	0.0231
Blackberry	40	3	0.0022
Blanched celery	76	9	0.0012
Blue berry	45	2	0.0022
Broad bean	23	1	0.0211
Broccoli	104	1	0.0007
Brussels sprouts	70	2	0.0179
Cabbage lettuce	635	160	0.0294
Carrot	235	25	0.0307
Cauliflower	177	3	0
Celeriac	32	5	0.0182
Celery leave	62	17	0.1781

Food (group)	Total n samples	n samples > LOR	Mean concentration (mg/kg)
Courgette	106	1	0.0083
Cucumber	306	8	0.0076
Curly kale	46	4	0.1843
Currant (red, white, black)	54	1	0.0013
Fennel	26	1	0.0006
Fig	24	4	0.1079
Grape	497	101	0.0123
Grapefruit	102	48	0.1321
Green beans	254	25	0.0373
Green/(garden) peas	36	3	0.0095
Guava	3	2	0.0605
Kaki	33	14	0.1241
Kiwi fruit	73	4	0.0198
Leek	237	1	0
Legume (fresh)	137	54	0.2256
Lemon	52	22	0.2669
Lime	44	5	0.0137
Litchi	21	3	0.137
Maize	9	1	0.0322
Mandarin, tangerines	275	208	0.2176
Mango	129	13	0.1266
Melon	167	4	0.0061
Mung bean sprouts	79	2	0.0062
Nectarine	103	37	0.0341
Oats	2	1	0.08
Onion, incl. pearl/cocktail onion	233	8	0.002
Orange	438	256	0.2392
Oxheart/conical cabbage	55	6	0.0126
Pak-choi cabbage	97	6	0.0054
Papaya	44	2	0.0156
Parsley	89	10	0.0084
Passion fruit	52	13	0.237
Peach	126	56	0.0562
Pear	274	23	0.0067
Pineapple	56	1	0.0001
Plum	85	9	0.0337

Food (group)	Total n samples	n samples > LOR	Mean concentration (mg/kg)
Pumpkin	16	1	0
Radicchio rosso	100	5	0.0001
Radish	44	2	0
Raisin	34	2	0.0013
Rice	46	2	0.0041
Rye	5	2	0.62
Savoy cabbage	23	1	0.0001
Spinach	212	5	0.0396
Strawberry	502	8	0.0007
String bean	117	49	0.7478
Sweet cherry	60	29	0.3821
Sweet pepper	534	51	0.0134
Tea	83	3	0.0045
Tomato	506	1	0.0001
Turnip tops/greens	47	3	0.0047
Watermelon	13	1	0.0769
Wheat	61	20	0.0485

[a] LOR = Limit of reporting

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Appendix B WHO toxic equivalence factors as used in the dietary exposure assessment of dioxins

Congener	WHO 2005 TEF[a]
Chlorinated dibenzo-p-dioxins	
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.0003
Chlorinated dibenzofurans	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.03
2,3,4,7,8-PeCDF	0.3
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.0003
Non-ortho-substituted PCBs	
3,3',4,4'-tetraCB (PCB 77)	0.0001
3,4,4',5-tetraCB (PCB 81)	0.0003
3,3',4,4',5-pentaCB (PCB 126)	0.1
3,3',4,4',5,5'-hexaCB (PCB 169)	0.03
Mono-ortho-substituted PCBs	
2,3,3',4,4'-pentaCB (PCB 105)	0.00003
2,3,4,4',5-pentaCB (PCB 114)	0.00003
2,3',4,4',5-pentaCB (PCB 118)	0.00003
2',3,4,4',5-pentaCB (PCB 123)	0.00003
2,3,3',4,4',5-hexaCB (PCB 156)	0.00003
2,3,3',4,4',5'-hexaCB (PCB 157)	0.00003
2,3',4,4',5,5'-hexaCB (PCB 167)	0.00003
2,3,3',4,4',5,5'-heptaCB (PCB 189)	0.00003

[a] Values are obtained from van den Berg et al. (2006).

Appendix C Relative potency factors for the pesticides for which concentrations above LOR were reported in Dutch monitoring programmes of 2005-2006

Compound	NOAEL (mg/kg)	BMD_{10}[a] (mg/kg)	Effect[b]	Source[c]	RPF
Acephate (index compound)		1	Rat/brain	JMPR02	1
	2.5		Rat/brain	JMPR02	1
	5		Rat/RBC	JMPR02	1
Azinphos-methyl		0.80	Rat/brain	EPA	1.25
Chlorfenvinphos	0.5[d]		Rat/brain	JMPR94	5
Chlorpyrifos		1.33	Rat/brain	EPA	0.75
Chlorpyrifos-methyl		16	Rat/brain	EPA	0.06
Diazinon		8	Rat/brain	EPA	0.13
Dichlorvos		2.67	Rat/brain	EPA	0.37
Dimethoate		0.25	Rat/brain	EPA	4.0
Ethion	0.6[d]		Dog/brain	JMPR90	4.2
Fenitrothion	0.36[e]		Human/RBC	JMPR00	0.03
Fenthion (sum)[g]		0.24	Rat/brain	EPA	4.2
Malathion		266.67	Rat/brain	EPA	0.004
Methamidophos		0.08	Rat/brain	EPA	12.5
Methidathion		0.25	Rat/brain	EPA	4.0
Mevinphos		0.11	Rat/brain	EPA	9.1
Monocrotophos	0.1		Rat/brain	HCN[f]	25
Omethoate		0.09	Rat/brain	EPA	11
Parathion	0.5		Rat/RBC	EPA	5
Parathion-methyl		0.67	Rat/brain	EPA	1.5
Phosalone		8	Rat/brain	EPA	0.125
Phosmet		4	Rat/brain	EPA	0.25
Pirimiphos-methyl		2	Rat/brain	EPA	0.5
Profenophos		20	Rat/brain	EPA	0.05
Tolclofos-methyl	790[d]		Rat/brain	JMPR94	0.003
Triazophos	1.5[d]		Rat/RBC	JMPR02	3.33

[a] BMD_{10} = Benchmark dose at which acetylcholinesterase activity was reduced by 10 %.
[b] RBC = Red blood cell count

[c] Source 'EPA' refers to (EPA, 2006). JMPR references refer to the 'Pesticide residues in food' reports of the respective years, available at <www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-docs/en>.
[d] Sub-chronic or chronic studies
[e] To calculate the RPF for fenitrothion we used for acephate an acute NOAEL of 0.01 mg/kg bw/d for RBC from a single dose in humans (FAO/WHO, 2002).
[f] HCN = Health Council of the Netherlands (2003)
[g] Fenthion (sum) includes fenthion, fenthion-sulfone and fenthion-sulfoxide.

Appendix D Details on linkage of analysed foods, food groups or raw agricultural commodities to foods entered in DNFCS Young Children 2005/2006

Acrylamide

Acrylamide concentration data were linked to food consumption data by creating food groups. All analysed and consumed foods were assigned to these food groups. The food groups consisted of comparable foods based on similarity of acrylamide concentrations and food characteristics, an approach that has been used before (Boon et al., 2005; Konings et al., 2003; Svensson et al., 2003). Especially in the group biscuits-cookies several food groups could be distinguished due to the varying concentrations. For the basic analysis, see the grouping in Table D-1. In this grouping, the analysed children's cookie (1 sample; Appendix A.) was only linked to the identical cookie coded in the food consumption survey, due to its divergent (= high) acrylamide level. Although this was only a single analysis, this concentration is not unlikely. This can be considered as a worst case assumption (when children eat this cookie the concentration will always be high). We performed a sensitivity analysis to study the effect of the grouping of biscuits/cookies on the exposure assessment. This analysis is described in Appendix J.

Based on the analyses performed in chocolate, two groups of 'chocolate containing foods' were created (Table D-1). Acrylamide concentrations were assigned to the foods in these groups based on the cacao content of the foods.

Table D-1. Linking of foods or food groups analysed for acrylamide to foods entered in DNFCS-Young Children 2005/2006.

	As entered in DNFCS Young children 2005/2006		
Food (group) name[a]	Food	Food in Dutch	%[b]
Crisps	Crisps	Chips	
	Potato crisps straws - salted	Frites sticks	
	Crisps light	Chips light	
Chips (French fries)	Rösti prepared without fat	Rösti bereid z vet	
	Potatoes frozen slices	Aardappelschijfjes diepvries onbereid	
	Oven-baked chips prepared	Frites oven-diepvries bereid	
	Chips pre-fried	Frites voorgebakken	
	Chips, commercial	Frites, commerc.	
Biscuits	Biscuit sweet (averaged)	Biscuit	
	Biscuit wholemeal	Biscuit volkoren-	
	Chocoprince vanilla	Chocoprince (vanille)	
	Biscuit breakfast Jamin	Biscuit ontbijt- Jamin	
	Liga evergreen biscuit with currants	Liga evergreen met krenten	
	Biscuit gluten-free biscuit glutafin Nutricia	Biscuit glutenvrij glutafin Nutricia	
	Liga evergreen biscuit several flavours	Liga evergreen ov smaken	
	Biscuit chocolate	Biscuit chocolade-	

Food (group) name[a]	As entered in DNFCS Young children 2005/2006		
	Food	Food in Dutch	%[b]
	Liga groot & sterk biscuit extra calcium	Liga groot & sterk extra calcium	
	Liga continue choc & cereals	Liga continue choc & granen	
	Biscuit coated[c]	Koekje met glazuur[c]	
	Biscuit normal[c]	Biscuit normaal[c]	
	Biscuit with cream filling[c]	Biscuit fourre[c]	
	Biscuit with chocolate[c]	Biscuit chocolade[c]	
	Biscuit n.s.[c]	Biscuit n.s.[c]	
Breakfast cereals	Breakfast cereal rice krispies Kellogg's	Ontbijtproduct rice krispies Kellogg's	
	Breakfast cereals n.s.	Ontbijtgranen n.s.	
	Breakfast cereal choco krispies Kellogg's	Ontbijtproduct choco krispies Kellogg's	
	Breakfast cereal fruit 'n fibre Kellogg's	Ontbijtproduct fruit 'n fibre Kellogg's	
	Breakfast cereal smacks Kellogg's	Ontbijtproduct smacks Kellogg's	
	Breakfast cereal honey loops Kellogg's	Ontbijtproduct honey loops Kellogg's	
	Albona 7 grain energy breaksfast cereals	Albona 7-granen energie ontbijtproduct	
	Breakfast cereal all bran Kellogg's	Ontbijtproduct all bran Kellogg's	
Crisp bread	Crispbread average	Knäckebröd gemiddeld	
	Crispbread gold-brown Wasa	Knäckebröd goudbruin Wasa	
	Crispbread sesame Wasa	Knäckebröd sesam Wasa	
	Crispbread high fibre Wasa	Knäckebröd vezelrijk Wasa	
	Crispbread wholemeal Wasa	Knäckebröd volkoren Wasa	
	Weetabix	Weetabix	
Spiced biscuits	Spiced biscuit	Speculaas	
	Shortbread biscuit Bastogne	Koek Bastogne	
	Spiced biscuit, almond paste filled	Speculaas gevulde	
	Spiced biscuit with chocolate milk	Kruidnoten met chocolade melk	
	Spiced biscuit filled, butter	Speculaas gevuld, roomboter	
	Spiced biscuit flakes	Schuddebuikjes	
Bread	Soft white roll	Broodje luxe- witte	
	Bread currant	Brood krenten-	
	Bread wheat	Brood tarwe-	
	Bread white made with milk	Brood wit- melk	
	Bread rye light	Brood rogge- licht	
	Bread raisin	Brood rozijnen-	
	Bread wholemeal	Brood volkoren-	
	Bread white made with water	Brood wit- water	
	Bread wheat malt Tarvo	Brood mout- Tarvo	
	Croissants prepared in tin	Croissants bereid blik	
	Bread wholemeal rye/wheat	Brood tarwerogge volkoren	

Food (group) name[a]	As entered in DNFCS Young children 2005/2006		
	Food	Food in Dutch	%[b]
	Bread white Turkish	Brood wit- turks	
	Bread gluten-free	Brood glutenvrij	
	Roll with pudding	Broodje pudding-	
	Bread unknown	Brood onbekend	
	Bread linseed	Brood lijnzaad	
	Bread sovital	Brood sovital	
	Bread casino white	Brood casino wit	
	Bread raisin with almond paste	Brood krente met spijs	
	Bread muesli	Brood muesli	
	Bread sugar	Brood suiker	
	Bread brown with fruits and nuts	Brood bruin met vruchten en noten	
	Bread brown, sunflower seeds	Brood bruin, zonnebloempitten	
	Baguette cheese-onion	Stokbrood kaas-uien	
	Bread wholemeal, sunflower, seeds	Brood volkoren, zonnebloempitten	
	Bread white, sunflower seeds	Brood wit, zonnebloempitten	
	Tortilla	Tortilla	
	Croissant ham - cheese	Croissant ham - kaas	
	Croissant cheese	Croissant kaas	
	Cheese bread roll	Broodje kaas - broodbasis	
Dutch spiced cake	Dutch spiced cake	Koek ontbijt-	
	Dutch spiced cake wholemeal Peijnenburg	Koek ontbijt- volkoren Peijnenburg	
	Dutch spiced cake filled with fruit	Koek ontbijt- m vruchtenvulsel	
	Dutch spiced cake with candy	Koek ontbijt met kandij	
	Dutch spiced cake bar	Koekreep	
	Dutch spiced cake with raisins	Koek ontbijt met rozijnen	
Children's biscuits	Liga second step 6 months	Liga tweede stap 6 mnd	
	Liga groot & sterk 12 months	Liga groot & sterk 12 mnd	
	Nutricia Bambix children's biscuit - 12 months	Nutricia Bambix berenkoekjes - 12 mnd	
	Nutricia Bambix children's biscuit - 15 months	Nutricia Bambix beestenboel koekjes - 15 mnd	
Peanuts	Peanuts unknown	Pinda's onbekend	
	Peanuts coated	Borrelnoten	50
Coffee	Coffee ready to drink	Koffie bereid	
Snacks	Biscuit salted	Biscuit zoute	
	Sticks salted	Stokjes zoute	
	Ringlings, Smith	Ringlings, Smith	
	Nibbits, Smith	Nibbits, Smith	
	Wokkels, Smith	Wokkels, Smith	
	Cheese biscuit average	Koekje kaas gemiddeld	
	Crisps tortilla plain	Chips tortilla naturel	
	Bread sticks	Soepstengels	
	Chipitos	Chipito's	

Food (group) name[a]	As entered in DNFCS Young children 2005/2006		
	Food	Food in Dutch	%[b]
	Bugles	Bugles	
Cookies	Biscuit average	Koekje gemiddeld	
	Liga fruitkick	Liga fruitkick	
	Biscuit muesli	Koek muesli-	
	Biscuit sugar-free	Koekje suikervrij	
	Biscuit sugar-coated long egg	Lange vingers	
	Biscuit Dutch short	Spritsstukken	
	Cracknel	Krakeling	
	Cake orange Pim's	Cake orange Pim's	
	Meringue cakes	Bokkenpootje	
	Biscuit coconut flavoured	Koek kokos- klapper	
	Waffle treacle	Wafel stroop-	
	Shortbread	Zandtaartjes	
	Biscuit fruit	Vruchtenkoekjes	
	Bar muesli with chocolate	Reep muesli- met chocolade	
	Liga milkbreak - all flavours	Liga milkbreak - alle smaken	
	Biscuit assorted with butter	Koekje roomboter - gemiddeld	
	Shortbread chocolate	Sprits chocolade	
	Gingersnap	Kletskop	
	Cake wrapped in marzipan and chocolate	Mergpijpje	
	Biscuit peanut	Koek pinda	
	Biscuit nut and choc/choc chips	Koek met noten en choc/choc chips	
	Waffle galette	Wafel galette	
	Biscuit orange	Koek oranje	
	Shortbread with chocolate	Zandkoekje met chocolade	
	Biscuits (digestive) n.s.	Liga-achtigen n.s.	
	Biscuit fruit sultana	Fruitbiscuit sultana e.d.	
	Bar muesli with chocolate	Reep mueslireep met chocolade	
	Bar cereal/fruit	Reep granen/vruchten	
	Muesli bar average	Reep muesli gewoon	
	Waffles	Wafels	
	Cookie with nuts and chocolate	Koek met noten en chocolade	
	Brinta fruitvit	Brinta fruitvit	
Crackers/toast	Crispbread wholemeal Cracottes	Cracker volkoren Cracottes	
	Crackers cream	Cracker cream-	
	Cracker matzo	Cracker tea- matses hollandia	
	Toast	Brood geroosterd	
	Crispbread Cracottes	Toast naturel Cracottes	
	Cracker gluten-free glutafin Nutricia	Cracker glutenvrij glutafin Nutricia	
	Cracottes n.s.	Cracottes n.s.	
	Dutch rusk	Beschuit	
	Dutch rusk wholemeal	Beschuit volkoren	
	Dutch rusk multigrain	Beschuit meergranen	

Food (group) name[a]	As entered in DNFCS Young children 2005/2006		
	Food	Food in Dutch	%[b]
Rye bread	Bread rye dark	Brood rogge- donker	
	Bread rye	Brood rogge-	
Cornflakes	Breakfact cereal corn flakes Kellogg's	Ontbijtproduct cornflakes Kellogg's	
	Breakfast cereal frosties Kellogg's	Ontbijtproduct frosties Kellogg's	
	Breakfast cereal special K Kellogg's	Ontbijtproduct special K Kellogg's	
	Breakfast cereal cornflakes	Ontbijtproduct cornflakes	
	Breaksfast cereal cornflakes n.s.	Ontbijtproduct cornflakes n.s.	
Chocolate (milk)	Chocolate milk	Chocolade melk-	100
	Chocolate milk without sugar	Chocolade melk- zonder suiker	100
	Chocolate milk butterscotch	Chocolade melk butterscotch	100
	Chocolate filled with fruits	Chocolade gevuld met vruchten	100
	Chocolate M & M's	Chocolade M & M's	100
	Cocoa powder sweetened Benco	Cacaopoeder gezoet Benco	100
	Chocolate sprinkles average	Hagelslag chocolade- gem	100
Chocolate (plain)	Chocolate plain	Chocolade puur	100
	Chocolate plain without sugar	Chocolade puur zonder suiker	100
	Chocolate plain with nuts	Chocolade puur met noten	100
Peanut butter	Peanut butter	Pindakaas	
Chocolate containing products (milk)	Chocolate milk with nuts	Chocolade melk met noten	50
	Candy bars n.s.	Candy bars n.s.	50
	Mars	Mars	50
	Milky way	Milky way	50
	Bounty	Bounty	50
	Snickers	Snickers	50
	Desert sauce chocolate	Dessertsaus chocolade-	50
	Nuts	Nuts	50
	Chocolate with peanuts M & M's	Chocolade met pinda's M & M's	50
	Twix	Twix	50
	Chocolate praline	Bonbon	50
	Chocolate sprinkles milk De Ruijter	Hagelslag chocolade- melk De Ruijter	50
	Chocolate pasta milk De Ruijter	Pasta chocolade- melk De Ruijter	25
	Raisins in milk chocolate wrap	Rozijnen met melkchocolade omhuld	50
	Chocolate pasta coconut	Chocoladepasta cocos	25
	Chocolate frogs/mice	Chocolade kikkers/muizen	50
Chocolate containing products (plain)	Chocolate pasta plain De Ruijter	Pasta chocolade- puur De Ruijter	15
	Chocolate sprinkles plain De Ruijter	Hagelslag chocolade- puur De Ruijter	66

Food (group) name[a]	As entered in DNFCS Young children 2005/2006		
	Food	Food in Dutch	%[b]
	Marshmellow & biscuit wrapped in chocolate	Negerzoen	50
	Cocoa powder	Cacaopoeder	200
Coffee product	Cappuccino, instant ready to drink	Cappuccino oplos bereid	
Mixed nuts	Nuts mixed unsalted	Noten gemengd ongezouten	
Chocolate drink and pudding	Mousse chocolate	Mousse chocolade-	
	Pudding choclate with cream sauce Mona	Pudding chocolade- m roomsaus Mona	
	Milk chocolate semi-skimmed	Melk chocolade- halfvolle	
	Milk chocolate semi-skimmed chocomel Nutricia	Melk chocolade- halfvolle chocomel Nutricia	
	Milk chocolate semi-skimmed made of instant powder	Melk chocolade halfvolle van instantpoeder	
	Milk chocolate full-cream	Melk chocolade- volle	
	Milk chocolate skimmed	Melk chocolade- magere	
	Custard chocolate full-cream	Vla chocolade- volle	
Children's cookie	Children's biscuit	Kinderkoek	

[a] Food (group) names are those listed in Table A-1 (Appendix A).
[b] The percentages listed express for food 'borrelnoten' the amount of peanut in the food, and for chocolate containing foods the cocoa amount of these foods relative to the cocoa amount of chocolate. The percentages were combined with the respective acrylamide concentrations of the food groups.
[c] These biscuits could also be seen as typically children's biscuits. We identified them as such in scenario 2 as described in Appendix J.

Dioxins
Most dioxin analyses were performed in raw agricultural commodities (RACs). To link these concentrations to consumed foods the food conversion model (van Dooren et al., 1995) was used. This model is described in detail in section 2.5. The dioxin concentrations in animal products, excluding fish, shellfish and vegetable oils, were linked to consumed foods based on fat weight, while dioxin concentrations in vegetables, fruits and cereals were linked to consumed foods on product weight. Different from the general procedure in the food conversion model, cheese and butter were directly linked to dioxin concentrations in cheese and butter (and not via milk), since concentrations in these two foods were available.

For four types of coded fish in the food consumption survey (namely anchovy, sardines, crab fish and semi-fat fish) no analytical data were available. To link these fish types to dioxin concentrations, the following assumptions were made based on the fat properties of these fish types: anchovy and sardines were both assigned the concentration of herring, crab fish was assigned the concentration of shrimps and semi-fat fish was assigned the average of the dioxin concentrations in sole, plaice and perch.

A very limited number of vegetables and fruits had been analysed (Table A-2; Appendix A). To use these levels in the best way, the concentration levels of the analysed vegetables were linked to coded vegetables in the food consumption survey based on product characteristics. This resulted in assigning the concentration in beets to all root vegetables, while the mean level of endive, head cabbage, little gem, eggplant, French beans, pepper and tomato was linked to all other coded vegetables. For fruits

only grapefruit, mandarin/clementine and pear were analysed. The mean level analysed in these fruit types was linked to all coded fruits in the food consumption survey.

There were two samples of non-specified vegetable oils and one sample of sunflower oil analysed. The difference in dioxin concentrations between the two non-specified oil samples was significantly (0.046 vs. 0.23 pg WHO-TEQ/kg fat (lower bound estimate)). The origin of the non-specified oil samples could however not be determined. We could therefore not conclude if the concentrations were realistic. Based on a more recent analysis of a mixed sample of 14 different vegetable oils within the monitoring programme, we concluded that only the sample with the lower concentration should be included in the present exposure assessment (data not shown). All coded vegetable oils in the food consumption survey, except for sunflower oil, were assigned the concentration of the non-specified vegetable oil sample with the lowest dioxin concentration.

Mycotoxins

Aflatoxin B_1

Aflatoxin B_1 analyses performed in composite foods, such as biscuits, dried apricots, children's food (porridge and jars), peanut butter, vegetable oil and popcorn (Table A-3; Appendix A), were directly linked to consumption levels of these foods. For the composite foods which were not analysed themselves, the analyses of aflatoxin B_1 in RACs were used to calculate the concentration.

Table D-2. Grouping of analysed foods with limited concentration data in food groups for the dietary exposure to aflatoxin B_1.

Food group name	Food name	Food name in Dutch
Rice	Rice flour	Rijstmeel
	Rice	Rijst
Maize	Maize meal	Maismeel
	Popcorn	Gepofte mais
	Maize starch	Maiszetmeel
	Maize powder	Maizena
	Maize	Mais
Nuts	Almond	Amandel
	Cashew	Cashewnoot
	Coconut	Kokosnoot
	Hazelnut	Hazelnoot
	Macadamia nut	Macadamianoot
	Brazil nut	Paranoot
	Pecan	Pecannoot
	Peanut	Pinda
	Pistachio nut	Pistache noot
	Walnut	Walnoot
Children's food	Children's food	Kindervoeding
	Children's food (porridge)	Kindervoeding (pap)
	Children's food (jar)	Kindervoeding (potje)

For some foods VWA only provided limited concentration data. To preserve the data the following food groups were created consisting of similar foods: rice, maize, nuts and children's food. For details, see Table D-2.

DON
DON analyses performed in composite or processed foods, *i.e.,* toast/crackers, bread, biscuits, dried apricots, children's food (porridge and jars), couscous, pasta, maize crisps, peanut butter and popcorn,

Table D-3. Grouping of analysed foods with concentration data in food groups for the dietary exposure to DON.

Food group name	Food name	Food name in Dutch
Toast	Toast	Toast
	Rusk	Beschuit
	Crackers	Crackers
Maize	Maize	Mais
	Semolina	Maisgriesmeel
	Maize flour	Maismeel
	Corn starch	Maiszetmeel
Wheat	Self-raising flour	Bakmeel
	Gluten	Gluten
	Bread crumbs	Paneermeel
	Wheat	Tarwe
	Wheat flour	Tarwebloem
	Wheat germs	Tarwekiemen
	Wheat meal	Tarwemeel
	Wheat bran	Tarwezemelen
	Wheat starch	Tarwezetmeel
Wheat bread	Wheat bread, brown	Tarwebrood(jes), bruin
	Wheat bread, multi cereal	Tarwebrood(jes), meergranen
	Wheat bread, wholemeal	Tarwebrood(jes), volkoren
	Wheat bread, white	Tarwebrood(jes), wit
	Wheat and rye bread	Tarweroggebrood
Biscuits	Biscuits	Biscuitjes
	Cookies	Koekjes
Nuts	Almond	Amandel
	Cashew nut	Cashewnoot
	Coconut	Cocosnoot
	Hazelnut	Hazelnoot
	Macadamia nut	Macadamianoot
	Para nut	Paranoot
	Pecan nut	Pecannoot
	Peanut	Pinda
	Pistachio nut	Pistache noten
	Walnut	Walnoot
Children's food	Children's food	Kindervoeding
	Children's food (porridge)	Kindervoeding (pap)
	Children's food (jar)	Kindervoeding (potje)

were directly linked to consumption levels of these foods. For the composite foods which were not analysed themselves, the analyses of DON in RACs were used to calculate the concentration. The data on blancmange powder, the only food sampled less than five times while finding concentration(s) above LOR, were omitted.

For some foods VWA only provided limited concentration data. To preserve the data the following food groups were created consisting of similar foods: toast, maize, wheat, wheat bread, biscuits, nuts and children's food. For more details, see Table D-3.

Fumonisin B_1
Fumonisin B_1 analyses performed in composite foods, such as toast, biscuits, children's food, couscous, pasta and popcorn were directly linked to consumption levels of these foods. The fumonisin B_1 data on blancmange powder, the only product in sampled less than five times while finding concentration(s) above LOR, were omitted.

For some foods VWA only provided limited concentration data. To preserve the data the following food groups were created consisting of similar foods: toast, maize, wheat, biscuits and children's food. For more details, see Table D-4.

Table D-4. Grouping of analysed foods with limited concentration data in food groups for the dietary exposure to fumonisin B_1.

Food group name	Food name	Food name in Dutch
Toast	Crackers	Crackers
	Toast	Toast
Biscuits	Biscuits	Biscuitjes
	Cookies	Koekjes
Maize	Maize	Mais
	Semolina	Maisgriesmeel
	Maize meal	Maismeel
	Maize starch	Maiszetmeel
	Sweet maize	Zoete mais
Wheat	Self-raising flour	Bakmeel
	Gluten	Gluten
	Breadcrumbs	Paneermeel
	Wheat	Tarwe
	Wheat flour	Tarwebloem
	Wheat bread, brown	Tarwebrood(jes), bruin
	Wheat bread, multi-cereal	Tarwebrood(jes), meergranen
	Wheat bread, wholemeal	Tarwebrood(jes), volkoren
	Wheat germs	Tarwekiemen
	Wheat meal	Tarwemeel
	Wheat bran	Tarwezemelen
	Wheat starch	Tarwezetmeel
Children's food	Children's food	Kindervoeding
	Children's food (porridge)	Kindervoeding (pap)
	Children's food (jar)	Kindervoeding (potje)

Table D-5. Grouping of analysed foods with limited concentration data in food groups for the dietary exposure to OTA.

Food group name	Food name	Food name in Dutch
Toast	Toast	Toast
	Crackers	Crackers
Fruit drink	Fruit lemonade	Vruchtenlimonade
	Apple juice	Appelsap
	Concentrated fruit juice	Geconcentreerd vruchtensap
	Pear juice	Perensap
	Orange juice	Sinaasappelsap
	Fruit juice	Vruchtensap
Rice	Rice flour	Rijstmeel
	Rice	Rijst
Buckwheat	Buckwheat flour	Boekweitmeel
	Buckwheat	Boekweit
Wheat	Self-raising flour	Bakmeel
	Gluten	Gluten
	Wheat	Tarwe
	Wheat flour	Tarwebloem
	Wheat germs	Tarwekiemen
	Wheat meal	Tarwemeel
	Wheat bran	Tarwezemelen
	Wheat starch	Tarwezetmeel
	Wheat bread, wholemeal	Tarwebrood(jes), volkoren
Tea	Tea	Thee
	Tea with supplements	Thee met toevoegingen
Nut	Almond	Amandel
	Cashew	Cashewnoot
	Coconut	Kokosnoot
	Hazelnut	Hazelnoot
	Macadamia nut	Macadamianoot
	Brazil nut	Paranoot
	Pecan	Pecannoot
	Peanut	Pinda
	Pistachio nut	Pistache noot
	Walnut	Walnoot
Seed	Pine nut	Pijnboompit
	Pumpkinseed	Pompoenpit
	Sunflower, seed	Zonnebloempit
	Linseed	Lijnzaad
	Mustard, seed	Mosterdzaad
	Sesame, seed	Sesamzaad
Children’s food	Children’s food	Kindervoeding
	Children’s food (porridge)	Kindervoeding (pap)
	Children’s food (jar)	Kindervoeding (potje)

OTA
OTA data analysed in composite foods, such as toast/crackers, bread, dried apricots, children's food (porridge and jars), couscous, pasta, peanut butter, nuts and biscuits were directly linked to consumption levels of these foods. The data on blancmange powder, which is the only product in sampled less than five times while finding concentration(s) above LOR, were omitted.

For some foods VWA only provided limited concentration data. To preserve the data the food groups as listed in Table D-5 were created consisting of similar foods.

Patulin
The concentrations in apple juice and apple sauce were directly linked to consumption amounts of these foods. Because the analyses of fruit juices were all non-detects, replacing the non-detects by ½LOR or LOR resulted in unrealistically high concentrations. For that reason, it was decided to calculate the concentrations in fruit juices based on the amount of apple juice in a certain fruit juice rather than on the direct measurements. The food conversion table (van Dooren et al. 1995) was used to perform these calculations (for more details on this model, see section 2.5).

rivm

Appendix E Long-term dietary exposure assigning either zero, ½LOR or LOR to samples with a concentration below LOR

Acrylamide

Table E-1. Percentiles of long-term dietary exposure of children aged 2 to 6 years to acrylamide assigning either zero, ½LOR[a] or LOR to samples with an analysed concentration below LOR[b].

Age (years)	Exposure (µg/kg bw/d)								
	P50			P95			P99		
	0	½LOR	LOR	0	½LOR	LOR	LOR	½LOR	LOR
2-6[c]	0.6	0.7	0.7	1.1	1.2	1.2	1.4	1.5	1.5

[a] LOR = Limit of reporting
[b] For acrylamide only bread samples contained concentrations below the LOR of 8 µg/kg.
[c] Age dependency was not significant.

Dioxins

Congeners with concentration below LOR were assigned zero, ½LOR or LOR, except for vegetables, fruits and cereals. In these three food groups, congener levels below the LOR were assigned zero. We chose to do this, because middle and high bound levels in these food groups very likely overestimate the true levels. Dioxins are associated with the fat part in foods and most fruits, cereals and vegetables contain little to no fat. For more details on LOR levels, see Appendix A.

Table E-2. Percentiles of long-term dietary exposure of children aged 2 to 6 years to dioxins assigning either zero, ½LOR[a] or LOR to samples with an analysed congener concentration below LOR.

Age (years)	Exposure (pg/kg bw/d)								
	P50			P95			P99		
	0	½LOR	LOR	0	½LOR	LOR	0	½LOR	LOR
2	1.2	1.5	1.8	1.9	2.4	2.8	2.4	2.8	3.3
3	1.1	1.4	1.7	1.8	2.2	2.7	2.2	2.7	3.2
4	1.0	1.3	1.6	1.7	2.1	2.5	2.1	2.5	3.0
5	1.0	1.2	1.5	1.6	2.0	2.4	2.0	2.4	2.9
6	0.9	1.2	1.4	1.5	1.9	2.3	1.9	2.3	2.7

[a] LOR = Limit of reporting

Mycotoxins

Aflatoxin B_1

The presence of aflatoxin B_1 can be expected in foods containing maize, groundnuts, dried fruit, tree nuts, spices, figs, crude vegetable oils, cocoa beans, rice and copra. Samples with a concentration below LOR that may contain aflatoxin B_1 (including maize, maize containing foods, rice, rice containing foods, figs, oilseed, vegetable oil, nuts, nut-containing foods, peanut butter, spice, children's food and popcorn) were assigned either zero or LOR, respectively. The LOR of aflatoxin B_1 is 1 µg/kg,

Table E-3. Percentiles of long-term dietary exposure of children aged 2 to 6 years to different mycotoxins assigning either zero, ½LOR[a] or LOR to samples with an analysed concentration below LOR.

Age (years)	Exposure (ng/kg bw/d)								
	P50			P95			P99		
	0	½ LOR	LOR	0	½ LOR	LOR	0	½ LOR	LOR
Aflatoxin B_1 (in ng/kg bw/d)									
2-6[b]	0.5	0.8	1.0	1.4	1.9	2.5	1.9	2.7	3.4
DON (in µg/kg bw/d)									
2	0.1	0.3	0.5	0.3	0.5	0.7	0.4	0.6	0.9
3	0.1	0.3	0.4	0.3	0.4	0.7	0.4	0.5	0.8
4	0.1	0.3	0.4	0.2	0.4	0.6	0.3	0.5	0.8
5	0.1	0.3	0.4	0.2	0.4	0.6	0.3	0.5	0.7
6	0.1	0.2	0.4	0.2	0.4	0.6	0.3	0.4	0.7
Fumonisin B_1 (in µg/kg bw/d)									
2-6[b]	0.1	0.3	0.4	0.5	0.7	0.9	0.8	1.0	1.3
OTA (in ng/kg bw/d)									
2-6[b]	7.6	11	15	21	25	29	28	32	38
Patulin (in µg/kg bw/d)									
2-6[b]	0.003	0.03	0.06	0.02	0.1	0.3	0.03	0.2	0.4

[a] LOR = Limit of reporting
[b] Due to multimodal distribution of daily exposure, the ISUF method was used to model long-term exposure. This approach does not give information on age dependency of exposure.

except for children's food for which the LOR is 0.005 µg/kg (T. van der Horst (VWA), personal communication).

DON
The presence of DON can be expected in foods containing cereals or grains. The LOR of DON is 50 µg/kg (T. van der Horst (VWA), personal communication).

Fumonisin B_1
The presence of fumonisin B_1 can be expected in maize, wheat, sorghum, asparagus, rice, mung beans and tea. The LOR of fumonisin B_1 is 50 µg/kg (T. van der Horst (VWA), personal communication).

OTA
The presence of OTA can be expected in foods containing cereals or grains, dried fruit (raisins), pulses, coffee, peanuts. The LOR of OTA is 2 µg/kg (T. van der Horst (VWA), personal communication).

Patulin
The presence of patulin can be expected in products with processed apples, such as apple juice and apple sauce and not in the raw fruit, since rotten fruit will not be consumed. As apple juice is used in

several multi-fruit juices present on the Dutch market, these juices may contain patulin as well. The LOR of patulin is 50 µg/kg (T. van der Horst (VWA), personal communication).

Nitrate

For nitrate only a very limited number of samples had nitrate concentrations below LOR. Therefore the results of analyses for the lower, middle and upper bound scenarios were almost similar (Table E-4). For details on LOR levels, see Appendix A.

Table E-4. Percentiles of long-term dietary exposure of children aged 2 to 6 years to nitrate assigning either zero, ½LOR[a] or LOR to samples with an analysed concentration below LOR.

Age (years)	Exposure (mg/kg bw/d)								
	P50			P95			P99		
	0	½LOR	LOR	0	½LOR	LOR	0	½LOR	LOR
Summer									
2	1.9	1.9	1.9	3.6	3.6	3.6	4.7	4.7	4.7
3	1.8	1.8	1.8	3.4	3.4	3.4	4.4	4.4	4.4
4	1.6	1.6	1.7	3.1	3.1	3.1	4.0	4.1	4.1
5	1.5	1.5	1.5	2.9	2.9	2.9	3.8	3.8	3.8
6	1.4	1.4	1.4	2.7	2.7	2.7	3.5	3.5	3.5
Winter									
2	1.7	1.7	1.7	2.9	2.9	2.9	3.6	3.6	3.6
3	1.6	1.6	1.6	2.6	2.6	2.6	3.3	3.3	3.3
4	1.4	1.4	1.4	2.4	2.4	2.4	3.0	3.0	3.0
5	1.3	1.3	1.3	2.2	2.2	2.2	2.7	2.7	2.7
6	1.2	1.2	1.2	2.0	2.0	2.0	2.4	2.4	2.4

[a] LOR = Limit of reporting

Appendix F Modelling of processing

Processing factors were applied in the dietary exposure assessment of OPs and all mycotoxins, except patulin. Processing factors are relevant when analyses are perform in raw agricultural commodities which generally undergo a certain form of processing before consumption, such as peeling, cooking, washing, juicing, etc. Processing is known to affect concentrations (mainly reduction). Ignoring the effect of processing may often result in an overestimation of the exposure, but may at times also underestimate the levels of a pesticide present in foods as consumed (*e.g.*, dried products like raisins).

Below we will address how processing was included in the assessment of OPs and mycotoxins.

A. Processing factors used.

The information on processing effect for OPs was obtained from (van Klaveren et al., 2006). In this report, processing factors are reported for OPs to be applicable to a whole group of fruit or vegetables, as well as all OPs addressed (Table F-1). This was due to the limited information available per RAC-compound-processing type combination. Applying these processing factors is a simplification of reality, because in practice processing factors will differ per compound and RAC.

Table F-1. Processing factors used to assess the acute cumulative exposure of children aged 2 to 6 years to organophophorus insecticides.

	Processing factor[a]	
Type of processing	Nominal value[b]	Upper value[b]
Washing		
Fruit/vegetables	0.76	0.94
Peeling		
Fruit (citrus/exotic)	0.44	0.99
Other fruits/vegetables[c]	0.76	0.94
Cooking/canning[d]		
Vegetables	0.74	0.99
Drying		
Grape	0.49	3.18
Sauces		
Apple	0.67	0.92

[a] Processing effects were assumed to be variable between occasions and defined as a normal distribution either after a logistic transformation (for processing factors restricted between 0 and 1) or after a lognormal transformation (for processing factors that can be above 1). Mean and standard deviation of the normal distribution are derived from nominal and upper values specified at the natural scale, which are considered as 50th and 95th percentile of the distribution.
[b] Processing factor nominal and upper values were based on an assessment of data available for all organophosphorus pesticides.
[c] No data available. We therefore decided to equal the processing factors for peeling other fruits/vegetables to those for washing
[d] Except canned sauerkraut, which was considered as washed (factor = 0.75).

For **aflatoxin B_1, DON, fumonisin B_1 and OTA** processing factors were available for certain types of processing. See Table F-2 for a list of the factors used in the dietary exposure assessments.

Table F-2. Processing factors used to assess the long-term dietary exposure of children aged 2 to 6 years to aflatoxin B_1, DON, fumonisin B_1 and OTA.

Food and mycotoxin	Type of Processing	Processing factor				Source
		Nominal value	Upper value	Uncertainty about mean (P95 of mean)	Uncertainty about variance (df of variance)	
Aflatoxin B_1						
Uncooked rice	Cooking in water	0.66	0.69	0.999	20	(Park et al., 2005b)
Whole grain maize	Preparing dough and baking of tortilla	0.69 fixed value	NA[a]	NA	NA	(Mendez-Albores et al., 2004; Price and Jorgensen, 1985)
DON						
Uncooked pasta and noodles	Cooking in water	0.53	0.57	0.56	0.2	(Nowicki et al., 1988)
Uncooked rice	Cooking in water	0.5	0.6	0.7	0.1	Estimated based on pasta
Fumonisin B_1						
Tortilla	Preparing dough and baking of tortilla	0.35	0.55	0.5	17	(Dombrink-Kurtzman et al., 2000; Palencia et al., 2003; Saunders et al., 2001; Voss et al., 2001)
Corn flakes	Extrusion and baking of cornflakes	0.3	0.5	0.4	0.1	(Saunders et al., 2001)
Cooked maize	Cooking in water	0.9	0.999	0.999	20	(Bullerman et al., 2002; Jackson et al., 1996; Saunders et al., 2001)
Cake/muffins	Preparing dough and baking of cake/muffins	0.71	0.999	0.999	20	(Humpf and Voss, 2004)
Bread	Preparing dough and baking of bread	0.7	0.999	0.999	20	Based on muffin data
OTA						
Uncooked pasta and noodles	Cooking in water	0.7	0.8	0.999	20	Estimate based on rice data
Uncooked rice	Cooking in water	0.72	0.75	0.999	20	(Park et al., 2005a)

[a] NA = Not available

For **nitrate** only limited information was available on the effects of processing on nitrate concentrations in fruits and vegetables (Dejonckheere et al., 1994; Meah et al., 1994). Meah et al. (1994) studied processing effects in a variety of vegetables and some fruits (Table F-3). The reported processing factors were applied to calculate nitrate content of cooked vegetables and potatoes. For cooked vegetables and potatoes with unknown processing factor, an average processing factor 0.49 was assumed.

Table F-3. Processing factors used to assess the long-term dietary exposure of children aged 2 to 6 years to nitrate.

Food	Processing	Nominal value[a]
Chicory	Cooking in water	0.42
Endive	Cooking in water	0.16
Celery leaves	Cooking in water	0.83
Spinach	Cooking in water	0.31
Rhubarb	Cooking in water	0.6
Asparagus	Cooking in water	0
Blanched celery	Cooking in water	0.83
Broccoli	Cooking in water	0.4
Cauliflower	Cooking in water	0.02
Red cabbage	Cooking in water	0.43
White cabbage	Cooking in water	0.2
Brussels sprouts	Cooking in water	0.59
Kohlrabi	Cooking in water	0.55
Chinese cabbage	Cooking in water	0.2
Savoy cabbage	Cooking in water	0.28
Onion, including pearl/cocktail onion	Cooking in water	0
Leek	Cooking in water	0.54
Fennel	Cooking in water	0.6
Carrot	Cooking in water	0.55
Bunched carrot	Cooking in water	0.55
Beetroot	Cooking in water	0.73
Celeriac	Cooking in water	0.41
Tomato	Cooking in water	0.86
Courgette	Cooking in water	1.11
Sweet pepper	Cooking in water	0.11
Slicing beans	Cooking in water	0.64
Pumpkin	Cooking in water	0.73
Mushroom	Cooking in water	0.64
Cantharelle	Cooking in water	0.64

Food	Processing	Nominal value[a]
Banana	Peeling	0.38

[a] All processing factors were obtained from Meah et al. (1994), except for the processing factor for peeling banana. This last factor was obtained from Dejonckheere et al. (1994).

B. Modelling of processing

All factors are reported as a (logistic) distribution. The mean and variance of this distribution are described by its P50 and P95 respectively. The uncertainty about the mean and variance are described by the P95 of the mean and the number of degrees of freedom of the variance. For details about this approach, see MCRA manual 6, section 9.4.5 (de Boer and van der Voet, 2007).

Shortly, the effects of processing on OPs and mycotoxin concentrations were assumed to be variable by defining a distribution rather than a point estimate for each processing factor (de Boer and van der Voet, 2007). We assumed normal distributions for either the logistic (mycotoxins and OPs) or the log (OPs) transform of the processing factors, depending on the processing type. In a logistic-normal distribution the sampled processing factors will always fall between 0 and 1, while in the log-normal distribution sampled processing factors are positive, but can exceed 1, as is appropriate for, for example, drying. The distributions are specified by two parameters, for which the nominal value (PFm) and the upper 97.5 % confidence limit (PFupp), obtained in an assessment of available data, were taken. Using these two parameters, a normal distribution for the transformed processing factors is specified by a mean equal to logit(PFm) or ln(PFm), and a standard deviation equal to (logit(PFupp)-logit(PFm))/1.645 or (ln(PFupp)-ln(PFm))/1.645, respectively.

For mycotoxins also the uncertainty about the mean and variance were described by the P95 of the mean and the number of degrees of freedom of the variance. For details, see MCRA manual 6, section 9.4.5 (de Boer and van der Voet, 2007).

Due to the limited information, processing factors for nitrate were considered to be fixed.

Appendix G Variability between individual units within composite samples as applied in the cumulative exposure to OPs

When acute dietary exposure calculations are performed for single pesticides with monitoring concentrations derived from composite samples variability factors (also referred to as homogeneity factors) should be included in the assessment (EC, 2001b; FAO, 2002). These factors account for the fact that concentrations analysed in a mixed sample can originate from one individual unit of the commodity, and that consumers may be confronted with a concentration in a single unit (*e.g.*, an apple) rather than the averaged concentration as analysed in composite samples (of *e.g.,* 12 apples). Which variability factor to use for concentrations derived from monitoring samples is unclear. Based on two studies (Ambrus, 2006; Hamilton et al., 2004), a factor of 3 was used in this study for all raw agricultural commodities (RACs) addressed. This factor has been adopted at international level by JMPR for the deterministic estimation of the acute dietary exposure to pesticides (FAO/WHO, 2004). However, when establishing maximum residue levels (MRLs) of pesticides within Europe, the European Commission, depending on the unit weight of the commodity, uses the variability factors 3, 5 or 7 (EFSA, 2005c). To demonstrate the effect of using other variability factors than 3, also a sensitivity analysis was performed in which the cumulative exposure was calculated using these variability factors.

In the deterministic approach, used for worst case approaches in a preventive paradigm, one fixed level of the variability factor is applied. In the probabilistic approach however, where all RACs are considered together for the calculation of the total exposure, it is unlikely that one would eat the unit of every RAC containing the highest concentrations. We therefore developed an approach which simulates the variability of concentrations on units within a composite sample by defining the variability factor as a model parameter. This parameter, together with information on the number of samples in a composite sample, is used to specify a distribution of concentrations in the units composing a composite sample. Among several possibilities to define this model, the model based on a beta distribution (de Boer and van der Voet, 2007) was used here. The beta distribution is a flexible model for values bounded between two limit values. In the context of this report, the minimum limit value is 0 mg/kg. For the maximum level, the highest concentration possible for one of the composing units of the composite sample was used. The maximum level is calculated as the composite sample concentration multiplied with the number of units in the sample. Apart from these limit values the beta distribution has two parameters. For our purpose it is most convenient to use as parameters the composite sample mean concentration and the variability factor. Together these parameters determine the shape of the distribution. For a variability factor very close to 1 the distribution resembles a single spike around the composite mean concentration. For larger variability factors the distribution will become broader, but is still restricted by the lower and upper limit values.

Another option to model variability is the model based on a lognormal distribution, the approach used in the EFSA opinion on acute dietary intake assessment (EFSA, 2007c). The main difference between this approach and the beta distribution is that there is no upper limit anymore to the maximum concentration to be sampled. As in the beta model, the composite sample mean and the variability factor were used to describe the lognormal distribution. To establish the effect of assuming either a beta or lognormal distribution of concentrations within a composite sample, also a sensitivity analysis was performed in which the lognormal distribution was applied.

Table G-1. Percentiles of acute cumulative dietary exposure (µg/kg bw/d) of children aged 2 to 6 years according to three different scenarios of applying variability factors in a probabilistic assessment.

Percentiles of exposure	Var fac[a] = 3; Beta distribution[b] (Basis)	Var fac = 3; Lognormal distribution[b]	Var fac = 3, 5 or 7; Beta distribution
P50	0.6	0.6	0.6
P90	1.7	1.8	1.7
P95	2.5	2.6	2.4
P99	6.7	7.1	7.2
P99.9	27	31	41

[a] Var fac = Variability factor
[b] Variability was either modelled according to a beta or lognormal distribution. For more details, see text of Appendix G.

In both the beta and lognormal approach, variability was applied to each individual unit consumed. Variability was not applied to processed foods, such as fruit juices (except freshly squeezed juices) and apple sauce. For the number of units in a composite sample, figures mentioned in the EU guidance document 'Guidelines for the generation of data concerning residues as provided in Annex II part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning placing plant protection products on the market', Appendix B[12] were used. For the unit weights of the products, those collected by WHO and published on the WHO website (updated May 1 2003)[13] were used. For missing products, the most likely unit weight of a similar product was selected.

Results of either applying variability factors of 3, 5 or 7 instead of 3 or assuming a lognormal distribution as opposed to a beta distribution are listed in Table G-1 using Approach 2 to calculate the cumulative exposure (section 2.6). For reasons of comparison also the basic assessment (beta distribution and variability factors always 3) was included.

Assuming a lognormal distribution as opposed to a beta distribution did not affect the percentiles of exposure up to P99 (Table G-1). The P99.9 tended to be higher when applying a lognormal distribution. The same was true for applying higher variability factors. The highest P99.9 calculated (variability factor 3, 5 or 7 and beta distribution) was however still far below the ARfD of acephate (100 µg/kg bw/d; section 7.3).

[12] ec.europa.eu/food/plant/protection/resources/app-b.pdf
[13] www.who.int/foodsafety/chem/acute_hazard_db3.pdf

Appendix H 95 % confidence intervals (in brackets) around the best estimates of dietary exposure (samples with a concentration below LOR were assigned ½LOR)

Acrylamide

Age (years)	Percentiles of long-term dietary exposure (µg/kg bw/d)		
	P50	P95	P99
2-6	0.7 (0.6-0.8)	1.2 (1.0-1.4)	1.5 (1.2-1.7)

Dioxins

Age (years)	Percentiles of long-term dietary exposure (pg/kg bw/d)		
	P50	P95	P99
2	1.5 (1.3-1.6)	2.4 (2.0-2.6)	2.8 (2.4-3.4)
3	1.4 (1.2-1.5)	2.2 (1.9-2.5)	2.7 (2.3-3.1)
4	1.3 (1.2-1.4)	2.1 (1.8-2.4)	2.5 (2.1-2.9)
5	1.2 (1.1-1.4)	2.0 (1.7-2.3)	2.4 (2.1-2.8)
6	1.2 (1.1-1.3)	1.9 (1.6-2.1)	2.3 (1.9-2.7)

Mycotoxins

Age (years)	Percentiles of long-term dietary exposure		
	P50	P95	P99
Aflatoxin B_1 (in ng/kg bw/d)			
2-6[a]	0.8 (0.6-0.9)	1.9 (1.5-2.5)	2.7 (2.0-3.6)
DON (in µg/kg bw/d)			
2	0.3 (0.28-0.34)	0.5 (0.42-0.54)	0.56 (0.49-0.66)
3	0.3 (0.25-0.31)	0.4 (0.39-0.48)	0.5 (0.45-0.59)
4	0.3 (0.25-0.30)	0.4 (0.37-0.47)	0.5 (0.43-0.57)
5	0.3 (0.23-0.28)	0.4 (0.34-0.45)	0.5 (0.40-0.54)
6	0.2 (0.22-0.27)	0.4 (0.33-0.42)	0.4 (0.39-0.52)

Age (years)	Percentiles of long-term dietary exposure		
	P50	P95	P99
Fumonisin B_1 (in µg/kg bw/d)			
2-6[a]	0.3 (0.2-0.3)	0.7 (0.5-0.9)	1.0 (0.8-1.4)
OTA (in ng/kg bw/d)			
2-6[a]	11.3 (7.3-15.8)	25 (14.8-46)	32 (19.9-68)
Patulin (in µg/kg bw/d)			
2-6[a]	0.03 (0.03-0.04)	0.1 (0.1-0.2)	0.2 (0.2-0.3)

[a] Due to multimodal distribution of daily exposure, the ISUF method was used to model long-term exposure. This approach does not provide information on the age dependency of exposure.

Nitrate

Age (years)	Percentiles of long-term dietary exposure (mg/kg bw/d)		
	P50	P95	P99
Summer			
2	1.9 (1.8-2.3)	3.6 (3.3-4.5)	4.7 (4.3-6.0)
3	1.8 (1.5-2.0)	3.4 (2.7-3.8)	4.4 (3.4-5.0)
4	1.6 (1.5-1.9)	3.1 (2.8-3.8)	4.1 (3.5-5.0)
5	1.5 (1.4-1.9)	2.9 (2.5-3.5)	3.8 (3.3-4.6)
6	1.4 (1.2-1.7)	2.7 (2.3-3.2)	3.5 (2.9-4.3)
Winter			
2	1.8 (1.6-2.0)	2.9 (2.5-3.4)	3.6 (2.9-4.3)
3	1.6 (1.3-1.7)	2.6 (2.1-2.9)	3.3 (2.5-3.8)
4	1.4 (1.3-1.6)	2.4 (2.0-2.9)	3.0 (2.4-3.6)
5	1.3 (1.1-1.4)	2.2 (1.7-2.5)	2.7 (2.1-3.3)
6	1.2 (1.1-1.3)	2.0 (1.7-2.4)	2.4 (2.0-3.1)

OPs

Percentiles of acute dietary exposure (µg/kg bw/d)	Approach 1[a]
P50	0.2 (0.1 - 0.3)
P90	1.1 (0.7 - 1.4)
P95	1.6 (1.1 - 2.1)
P99	4.1 (3.4 - 4.7)
P99.9	19 (15 - 24)

[a] Approach 1: OP levels are summed per sample. With Approach 2 uncertainty analyses have not yet been implemented (for more details, see section 2.6).

rivm

Appendix I Deviations from the decision tree of the panel 'Children and chemical substances' of the Dutch Food and Consumer Product Safety Authority

1. Characterize the exposure of the age group

In the present study only the exposure via the diet was taken into account, whereas the panel's decision tree refers to all sources of exposure. Furthermore, several percentiles of exposure (median, P95 and P99) were calculated and not the average as suggested. A realistic exposure was estimated.

Option 1a: The decision tree indicates that if the exposure is below the ADI/TDI, it can be concluded that there is no additional health risk. Nevertheless, also for these substances the toxicity database (including those studies on which the ADI/TDI was based, as well as possible other relevant studies) was evaluated, to assure that health risks were really not present in those cases.

2. Determine if reduction of exposure is possible

The performance of step 2 was performed after step 5, due to practical reasons. Before reduction measures will be developed, one will need to know if a health risk is actually present.

3. Evaluate the toxicity database and check availability of adequate data on reproduction toxicity

Step 3 was followed. The results are reported in the sections 'Adequacy of the toxicity database' at the beginning of the toxicological profile per compound (see Appendices K to O). In practice, the approach of step 3 as proposed by the panel was not always followed in detail or in the same order. Nevertheless, this has not affected the outcome of the risk assessment.

4. Evaluate the health risks of an exceedance of the ADI or TDI

In those cases where the ADI/TDI was exceeded, we intended to use the tiered approach as indicated. However, the first tier mentioned in the decision tree is a deterministic risk assessment. As the exposure assessments were performed in a probabilistic manner, we already started in a relatively high tier. For the hazard assessment, we started with ADI/TDI based on NOAELs (or LOAELs). If the probabilistic exposure was still higher than the ADI/TDI, we examined whether an additional safety factor of two was present in the derivation of the TDI (section 2.7). A BMD approach was used if the data to perform such an analysis were available.

5. Conclusions

Ad step 5. The conclusion 'there is no additional health risk' was rephrased to 'there is a negligible health risk' which was considered a better description.

Appendix J Effect of grouping analysed biscuits and cookies on the dietary exposure to acrylamide

A variety of biscuits and cookies had been analysed (Appendix A). Among the analysed foods we could distinguish biscuits, cookies (containing more fat or stuffing), children's biscuits and one children's cookie. In the basic analysis, the analysed biscuits and cookies were linked as good as possible to those coded in the children's food consumption survey (Appendix D).

We performed three additional analyses to examine the effect of linking concentration data on the exposure to acrylamide regarding the grouping of biscuits and cookies.

1. In a first scenario, the one 'high' children's cookie level was grouped with the children's biscuits group, under the assumption that the high concentration can also occur in the biscuits of the food group 'children's biscuits'. By doing so the average acrylamide concentration of the food group 'children's biscuits' increased from 143 to 278 µg/kg (Appendix A).
2. In the second scenario some of the biscuits present in the food group 'biscuits' were moved to the food group 'children's biscuits', because, based on the description, they could also be identified as children's biscuits (Appendix D).
3. In a third scenario, the same approach was taken as described in scenario 2, except that the 'high' children's cookie was also added to the children's biscuit group.

The results show that the exposure in all three scenarios was 3 to 6 % lower than the basic scenario (< LOR = ½LOR), showing that the basic scenario, in relation to grouping of biscuits/cookies, resulted in the most conservative estimate of exposure (Figure J-1).

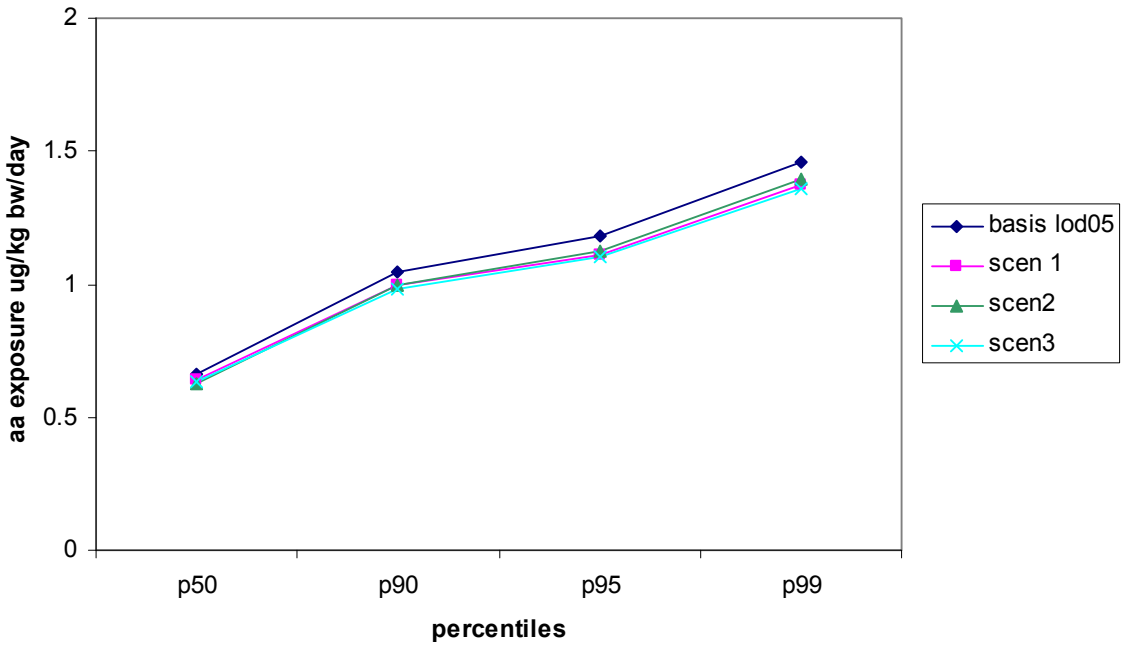

Figure J-1. Effect of different scenarios of grouping biscuits and cookies on the percentiles of long-term dietary exposure of children aged 2 to 6 years to acrylamide (aa).

Appendix K Toxicological profile of acrylamide

Introduction
Acrylamide (CH_2=CH-CO-NH_2)[14] is an important industrial chemical used since the mid 1950s as a chemical intermediate in the production of polyacrylamides, which are used as flocculants for clarifying drinking water and other industrial applications (*e.g.*, grouting). Studies conducted in Sweden in 2002 showed that high levels of acrylamide were formed during the frying or baking of a variety of foods.

Toxicology
In experimental animals, acrylamide is rapidly and extensively absorbed from the gastrointestinal tract following oral administration, and is widely distributed to the tissues as well as the foetus. It has also been found in human milk. Acrylamide is metabolized to a chemically reactive epoxide, glycidamide, in a reaction catalyzed by cytochrome P450 E21. An alternate pathway for metabolism of acrylamide is conjugation with glutathione. Acrylamide and its metabolites are rapidly eliminated in urine, primarily as mercapturic acid conjugates of acrylamide and glycidamide.

The absolute bioavailability of acrylamide (*i.e.*, the fraction entering the circulation as parent compound) is in the range of 23 to 48 % in rodents for a dose of 0.1 mg/kg bw administered in the diet over a 30-minutes period. Extensive first-pass metabolism of acrylamide to glycidamide leads to much higher relative internal exposures to glycidamide after dietary administration compared to intravenous administration.

Both acrylamide and glycidamide bind covalently to amino acids in hemoglobin (Hb), and adducts with the N-terminal valine residue have been used to estimate internal exposures in human biomonitoring studies. Studies measuring concentrations of acrylamide- and glycidamide-Hb adducts in rodents and humans with background exposure to acrylamide through the diet suggested that there may be species differences in the relative formation of glycidamide, with mouse > rat > human. However, the long half-life of Hb means that the measured adduct levels reflect a time-weighted average over the lifetime of the erythrocyte. Thus, similar levels of adducts could arise from the same total exposure over an extended time period as over a short period, which limits the utility of these biomarkers for dose-response modelling.

Single oral doses of acrylamide produced acute toxic effects only at doses above 100 mg/kg bw, and reported LD_{50}s are generally above 150 mg/kg bw. Many studies conducted in a number of animal species have shown that the nervous system is a principal site of acrylamide-toxicity. Repeated exposure to acrylamide causes a degenerative peripheral nerve changes that result from an accumulation of damage at the sites of toxicity. For example, the same degree of neurotoxicity was observed in rats given acrylamide at a dose of 50 mg/kg bw i.p. for 11 days as in rats given drinking water containing acrylamide at a dose of 21 mg/kg bw for 40 days. Continued dosing with acrylamide has been shown to induce nerve terminal degeneration in brain areas critical for learning, memory and other cognitive functions (*i.e.*, cerebral cortex, thalamus and hippocampus) and these lesions may precede the morphological changes in nerves. In rats exposed to acrylamide in drinking water for 90 days, the NOEL for morphological changes in nerves detected using electron microscopy was

[14] The summary of the toxicology and toxicity of acrylamide in this paragraph is largely taken from the report of the meeting on acrylamide of the FAO/WHO Joint Expert Committee on Food Additives and Contaminants held in Rome, February 2005 (FAO/WHO, 2006).

0.2 mg/kg bw/d and no exposure-related non-neoplastic lesions were found at other tissues at dose levels below 5 mg/kg bw/d.

In reproduction studies, male rodents showed reduced fertility, dominant lethal effects, and adverse effects on sperm count and morphology at oral doses of acrylamide > 7 mg/kg bw/d. In female rodents, no adverse effects on fertility or reproduction have been observed, apart from slight reductions in rat offspring body weight at oral doses of 2.5 mg/kg bw/d (LOEL) and above. In developmental toxicity studies, acrylamide was foetotoxic in mice only at a maternally toxic oral dose of 45 mg/kg bw/d, and was not teratogenic in mice or rats. In a developmental neurotoxicity study, in which acrylamide was dosed orally from gestational day six to lactation day 10, the NOEL for developmental neurotoxicity was 10 mg/kg bw/d. The overall NOEL for reproductive and developmental effects was 2 mg/kg bw/d (Tyl and Friedman, 2003). Obviously, the reproductive and developmental toxicity of acrylamide is expressed at substantially higher dosages compared to the dosages at which neurotoxicity and carcinogenicity is expressed.

Although acrylamide did not show mutagenicity in the Ames Salmonella assay, glycidamide clearly did. Acrylamide is both clastogenic and mutagenic in mammalian cells *in vitro* and *in vivo*. In addition, dominant lethality studies have shown acrylamide to be a germ cell mutagen in male rodents. The mutational spectra produced by acrylamide and glycidamide in transgenic mouse cells are consistent with formation of pro-mutagenic purine DNA-adducts *in vivo*. Glycidamide is much more reactive than acrylamide with DNA, and metabolism of acrylamide to glycidamide appears to be a prerequisite for the genotoxicity of acrylamide *in vitro* and in experimental animals.

In rodents dosed with acrylamide, glycidamide-DNA adducts are formed at comparable levels in all tissues examined and accumulate to apparent steady state levels from repetitive dosing regimens. DNA adducts have been found in liver, lung, testis, leukocytes and kidney of mice, and in liver, thyroid, testis, mammary gland, bone marrow, leukocytes and brain of rats treated with either acrylamide or glycidamide . Formation of DNA adducts in mice shows a monotonic dependence on acrylamide dose, from measurable adduct levels at background exposure, with evidence for saturation of adduct levels at higher doses.

Acrylamide in drinking water has been tested for carcinogenicity in two experiments in Fischer 344 rats; there were increases in tumour incidences at a variety of sites (Friedman et al., 1995; Johnson et al., 1986). Acrylamide was evaluated by the IARC in 1994 and classified as a group 2A carcinogen (probably carcinogenic to humans) (IARC group 2A), based on a positive cancer bioassay result and supported by evidence that acrylamide is efficiently biotransformed to a chemically reactive genotoxic metabolite, glycidamide , in both rodents and humans (IARC, 1994). The endocrine-responsive nature of several tumour sites from the two chronic bioassays of acrylamide in F344 rats has elicited speculation about neuroendocrine-mediated mechanisms. However, no published studies have linked hormonal changes with the carcinogenicity of acrylamide in any tissue, nor is there any indication of hormonal effects from reproductive studies. Moreover, the wide body of evidence supporting a genotoxic mechanism is not incompatible with hormonal dysregulation by acrylamide because it is clear that other factors beyond DNA damage are probably required for the observed target tissue specificity of tumorigenesis of acrylamide.

Observations in humans
Acrylamide adducts to Hb have been used as biomarkers of acrylamide exposure in humans. Although levels of acrylamide adducts were often higher among exposed workers and smokers, including a positive correlation with the amount smoked, some uncertainties remain precluding its current use as a marker of dietary acrylamide intake. Because analytical methods may vary between laboratories, there

is a need for improved and validated analytical methodology. A means to link biomarkers of acrylamide exposure in humans with toxicity measurements in experimental animals is not currently available.

Epidemiological studies have been carried out on workers occupationally exposed to acrylamide. Acrylamide exposure was not associated with overall cancer mortality or with any statistically significant dose-related to an increase in cancer risk at any organ site, except a statistically significant doubling of risk for pancreatic cancer for workers with the highest cumulative exposure. These studies, however, were based on low numbers of cases and measurements of dietary exposure to acrylamide were not made, and potential confounders such as tobacco smoking were not considered. For an overview of epidemiological studies carried out in recent years on the general population, see Table K-1. Of the studies listed, those of Olesen et al. (2008) and Hogervorst et al. (2008a, b) are most relevant because these deal with cohort studies and corrected for tobacco smoking. Furthermore, these studies included non-smokers, the relevant subgroup for children. However, the results of these studies are inconsistent.

Table K-1. Summary of epidemiological studies on cancer and the dietary intake of acrylamide.

Cancer	Country	Association between exposure and cancer	Reference
Oral cavity/pharynx, larynx, esophagus, breast	Italy/Switzerland	No[a]	(Pelucchi et al., 2006)
Colon/rectum, ovaries, prostate	Italy/Switzerland	No	(Pelucchi et al., 2006)
Kidney	Italy	No[a]	(Pelucchi et al., 2007)
Colon, kidney, bladder	Sweden	No	(Mucci et al., 2003a, b)
Kidney	Sweden	No[a]	(Mucci et al., 2004)
Breast	Sweden	No[a]	(Mucci et al., 2005)
Colon/rectum	Sweden	No	(Mucci et al., 2006)
Breast[b]	Denmark	Yes (significant)	(Olesen et al., 2008)
Endometrium, ovaries	Netherlands	Yes (significant)	(Hogervorst et al., 2007)
Breast	Netherlands	No	(Hogervorst et al., 2007)
Kidney	Netherlands	Yes (significant)	(Hogervorst et al., 2008a)
Esophagus, colon/rectum	Netherlands	No	(Hogervorst et al., 2008b)
Stomach, pancreas	Netherlands	No[a]	(Hogervorst et al., 2008b)

[a] Although a small positive association between the lowest and the highest quintiles of intake was observed, this association was not significant.

[b] Breast cancer in postmenopausal women; exposure measured as acrylamide-Hb and glycidamide -Hb adduct levels in blood.

Appendix L Toxicological profile of dioxins

Introduction

'Dioxins' is the generic term for a number of chemicals encompassing the polychlorinated di-benzo-p-dioxins (PCDDs), the polychlorinated dibenzofurans (PCDFs) and the coplanar polychlorinated biphenyls (coplanar or dioxin-like PCBs)[15]. The possible number of chlorine atoms in a dioxin results in 75 PCDD congeners (seven of which are toxicologically relevant), 135 PCDF congeners (10 of which are toxicologically relevant) and 209 PCB congeners (67 of which are coplanar, *i.e.*, non-ortho or mono-ortho substituted ones, and are thus characterized as 'dioxin-like'-PCBs; 12 of these are toxicologically relevant).It is generally assumed that the toxicity of dioxins is expressed through a common mechanism of action and all the compounds act through this mechanism, that is, interaction with the cytosolic aryl hydrocarbon receptor protein (Ah receptor). During the last few decades, data from many experimental studies with dioxins are consistent with an additive model. As a result of this generally accepted additivity, the toxic equivalency concept was developed during the mid 1980s. It uses the relative effect potency determined for individual PCDD, PCDF and dioxin-like PCB compounds for producing toxic or biological effects relative to a reference compound, usually 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is considered the most toxic dioxin. The total toxic equivalent (TEQ) is operationally defined by the sum of the products of the concentration of each compound multiplied by its TEF (toxic equivalency factor) value, and is an estimate of the total TCDD-like activity of the mixture (van den Berg et al., 2006). Thus, in the TEF scheme, a dose of TCDD of, for example, 1 pg TCDD per kg bw is considered to be equivalent to a toxic equivalence of 1 pg TEQ (properly addressed as 'WHO-TEQ') per kg bw.

Toxicology

Adequacy of toxicity database

There is little doubt regarding the most sensitive endpoints of dioxin-related toxicity, which appear to be reproductive and developmental toxic effects (adverse development effects in male offspring of rats, and immunological deficits in male and female offspring of rats). However, despite the large amount of information on the toxicity of dioxins, substantial uncertainties remain regarding:

- the estimate of TEFs for the poorly studied compounds (the vast majority of dioxin-compounds) in relation the well-studied TCDD: TCDD typically constitutes a small percentage of the total toxic equivalents in food;
- the problems in exactly establishing the dose in experimental animal studies: these doses are often in the same order of magnitude as the background exposure from food components;
- the uncertainties in relating the rather short-term exposure of experimental animals to long-term intake of humans;
- the uncertainties in estimating the body burden of experimental animals resulting from an intake of dioxins for a certain time period and its extrapolation to humans.

For these reasons the TWI and TMI as derived by SCF (2000c, 2001) and JECFA (FAO/WHO, 2001a), respectively, were characterized as 'provisional'. In conclusion: the database lacks many data on the PCDDs, PCDFs and dioxin-like PCBs other than TCDD.

[15] The summary of the toxicology of dioxins in this paragraph is largely taken from the report of the JECFA meeting on dioxins (FAO/WHO 2001b) and the opinions on dioxins of SCF (SCF 2000c, 2001).

Toxicokinetics and toxicodynamics

Experiments in humans and laboratory animals given an oral dose of TCDD showed 50 to 90 % absorption, comparable with the near-complete absorption of PCDDs, PCDFs and PCBs by nursing infants from their mothers' milk.

After absorption from the gut, TCDD enters the lymph in the form of chylomicrons and is cleared from the blood within 1 h, to appear mainly (74 to 81 % of an administered dose) in the liver and adipose tissue. The distribution of PCDDs and PCDFs between the blood and organs is governed by lipid partitioning and binding to plasma proteins. After entering liver cells, TCDD either dissolves in the lipid fraction or binds to the Ah receptor or to cytochrome P450 (CYP) proteins. In laboratory animals, PCDDs and PCDFs are excreted almost exclusively in the bile, excretion in the urine being a minor route. Whereas the parent compound is found primarily in the organs of rodents, only metabolites of PCDDs and PCDFs occur in bile, indicating hepatic metabolism, including hydroxylation and conjugation, of these compounds. Faecal excretion of unmetabolized PCDDs and PCDFs is also an important route of elimination in humans.

In rodents, the half-life of TCDD ranges from 8 to 24 days in mice to 16 to 28 days in rats. Humans eliminate PCDDs and PCDFs more slowly, the estimated mean half-life of TCDD ranging from 5.5 to 11 years. The half-lives of other PCDD congeners and of PCDFs and coplanar PCBs vary widely. These differences in the half-lives of different congeners are reflected in their TEFs. In laboratory animals, the acute toxicity of TCDD and related PCDDs and PCDFs varies widely between and among species. For example, the median lethal dose in guinea-pigs treated orally was 0.6 mg/kg bw, while that in hamsters was > 5,000 mg/kg bw. Explanations for this variation include differences in Ah receptor functionality, toxicokinetics and body fat content. While data on acute toxicity are available for various commercial PCB mixtures (median lethal doses usually > 100 mg/kg bw), the data on individual coplanar PCB congeners in mammals are limited.

One of the more common symptoms associated with lethality induced by PCDDs is a generalized delayed wasting syndrome characterized by inhibition of gluconeogenesis, reduced feed intake and loss of body weight. Other toxic effects observed after a single exposure to PCDDs include hemorrhages in a number of organs, thymic atrophy, reduced bone-marrow cellularity and loss of body fat and lean muscle mass.

TCDD and other PCDDs induced tumours at multiple sites in laboratory animal species of each sex. In a series of assays *in vivo* and *in vitro*, TCDD promoted the growth of transformed cells, consistent with observations of cancer promotion in whole animals *in vivo*. In a long-term study of carcinogenicity with TCDD in rats, the LOAEL (lowest observed adverse effect level) for hepatic adenomas in females was 10 ng/kg bw/d, and the NOAEL (no observed adverse effect level) was 1 ng/kg bw/d. Several studies have shown that TCDD promotes tumours in laboratory animals, in particular liver tumours. Several other PCDDs, PCDFs and coplanar PCBs also promoted liver tumours. In a long-term study in rats in which the incidence of liver tumours was increased over that in controls, the LOAEL of 10 ng/kg bw/d corresponded to a steady-state body burden of 290 ng/kg bw. In order for humans to attain a similar steady-state body burden, they would have to have a daily intake of 150 pg/kg bw. The results of several short-term assays for genotoxicity with TCDD, covering various endpoints, were negative. Furthermore, TCDD did not bind covalently to DNA from the liver of mice.

It can be concluded that TCDD is not a genotoxic carcinogen, but a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor. This receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals. The IARC classified TCDD as a human carcinogen

(Group 1); other PCDDs and PCDFs were considered not to be classifiable as to their carcinogenicity to humans (group 3; IARC, 1997). A number of biochemical changes, including induction of enzymes such as CYP, altered expression of growth factors and enhanced oxidative stress, have been noted in laboratory animals with body burdens of TCDD within a lower range of 3 to 10 ng/kg bw. These biochemical effects can be considered to be early markers of exposure to PCDDs, PCDFs and coplanar PCBs, or events induced by these compounds in animals and in humans that may or may not result in adverse effects at higher body burdens.

In a study by Ohsako et al. (2001), pregnant Holtzman rats were given a single oral dose of TCDD at 0 to 800 ng/kg bw on day 15 of gestation, and the male offspring were examined on days 49 and 120 after birth. No changes were seen in testicular or epididymal weight or in daily sperm production or sperm reserve at any dose. However, the weight of the urogenital complex, including the ventral prostate, was significantly reduced at doses of 200 and 800 ng/kg bw in rats killed on day 120. Moreover, the anogenital distance of male rats receiving doses ≥ 50 ng/kg bw and killed on day 20 was significantly decreased. Administration of TCDD at any dose resulted in a dose-dependent increase in 5α-reductase type 2 mRNA and a decrease in androgen receptor mRNA in the ventral prostate of rats killed at day 49 but not in those killed at day 120, with no adverse sequelae at the lowest dose of 12.5 ng/kg bw. Physiologically-based pharmacokinetic (PBPK) modelling indicates that the equivalent maternal body burden after multiple doses at this NOAEL would be 13 to 19 ng/kg bw. Likewise, the LOAEL of 50 ng/kg bw corresponds to an equivalent body burden of 51 to 80 ng/kg bw.

The lowest LOAEL reported for the reproductive system of male offspring was found in an experiment with Wistar rats by Faqi et al. (1998). In this study, the dams were treated subcutaneously before mating and throughout mating, pregnancy and lactation. They received an initial loading dose of ^{14}C-TCDD at 25, 60 or 300 ng/kg bw two weeks before mating, and then a weekly maintenance dose of TCDD at 5, 12 or 60 ng/kg bw. The size of the maintenance doses was determined on the basis of a reported elimination half-life for TCDD of three weeks in adult rats. The effects on male reproductive endpoints were studied on days 70 and 170 after birth. The number of sperm per cauda epididymis at puberty and in adulthood was lower in the offspring of all treated dams than in those of controls. Daily sperm production was permanently lower in offspring of treated dams than in those of controls, as was the sperm transit rate, thus increasing the time required by the sperm to pass through the cauda epididymis. Moreover, the offspring of the treated groups showed increased numbers of abnormal sperm when investigated in adulthood. The latency periods to mounting and intromission were significantly greater in offspring of dams at the lowest and highest doses, but not of those at the intermediate dose, than in offspring of controls. In the male offspring of dams at the highest dose, the concentration of serum testosterone was decreased in adulthood, and permanent changes found in the testicular tubuli included pyknotic nuclei and the presence of cell debris in the lumen. The fertility of the male offspring was not affected in any of the treated groups. PBPK modelling showed that a maternal body burden of 25 to 39 ng/kg bw at steady state would be required to arrive at the foetal body burden which resulted in adverse effects after an initial dose of 25 ng/kg bw and weekly maintenance doses of 5 ng/kg bw (LOAEL).

These studies provide evidence that adverse effects on the reproductive system are induced in male offspring of pregnant rats given TCDD. The studies show reductions in daily sperm production, in the number of sperm in the cauda epididymides and in epididymal weight as well as accelerated eye opening, a reduction in anogenital distance and feminized sexual behaviour in male offspring associated with maternal steady-state body burdens of TCDD of ≥ 25 ng/kg bw. Reductions in the weights of the testes and the size of the sex accessory glands, such as the ventral prostate, in male offspring, development of external malformations of the genitalia in female offspring and reduced fertility in males and females required higher maternal body burdens. It should be mentioned that the

most sensitive endpoints differed between studies. Perhaps this reflects strain differences in sensitivity and even minor differences in the experimental conditions, for example, the diet.

Observations in humans

In adults, effects such as chloracne are observed after exposure to PCDDs, PCDFs and coplanar PCBs at doses several orders of magnitude higher than those generally acquired via background contamination of foods. Following an industrial incident in Seveso[16], more female children than expected were born to fathers who had serum TCDD concentrations > 80 pg/g of lipid (16 to 20 ng/kg bw) at the time of conception.

In most of the epidemiological studies considered for the evaluation of the carcinogenicity of TCDD, mostly in occupational settings, exposure had been primarily to TCDD, with some exposure to mixtures of other PCDDs, as contaminants of phenoxy herbicides and chlorophenols. The studies involved persons with the highest recorded exposure to TCDD, the estimated geometric mean blood lipid concentrations after the last exposure ranging from 1,100 to 2,300 pg/g of lipid in the industrial cohorts; lower average concentrations were found in the population exposed in Seveso.

Excess risks of the order of 40 % were found for all neoplasms combined in all the studies of industrial cohorts in which the exposure assessment was adequate. The risks for cancers at specific sites were increased in some of the studies, but the results were not consistent between studies, and no single cancer site seemed to predominate.

Increasing risks for all neoplasms with time since first exposure were observed in those studies in which latency was evaluated. The follow-up of the Seveso cohort has so far been shorter than that of the industrial cohorts; however, the rate of death from all cancers has not been found to differ significantly from that expected in the general population. Excess risks were seen for cancers at some specific tissues among persons in the most heavily contaminated zones at the time of the incident, but there were few cases.

In these well-conducted cohort studies, the intensity of exposure could be ascertained with precision because of the long biological half-life of TCDD in human tissues, and the relative risks increased significantly with increasing exposure. Although the excess cancer risk at the highest exposure was statistically significant, these results must be evaluated with caution, as the overall risks are not high and the strongest evidence is for industrial populations whose exposure was two to three orders of magnitude greater than that of the general population, and who also had heavy exposure to other chemicals; furthermore, lifestyle factors such as smoking were not evaluated. In addition, it must be noted that there are few precedents of carcinogens that increase the risk for cancer at all tissues combined, with no excess risk for any specific tumour predominating.

Health based limit value

On the basis of the available toxicity data, SCF and JECFA derived a tolerable intake for TCDD based on the assumption that there is a threshold for all effects, including cancer. Carcinogenicity due to TCDD was not linked to mutagenicity or DNA binding, and it occurred at higher body burdens in animals than any other toxic effect. Hence, the assessment of a tolerable intake based on effects other than cancer would also cover any carcinogenic risk.

[16] In an industrial incident in Seveso (Italy) in 1976, several hundred grams up to a few kilograms of TCDD escaped into the atmosphere and settled over an area of approximately 18 km^2. As a result, several thousands of people living nearby the plant were exposed to high amounts of TCDD.

The lowest LOAEL and NOAEL were provided by the studies of Faqi et al. (1998) and Ohsako et al. (2001), respectively. With the toxicokinetic conversions described above, these two studies indicate maternal body burden LOAELs and NOAELs for effects on male rat offspring of 25 to39 ng/kg bw and 13 to 19 ng/kg bw, respectively.

In the studies used to estimate body burden on the basis of the distribution of TCDD after multiple dosing, radiolabeled material was used. Therefore, the background concentrations of TCDD and other PCDDs and PCDFs in the tissues of laboratory rats resulting from traces of these compounds in the feed were ignored. Studies aimed to predict the body burdens of rats resulting from the presence of coplanar compounds in laboratory feed indicated that 'unexposed' laboratory rats had toxic equivalent body burdens of 3/4 up to 12 ng/kg bw, depending on age. Correcting the body burdens calculated from the studies cited above resulted in estimated total toxic equivalent body burdens at steady state of 16 to 23 ng/kg bw for the NOAEL and 28 to 43 ng/kg bw for the LOAEL. By means of PBPK-modelling it was calculated that these body burdens correspond to equivalent human daily intakes (EHDIs) of 8 to 10 pg/kg bw/d and 14 to 20 pg/kg bw/d, respectively.

Use of body burdens to scale doses from studies in laboratory animals to equivalent human doses removes the need for assessment factors to account for differences in toxicokinetics between animals and humans. To account for inter-individual differences in toxicokinetics among humans, however, an assessment factor should be applied. In view of limited data on the toxicokinetics of TCDD in humans, the default factor of 3.2 was considered appropriate (Vermeire et al., 2007).

With regard to the potential differences in toxicodynamics between experimental animals and humans and within the human population, studies of Ah receptor binding affinity and adverse responses directly dependent on Ah receptor activation suggest that humans are less sensitive to TCDD than responsive rodent strains. However, studies of some biochemical or cellular effects, such as CYP1A1 and CYP1A2 induction, suggest a comparable sensitivity. Therefore, for some endpoints it cannot be excluded that the most sensitive humans might be as sensitive to the adverse effects of TCDD as experimental animals. Hence, an assessment factor for differences in toxicodynamics between experimental animals and humans and for interindividual variation among humans is not needed. However, if a LOAEL instead of a NOAEL is used for deriving a health based safety limit, an additional assessment factor is needed. As the LOAEL for the sensitive endpoint was considered to be close to a NOAEL and represented marginal effects, the application of a factor of 3 to account for use of a LOAEL instead of a NOAEL was considered appropriate. This resulted in an overall assessment factor of 9.6 (3 x 3.2). Thus a total assessment factor of 3.2 should be applied to the EHDI associated with the NOAEL, and a total assessment factor of 9.6 should be applied to the EHDI associated with the LOAEL. This results in values for a provisional tolerable daily intake (pTDI) of 2.5 to 3.1 pg/kg bw/d based on the NOAEL, and 1.5 to 2.1 pg/kg bw/d based on the LOAEL.

SCF (2000c, 2001) chose the midpoint of these ranges expressed on a weekly basis, and arrived at a provisional tolerable weekly intake (pTWI) of 14 pg/kg bw/week. JECFA (FAO/WHO, 2001a) chose the midpoint of these ranges expressed on a monthly basis and arrived at a provisional tolerable monthly intake (pTMI) of 70 pg/kg bw/month. Furthermore, both organisations concluded that these tolerable intakes should be applied to intake of PCDDs, PCDFs and coplanar PCBs, expressed as TEQs.

For pragmatic reasons a pTDI of 2 pg TEQ/kg bw/d can be used in, for example, intake calculations, if it is kept in mind that an incidental high intake on a particular day will have a small or even negligible effect on the overall body burden.

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Appendix M Toxicological profile of selected mycotoxins

Aflatoxin B_1

Introduction
The present description of the toxicological profile of aflatoxin B_1 is largely based on an EFSA opinion (EFSA, 2007b) and a JECFA evaluation (FAO/WHO, 2001b).

Toxicology

Toxicokinetics
Absorption of aflatoxins in the rat small intestine is a rapid. The liver is the major site of aflatoxin metabolism and the CYPs that are implicated are CYP3A4, 3A5 and 1A2 (Wild and Turner, 2002). Aflatoxin B_1 is metabolised to aflatoxin Q_1, aflatoxin M_1, aflatoxin P_1, aflatoxin B_1-*endo*-8,9-epoxide and the critical product aflatoxin B_1-*exo*-8,9-epoxide. Aflatoxin B_1-*exo*-8,9-epoxide may be subsequently metabolized by, among others, conjugation to glutathione and hydrolysis, which are the major detoxification pathways. The order of GSH conjugation to aflatoxin B_1 among species is mouse > rat > human with humans exhibiting comparatively low conjugation (Kirby et al., 1993; Raney et al., 1992). However, aflatoxin B_1-*exo*-8,9-epoxide is very reactive and may form DNA- and protein adducts. The metabolites are excreted in urine and bile. Aflatoxin M_1 appears as a metabolite in milk.

Toxicodynamics
Aflatoxins are well recognized as a cause of liver cancer but they have additional important toxic effects with a range of consequences: 1) large doses lead to acute toxicity and death; 2) chronic sublethal doses have nutritional and immunologic consequences.

Acute toxicity
In rats aflatoxin B_1 oral LD_{50}s of 5.5 to 17.9 mg/kg bw were reported. Aflatoxin B_1 causes acute hepatotoxicity in humans and experimental animals. In April 2004, an aflatoxicosis outbreak in Kenya resulted in 317 cases and 125 deaths.

Short-term toxicity
Aflatoxin B_1 induces thymic aplasia, reduced T-lymphocyte function and number, suppressed phagocytic activity and reduced complement activity (reviewed in (Williams et al., 2004)). Studies conducted in poultry, pigs and rats showed that aflatoxins result in suppression of the cell-mediated immune response and may impair the function of macrophages. The species differences noted for the acute toxicity and carcinogenicity also apply to the immune response. The overall NOAEL for mice and rats was 30 µg/kg bw/d. Studies on Gambian children (Turner et al., 2003) and Ghanaians (Jiang et al., 2005) indicate that dietary exposure to aflatoxin B_1 could result in impairment of cellular immunity that could decrease host resistance to infections.

Chronic aflatoxin exposure has major effects on nutritional status in animals. However a NOAEL for this effect was not determined. In children < 5 years of age in Benin and Togo, a dose-response relationship between aflatoxin exposure and the degree of stunting and body weight reduction was found (Gong et al., 2002, 2004).

Long-term toxicity and carcinogenicity

Studies in experimental animals have consistently shown aflatoxin B_1 to be both genotoxic and carcinogenic. Aflatoxin B_1 is mutagenic in bacterial systems and in eukaryotes, usually requiring an exogenous bioactivation system (IARC, 1993). Sufficient experimental evidence is available for the carcinogenicity of naturally occurring mixtures of aflatoxins, and of aflatoxin G_1 and aflatoxin M_1, whereas there is only limited evidence for aflatoxin B_2 and inadequate evidence for aflatoxin G_2. For the carcinogenic effect of aflatoxin B_1 there are differences in sensitivity between species and strains. These differences may reflect the differences in the rates and extents of metabolic activation and detoxification. In rodents, the principal tumours were in the liver, primarily hepatocellular carcinoma (HCC), but also the lung, kidney and colon. For liver tumours the effective dose of aflatoxin B_1 were 10 to 30 µg/kg food in fish and birds and 15 to 1,000 µg/kg food in rats. The mean TD_{50} value (*i.e.,* the daily dose throughout the lifespan that will halve the mortality-corrected estimate of the probability of remaining tumourless) was 3.2 µg/kg bw/d in male rats (CPDB, 2006). Dietary studies with aflatoxin B_1 in rats showed that the Fisher rat is the most sensitive strain and that males are slightly more sensitive than females.

In mice TD_{50}s of > 70 to 5300 µg/kg bw/d were calculated (some strains of mice showing no response at doses up to 150000 µg/kg diet). In tree shrews, the TD_{50} was 26.9 µg/kg bw/d. Squirrel monkeys developed liver tumours when fed aflatoxin B_1 at 2000 µg/kg diet for 13 months, and rhesus, African green and Cynomolgus monkeys developed a low (7 to 20 %) incidence of liver tumours when fed average doses of 99 to 1,225 mg/animal over 28 to 179 months. In these species, tumours in extrahepatic tissues (including tumours of the pancreas, gall bladder and the vascular system) were observed at much higher frequency than the liver tumours. The TD_{50} for liver tumours in rhesus monkeys was 156 µg/kg bw/d and for all tumours combined 8.2 µg/kg bw/d. In the Cynomolgus monkeys, the TD_{50} for liver tumours was 848 µg/kg bw/d and for all tumours combined it was 20.1 µg/kg bw/d (CPDB, 2006; FAO/WHO, 2001b; Wogan, 1992).

Aflatoxin M_1, a metabolic hydroxylation product of aflatoxin B_1, is considered to be a genotoxic agent, based on its activity *in vitro* and its structural similarity with aflatoxin B_1 (FAO/WHO, 2001b). Based on studies in rats, FAO/WHO (2001b) as a conservative estimate, considered the potency of aflatoxin M_1 to be 10 % that of aflatoxin B_1. The IARC classified aflatoxins as 'human carcinogens' (IARC 1993, 2002), and concluded in 1993 and 2002 that there was sufficient experimental evidence for the carcinogenicity of aflatoxin B_1 and aflatoxin M_1.

Human epidemiological data

Reasonably consistent associations were found between estimates of dietary exposure to aflatoxins and HCC rates in a number of countries in sub-Saharan Africa and south-east Asia (IARC, 1993). Two longitudinal cohort studies, in Shanghai (Qian et al., 1994; Ross et al., 1992) and Taiwan (Wang et al., 1996), reported increased risks of HCC in individuals positive for HBV infection and aflatoxin biomarkers alone, but a more than multiplicative interaction between the two risk factors. There was a statistically significant relationship between detectable aflatoxin B_1-albumin adducts and HCC risk (Sun et al., 2001; Wang et al., 1996). However, it is notable that none of the above studies provided direct quantitative relationships between dietary aflatoxin intakes, biomarkers and HCC risk. Overall, published studies show a positive correlation between population estimates of aflatoxin exposure and the proportion of HCC with a 249_{ser} mutation (Wild and Turner, 2002). This particular type of transversion mutation at guanine residues is consistent with that induced by aflatoxins in a variety of experimental models. In regions of China where aflatoxin exposure is reported as high, the 249_{ser} mutation was observed in more than 50 % of HCC compared to less than 10 % in low exposure regions. In geographic regions of expected low aflatoxin exposure (including Europe and North America), the prevalence of 249_{ser} mutations is extremely low (< 1 %). These data are consistent with a

multiplicative effect on HCC risk of the mutational effect of aflatoxin on TP53 and chronic infection with HBV.

DON

Introduction/characterization of exposure
Deoxynivalenol (DON or vomitoxin) is a mycotoxin belonging to the group of trichothecenes, and is produced by fungi of the *Fusarium* genus, *i.e., Fusarium culmorum* and *Fusarium graminearum.* These fungi are present in several cereal crops, predominantly in grains such as wheat, maize, barley, oats and rye. DON is also present in processed grain products like malt, bread, biscuits, cookies and beer. The amount of DON present on crops is strongly associated with the weather condition. Especially the moisture at the time of flowering and the timing of rainfall are critical factors (FAO/WHO, 2001b). All grains can be contaminated to some extent with DON. As grains and grain products such as bread are regular and valuable constituents of a child's diet, this means that exposure to (low levels of) DON will likely occur on a regular basis. In addition, annual variations in weather conditions may influence the DON content of grain significantly and as a result occasional high(er) intake levels of DON may occur.

Possible exposure reducing measures
Until 1999 in the Netherlands an 'action limit' of 1000 µg DON/kg wheat was in use. When the DON content of wheat exceeded this value, this was reason to investigate the underlying cause and if necessary to take the wheat of the market for human consumption. In 1998 and 1999, high levels of contamination with DON were detected in wheat and wheat containing food products in the Netherlands. An exposure assessment performed in this same period showed that a considerable percentage of young children exceeded the TDI of DON, in some cases even two-fold. In response to this observation, at the end of 1999 the 'action limit' was reduced to 500 µg DON/kg wheat, and some producers even maintain lower limits (Health Council of the Netherlands, 2001). The EU also provides regulation concerning the concentrations of DON in food. In an amendment to EU regulation EC466/2001 (EC, 2001a), maximum levels of contaminants, among which DON, in food groups are indicated (Table M-1).

Table M-1. Maximum levels of DON allowed in food groups (EC, 2005; 2006b).

Food groups	Maximum levels (µg/kg)
Unprocessed cereals other than durum wheat, oats and maize	1,250
Unprocessed durum wheat and oats	1,750
Unprocessed maize	1,750
Cereal flour, including maize flour, maize grits and maize meal	750
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500
Pasta (dry)	750
Processed cereal-based food for infants and young children and baby food	200

The most efficient strategy to reduce *Fusarium* infections in wheat is good agricultural practice. Techniques that contribute to the reduction of *Fusarium* infections are suitable crop rotation, appropriate use of fertilizers, irrigation and weed control. For instance, maize-wheat rotation increases the incidence of *Fusarium* infections, whereas removal or ploughing in of crop debris reduces the incidence. *Fusarium* infections may also be reduced by chemical treatment of crop, however, efficacy is limited and treatment could potentially result in spread of other, less sensitive fungi (Health Council of the Netherlands, 2001; FAO/WHO, 2001b).

The Health Council of the Netherlands states that the present concentration limits in the Netherlands are rarely exceeded and that, from a production point of view, the economical benefits of actively controlling *Fusarium* infections in wheat or the development of resistant species, are therefore limited (Health Council of the Netherlands, 2001). On the other hand, research by the Health Council of the Netherlands showed that further reduction of the 'action limit' to 100 μg DON/kg wheat is needed to assure that consumption of DON will not exceed the ADI of 0.5 μg/kg bw that was derived by the Health Council of the Netherlands (2001).

Toxicology

Adequacy of toxicity database

The toxicity database of DON is reasonably adequate but lacks unequivocal data concerning potential immune toxicity of DON. A 2-year feeding study in mice is available, which has been used to derive TDI values (critical effect reduced weight gain). Note that in a 2-year study the animals are aged 6 weeks at the start of the study, which corresponds to humans aged 12 year (VWA, 2008). As 12-year old humans are still growing, this study was considered suitable for the present risk assessment. In addition, adequate reproduction toxicity studies are present. There are indications that DON has adverse effects on serum IgA levels after short-term exposure to doses below the NOAEL that was used to derive the TDI values. However, in the 2-year feeding study no effect on serum IgA was observed at doses that do not influence body weight gain. Additional information on immune toxicity of DON is desirable to determine the effects of short- and long-term exposure to current dietary levels of DON on the immune system.

Toxicokinetics and toxicodynamics

After oral dosing of DON a high bioavailability of approximately 55 % was reported in pigs (FAO/WHO, 2001b; Goyarts and Danicke, 2006), but no values are available for rats, mice or humans. DON is rapidly absorbed after oral dosing in pigs, suggesting that the absorption takes place in the upper parts of the gastrointestinal tract (Danicke et al., 2004; FAO/WHO, 2001b; Goyarts and Danicke, 2006). For rats no absolute values are available, however two studies report recovery of 25 % and 37 % of an oral dose of DON in urine respectively 96 and 72 hours after administration (FAO/WHO, 2001b; Meky et al., 2003). Metabolism of DON in pigs and rats is thought to take place primarily in the intestine (Danicke et al., 2004; Worrell et al., 1989). The epoxy group of DON is considered to be essential for the toxicity of DON, and de-epoxidation of DON may thus be an important detoxification route. The intestinal microflora and/or the faeces of both rats and pigs were shown to possess de-epoxidation capacity, although study results in pigs were somewhat contradictory (FAO/WHO, 2001b; Goyarts and Danicke, 2006). In the faeces of ten human volunteers, no de-epoxidation capacity was found (Eriksen and Pettersson, 2003). Another major route of detoxification of DON is glucuronidation and DON-glucuronide is detectable in urine of humans exposed to DON (Meky et al., 2003). In experimental animals, it was shown that DON and its metabolites are eliminated via urine, faeces and bile (FAO/WHO, 2001b). A half-life of four to seven hours after oral ingestion was reported for DON in pigs (Danicke et al., 2004; FAO/WHO, 2001b; Goyarts and Danicke, 2006). Due to the rapid excretion of DON, no accumulation is expected.

One study in mice addressed possible differences in toxicokinetics of DON in weanling and adult mice. When given doses of 5 mg/kg bw/d by gavage the uptake of DON in plasma, spleen, liver, lung and kidney was approximately twice as high in weanling mice as compared to adult mice. Subsequently, DON levels in tissue and plasma of weanling mice approached the levels in adult mice within two hours, suggesting that differences in the clearance are less pronounced than differences in the uptake of DON (Pestka and Amuzie, 2008).

Oral exposure to DON can result in adverse effects on the gastrointestinal tract and the immune system. Acute toxic effects on the gastrointestinal tract result in nausea, vomiting, abdominal pain and diarrhea. This can be caused by both local and central effects of DON. Vomiting induced by DON is probably mediated by effects on the serotonin receptors in the gastrointestinal tract (FAO/WHO, 2001b). Besides, direct cytotoxic effects on intestinal cells and disruption of the epithelial barrier by DON have been reported (Maresca et al., 2002; Sergent et al., 2006). It was suggested that these effects may contribute to the occurrence of (inflammatory) diarrhea and intestinal bleeding. (Sub)chronic low dose toxicity of DON in experimental animals is characterized by a reduced weight gain, which can be ascribed to a decreased uptake of nutrients from the intestine and/or reduced apatite. Reduced weight gain is generally viewed as the critical effect of DON exposure. It is probably mediated via effects on the serotoninergic system and by reduced protein synthesis. The former is influenced in the brain and locally in the gastrointestinal tract (FAO/WHO, 2001b), but no systemic effects were observed (Prelusky, 1994). Reduced protein synthesis likely affects the transport systems in the intestinal epithelium that are responsible for nutrient uptake.

Carcinogenicity

DON is thought not to present a carcinogenic hazard. In 1993, IARC classified toxins derived from a number of *Fusarium* species, among which DON, in group 3 (not classifiable as to their carcinogenicity to humans). For DON no human or experimental data on potential carcinogenicity were available (IARC, 1993). More recent carcinogenicity studies in mice did not reveal any carcinogenic effects of DON nor initiator or promoter properties (FAO/WHO, 2001b).

Health based limit value

Several TDI evaluations for DON are available. In 1999, RIVM estimated a provisional TDI for DON of 1.1 µg/kg bw based on a NOEL of 0.11 mg/kg bw/d in a 2-year feeding study in mice and a uncertainty factor of 10 each for inter and intraspecies differences. The critical effect in this study was reduced weight gain (Pieters et al., 1999). In the same year, SCF allocated a temporary TDI of 1 µg/kg bw based on the same study, using a NOEL of 0.1 mg/kg bw and an uncertainty factor of 100 (SCF, 1999). The TDI was considered temporary pending an evaluation whether the establishment of a group TDI for trichothecenes would be appropriate and feasible. This evaluation was performed in 2002 and it was decided against allocation of a group TDI. A full TDI for DON was established at 1 µg/kg bw (SCF, 2002a). In 2001, JECFA allocated a provisional maximum TDI of 1 µg/kg bw to DON, based on the same study as used by SCF and an uncertainty factor of 100. JECFA concluded that intake at this level would not result in effects on the immune system, growth or reproduction (FAO/WHO, 2001b). The Health Council of the Netherlands used the same 2-year feeding study in mice to derive a TDI. However, it was decided to use an uncertainty factor of 210, composed of an uncertainty factor of 10 for intraspecies differences, an uncertainty factor of 3 for interspecies differences and a scaling factor of 7 for differences in energy use, as a measure for metabolism, between man and mice. The latter was considered appropriate by the Health Council of the Netherlands because exposure to DON may influence weight gain, which is related to the level of metabolism (Health Council of the Netherlands, 2001).

Relevance of the TDI values for young children

Decreased weight gain can be a critical toxic effect for children exposed to DON however it should also be considered whether effects for which children are potentially more sensitive than adults may occur at similar dose levels. Specifically, information on potential reproductive toxicity, immune toxicity, neurotoxicity and endocrine toxicity was evaluated. The result of this evaluation is indicated below.

Reproduction toxicity

In a number of oral developmental toxicity studies in which deoxynivalenol was administered during (part of) the gestation, overall NOAELs for fetotoxic or teratogenic effects of 0.5 mg/kg bw/d in mice, 0.2 mg/kg bw/d in rats, 0.6 mg/kg bw/d in rabbits and 0.04 mg/kg bw/d in pigs were reported (FAO/WHO, 2001b). In the latter two cases, naturally contaminated diets were used and it cannot be excluded that other contaminants in the diet contributed to the toxic effects. In addition, in the JECFA evaluation describing the study in pigs it is stated that the study report indicates that the (adverse) effects of DON could not be attributed to a direct physiological or toxicological effect of DON (FAO/WHO, 2001b). A 1-generation oral toxicity study in mice showed reduced feed and water intake and reduced body weight of male and female mice and a reduced number of live pups, postnatal survivors and body weight of the progeny after administration of DON. The LOAEL in this study was 0.38 mg/kg bw/d, which was the lowest dose tested (FAO/WHO, 2001b). In a same study in rats, no adverse effects of DON exposure on fertility or fetuses were observed at doses up to and including 1 mg/kg bw/d (FAO/WHO, 2001b). With respect to effects of DON on fertility two oral studies in rats showed respectively adverse effects on the male reproductive system at doses above 1 mg/kg bw/d (Sprando et al., 2005) and reduced fertility in male and/or female rats fed 2 mg/kg bw/d of DON (FAO/WHO, 2001b). In mice fed up to 2 mg/kg bw/d, no effect of DON on fertility was observed (FAO/WHO, 2001b).

From these studies it was concluded that DON has developmental and reproductive toxic effects in experimental animals. However, these effects are observed at oral doses higher than those that induce reduced weight gain in the above mentioned long-term toxicity study in mice. In short-term studies in mice, the NOAEL for developmental toxicity was 0.5 mg/kg bw/d. In a 1-generation study in mice, reduced fetal viability, observed at the lowest dose tested (0.38 mg/kg bw/d), was accompanied by maternal toxicity. The same study in rats showed no adverse effects of DON on fetuses or fertility at doses up to and including 1 mg/kg bw/d.

Immune toxicity

A number of oral studies in experimental animals showed adverse effects of DON on the immune system. These effects were ascribed to effects on protein and DNA synthesis and resulting effects on cytokine expression. It was suggested that trichotecenes stimulate or suppress the immune system depending on the dose that is administered (Pestka et al., 2004). In this respect, effects on antibody production, leukocyte count and resistance to pathogens are mentioned. The effects of DON on antibody production, most notably serum IgA, appear to be most sensitive.

A limited number of studies in pigs show effects of feeding DON on serum IgA levels. An 8-week feeding study showed a moderate, dose related increase in serum IgA levels in pigs fed 0 to 0.8 mg DON/kg bw/d (Drochner et al., 2004). However, no NOAEL could be derived from this study, and analysis of the data suggests that the differences in serum IgA levels may have occurred irrespective of DON treatment (personal observations/communication). In a second feeding study in pigs, no effects on serum IgA, IgG or IgM levels were observed in pigs fed 0.1 mg/kg bw/d during four weeks (Goyarts et al., 2006). After acute exposure to 0.1 mg DON/kg bw also no increase of serum IgA levels was seen, however serum IgG and IgM levels were significantly increased compared to pigs fed control diet (Goyarts et al., 2006). Ingestion of low doses of DON (~ 0.01 to 0.04 mg/kg bw/d) did not affect a number of hematological, biochemical and immunological parameters, among which immunoglobulin levels, in piglets after four weeks of treatment (Accensi et al., 2006). Finally, one study showed increased serum IgA levels and an altered vaccinal immune response in pigs fed feed containing 2.2 to 2.5 mg DON/kg for nine weeks (Pinton et al., 2008). It could not be determined from this study what daily doses of DON were ingested by the animals.

Mice appear to be particularly sensitive for DON induced changes in serum immunoglobulin levels, with observed increases in serum IgA levels after four weeks of treatment with doses as low as 0.071 mg/kg bw (NOAEL in this study 0.014 mg/kg bw) (Gouze et al., 2006). DON induced increased serum IgA levels in mice are associated with increased IgA deposition in the kidney, which may cause nephropathy (Pestka et al., 2004). Indeed, administration of DON is often used as experimental mouse model for immunoglobulin nephropathy. No NOAEL for kidney IgA deposition could be derived from the available oral toxicity studies. The overall LOAEL for kidney IgA deposition was 0.4 mg/kg bw/d (Greene et al., 1994). Although adverse effects of DON on immunoglobulin levels are observed at low doses in short-term toxicity studies in mice, in the 2-year feeding study in mice mentioned previously only a slight increase of serum IgA (56 %) and IgG (< 10 %) levels was observed in female, but not male, mice exposed to 0.7 mg DON/kg bw/d. In addition, the authors indicate that this effect was considered not to be biologically relevant The NOAEL in this study was 0.1 mg/kg bw/d (FAO/WHO, 2001b).

Two feeding studies in mice addressed respectively the effects of DON on weight gain and immunoglobulin levels after intermittent exposure (Banotai et al., 1999) and the reversibility of the adverse effects of DON (Dong and Pestka, 1993). Intermittent (*i.e.,* every other week for 13 weeks) exposure to 4 mg/kg bw/d of DON did not result in increased serum IgA levels and resulted in either no or less severe IgA accumulation in the kidney. In addition, the reduced weight gain caused by DON intake was partially compensated during the weeks in which DON was not administered. Hematuria as a measure of nephropathy was increased in the intermittent group, although less pronounced than in the continuously exposed mice. In addition, no hematuria was observed in the intermittent group when measured during a week in which no DON was administered (Banotai et al., 1999). When 5 mg/kg bw/d of DON was fed to mice for eight weeks, serum and kidney IgA remained elevated for at least 16 weeks, although less pronounced than in mice fed DON continuously for 24 weeks. In addition, the severity of hematuria diminished with time and body weight recovered after cessation of eigth weeks DON treatment (Dong and Pestka, 1993). These results suggest that the main adverse effects of DON are reversible.

Fumonisin B_1

Introduction/characterization of exposure
Fumonisins, among which fumunisin B_1, are mycotoxins that are formed by the fungi *Fusarium verticillioides* and *Fusarium proliferatum*, which grow on maize. Fumonisin B_1 is the most significant fumonisin in terms of toxicity and occurrence (Bakker et al., 2003). Fumonisin B_1 is not present in milk, meat or eggs from animals fed feed containing fumonisin B_1 at levels that would not affect the health of the animals (WHO-IPCS, 2000). Infrequently, fumonisins occur in other foods, such as wheat, sorghum, asparagus, rice and mung beans (Bakker et al., 2003). The present analysis of food consumption by children and evaluation of the fumonisin B_1 concentration in these foods shows that potential sources of fumonisin B_1 other than (foods containing) maize did not contribute significantly to exposure. All maize can be contaminated to some extent with fumonisin B_1. As maize and maize-based products are regular constituents of a child's diet, this means that exposure to (low levels of) fumonisin B_1 will likely occur on a chronic basis. In addition, variations in harvest quality may influence the fumonisin B_1 levels in maize significantly and as a results occasional high(er) intake levels of fumonisin B_1 may occur.

Possible exposure-reducing measures
In the JECFA evaluation of fumonisin B_1 some strategies to limit the fumonisin B_1 content of maize after harvesting are indicated (FAO/WHO, 2001b). First, as fungi grow in moist conditions, drying of the maize kernels reduces the accumulation of mycotoxins after harvest. Second, fumonisin B_1 can be

decontaminated by ammoniation under high temperature or pressure, reducing the concentration by 79 %. However, it is indicated that several parameters might affect the efficacy of ammoniation and their role, as well as the safety of the process, should be further studied. Other approaches towards decontamination that showed (some) positive effects when tested are reaction with fructose and gamma irradiation. Finally segregation of mouldy kernels can reduce the fumonisin B_1 content of maize batches. Except under extreme conditions, the concentrations of fumonisins in food do not increase during storage. Fumonisins are not destroyed during the production of grits, bran, germ, meal and flour, which are produced by dry milling. In contrast, wet milling, which is used to produce germ, fibers, gluten and starch, reduces the amount of fumonisin B_1 by 60 to 90 %, depending on the product (FAO/WHO, 2001b). Fumonisin B_1 is not affected by food processing methods that are used in North America and Western Europe. Treating maize with base or water lowers the fumonisin B_1 concentration, however, toxicity remains evident (WHO-IPCS, 2000; FAO/WHO, 2001b).

In EU regulation EC 856/2005 (EC, 2005) it is indicated that if no specific maximum level for fumonisins in food is fixed before 1 October 2007, the maximum levels of the sum of fumonisin B_1 and B_2 as indicated in Table M-2 will apply from this date. As no other European regulations concerning fumonisins were located it is assumed that currently these levels apply. In 2004-2006, VWA determined fumonisin B_1 concentrations in several batches of food. In the successive years, respectively 45.9 %, 12.0 % and 16.7 % of the maize samples examined by VWA exceeded the concentration limits. In addition, exceedances were observed in baby food (VWA, 2007b).

Table M-2. Maximum levels of the sum of fumonisin B_1 and B_2 allowed in food groups (EC, 2005)

Food groups	Maximum levels (µg/kg)
Unprocessed maize	2,000
Maize grits, maize meal and maize flour	1,000
Maize-based foods for direct consumption	400
Processed maize-based foods for infants and yound children and baby food	200

Toxicology

Adequacy of toxicity database

The toxicity database of fumonisin B_1 was adequate for the present risk evaluation. A subchronic (90 days) and a chronic (2-year) oral toxicity study in rats are available, which have been used to derive TDI values. These studies identified kidney toxicity as the most sensitive effect of fumonisin B_1. Besides, adequate reproduction toxicity studies are present. Information concerning immunotoxicity and neurotoxicity of fumonisin B_1 is limited; however the available data indicate that these effects are not likely to occur at doses below the NOAEL used to derive the TDIs. Given that the current dietary intake only slightly exceeds the TDIs for a small percentage of children, overall the information was considered sufficient.

Toxicokinetics and toxicodynamics

There are no kinetic data available for fumonisin B_1 in humans. In many animal species, including swine, rat, mouse and non-human primates, fumonisin B_1 is poorly absorbed (< 6 %) after oral intake. Distribution and elimination of fumonisin B_1 occurs rapidly and it is recovered mainly unmetabolized in the faeces. Small amounts are excreted in the urine. Studies have shown that in several animal species it is not transferred into milk and does not cross the placenta. A small amount of fumonisin B_1 was shown to be retained in the liver and the kidney (WHO-IPCS, 2000; SCF, 2000b).

The toxicity of fumonisins is ascribed to their interference with sphingolipid biosynthesis and metabolism, which can result in disturbances of a number of cellular processes such as growth and differentiation and can lead to apoptosis induction. Interference of fumonisins with the biosynthesis of sphingolipids is reflected by changes in the sphinganine/sphingosin (Sa/So) ratio. It is indicated that effects on the Sa/So ratio are observed at fumonisin doses of 0.2 mg/kg bw and higher (SCF, 2000b). A study in rats showed that daily gavage of 0.01 to 5 mg/kg bw/d of fumonisin B_1 resulted in alterations in tissue Sa/So ratios at doses of 1 mg/kg bw/d and higher with the kidney and liver most affected. After cessation of treatment the Sa/So ratio in the kidney returned to normal in one to three weeks (Garren et al., 2001). Fumonisin B_1 causes apoptosis in several tissues in experimental animals, which is thought to play an important role in fumonisin toxicity (SCF, 2000b). The major target organs of fumonisin B_1 are the kidney and the liver. In addition, neurotoxic and immunosuppressive effects of fumonisin B_1 have been reported in livestock and experimental animals (SCF, 2000b).

Carcinogenicity

Fumonisin B_1 is a carcinogen, causing both kidney and liver cancer in exposed experimental animals. In 1993 and 2002, IARC evaluated fumonisin B_1 and classified it as a group 2B carcinogen (possibly carcinogenic to humans), based on sufficient evidence in experimental animals but no adequate evidence in humans (IARC, 1993, 2002). There is no evidence that fumonisin B_1 is carcinogenic via a genotoxic mechanism (SCF, 2000b). In experimental animals, kidney or liver toxicity are a prerequisite for the development of cancer in the respective organ, indicating that there is likely a threshold for carcinogenesis induced by fumonisin B_1.

Health based limit value

In 2000, SCF allocated a TDI of 2 µg/kg bw/d to fumonisin B_1 based on the overall NOAELs for kidney toxicity of 0.2 and 0.25 mg/kg bw/d respectively in a subchronic (90 days) and a chronic (2-year) oral toxicity study in rats. A safety factor of 100 was applied (SCF, 2000b). In 2001, JECFA allocated a provisional maximum TDI of 2 µg/kg bw to fumonisins B_1, B_2 and B_3, alone or in combination. This value was based on the NOEL for renal toxicity of 0.2 mg/kg bw/d derived from the above mentioned studies in rats and a safety factor of 100 (FAO/WHO, 2001b). In response to the publication of JECFA, the opinion of SCF was updated in 2003 and it was decided to expand the TDI for fumonisin B_1 and establish a group TDI of 2 µg/kg bw for the total of fumonisin B_1, B_2 and B_3, alone or in combination (SCF, 2003).

Relevance of the TDI values for young children

Kidney toxicity can be a critical toxic effect for children exposed to fumonisin B_1 however it should also be considered whether effects for which children are potentially more sensitive than adults may occur at similar dose levels. Specifically, information on potential reproductive toxicity, immune toxicity, neurotoxicity and endocrine toxicity was evaluated. The result of this evaluation is indicated below.

Reproduction toxicity

WHO and JECFA indicate in their evaluations that despite initial concern regarding potential reproduction toxicity of fumonisins, the conclusion that fumonisins act as a developmental or reproductive toxicant in farm animals or humans is not supported by experimental data. Fumonisins do not cross the placental barrier in rats, mice and rabbits. The embryotoxic effects that were observed in reproduction toxicity studies (reduced weight gain and growth abnormalities) are thought to be secondary effects of maternal toxicity. Overall, there is a reasonable amount of reproductive studies in experimental animals covering exposure prior to and during pregnancy and during lactation (WHO-IPCS, 2000; FAO/WHO, 2001b). No effects on breeding capacity of mice and rats were observed in reproduction toxicity studies. A limited number of studies have investigated the effects of fumonisin B_1

on weanling piglets/pubertal boars. Although effects were observed on sperm reserves and production, concentrations fumonisin B_1 that caused these effects were considerably higher than the NOAEL for kidney toxicity (Gbore and Egbunike, 2008).

Immune toxicity
A number of studies showed potential immunosuppressive or modulating effects of fumonisin B_1 *in vitro* and in experimental animals. However, no NOAEL could be derived from the studies addressing effects on the immune system. SCF mentions that toxic effects of fumonisin B_1 on the immune system are observed at higher dose levels than kidney toxicity and liver toxicity (SCF, 2000b).

Neurotoxicity
Neuronal degradation and disrupted sphingolipid metabolism in the brain was observed after intracerebral infusion of 0.1 mg/d of fumonisin B_1 in mice (Osuchowski et al., 2005). Subcutaneous infusion of the same dose of fumonisin B_1 did not result in neurotoxic effects. Subcutaneous administration of 0.4 to 8 mg/kg bw/dag of fumonisin B_1 to juvenile rats resulted in increased Sa levels and Sa/So ratios in the brain of treated rats and decreased myelin deposition (Kwon et al., 1997). In horses and ponies, the most relevant effect of fumonisin B_1 is lethal neurotoxicity (equine leukoencephalomalacia syndrome, characterised by necrotic lesions in the white matter of the cerebrum). In a short-term toxicity study, this effect was associated with increased Sa/So ratios and had an estimated NOAEL of 0.2 mg/kg bw (SCF, 2000b). It is indicated that the neurotoxic effects in horses and ponies may be secondary to adverse cardiovascular effects caused by fumonisin B_1 (SCF, 2000b).

Ochratoxin A

Introduction

The present description of the toxicological profile of ochratoxin A (OTA) is largely based on an EFSA opinion (EFSA, 2006b) and a JECFA evaluation (FAO/WHO, 2001b).

Toxicology

Adequacy of toxicity database
The critical studies on which the TWI for OTA is based was performed in pigs aged 8 to 12 weeks (Krogh and Elling, 1977; Krogh et al., 1988). No adequate study of reproductive toxicity is available. Such a study could reveal health effects of early exposure of pups to OTA, and therefore the lack of such a study could be considered a major data deficiency. It is considered that the study in young pigs mentioned above is adequate for the risk assessment of OTA exposure in children aged 2 to 6 years. Therefore, despite the lack of a reproductive toxicity study the database is considered sufficient to perform a risk assessment of OTA for young children.

Toxicokinetics
Following oral administration, OTA is relatively rapidly, although incompletely (40 to 66 %) absorbed. OTA is extensively bound to plasma proteins (albumin and other macromolecules; up to 99.98 % in human blood). OTA is distributed mainly to the kidneys, and to a lesser extent to liver, muscle and fat. OTA can cross the placenta. OTA is hydroxylated in the liver only to a minor extent resulting in the R- and S-epimers of 4-OH-OTA. In some animal species, also 10-OH-OTA production has been described. Furthermore pentose and hexose conjugates of OTA may be formed. In addition to hepatic metabolism, bacterial metabolism in the gastrointestinal tract yields the cleavage product, ochratoxin α, which can be absorbed from the lower gastro-intestinal tract.

In a human volunteer study the major analyte in blood serum was the parent compound, and only small concentrations of OTA metabolites and/or conjugates could be measured (Studer-Rohr et al., 2000). In urine samples, however, only about 50 % of the radioactivity was parent OTA, suggesting the presence of OTA metabolites (particularly ochratoxin α) and/or OTA glucuronic acid conjugates.

In many species, including monkeys and humans, the major route of excretion is renal elimination, whereas in rodents biliary excretion seems to prevail. Biliary excretion and entero-hepatic recirculation of OTA-glucuronides may account for the interindividual and interspecies variability of kinetic parameters observed in the various kinetic studies. In all species studied to date (humans, rodents, pigs, rabbits, fish, birds), OTA kinetics has been characterized by an open two-compartment model. In rats and pigs, elimination half-lives of five and six days, respectively were reported. In vervet monkeys, the elimination half-life of OTA monkeys was 19 to 21 days. In a human volunteer study with oral administration of OTA, the toxicokinetics were best described by a two-compartment model. The model indicated a fast elimination and distribution phase (half-life about 20 hours) followed by a slow elimination phase (plasma half-life of 35 days).

In addition to renal clearance, OTA was detected in human, rat and rabbit milk. Recent evidence suggests that this excretion is supported by BCRP (breast cancer related protein), a member of the ATP-dependent efflux transporter family, known to be responsible for the excretion of various xenobiotics into milk (Jonker et al., 2005; Schrickx et al., 2006).

Mechanisms involved in renal cellular uptake

OTA accumulates in the kidneys, through carrier-mediated processes and possibly also via passive diffusion. *In vitro* data with cell cultures indicate that OTA is a substrate for the family of organic anion transporter proteins. In humans, the best characterized of these transporters is the SLC22A (formerly OAT1) family that has a wide substrate specificity. Sex- and species-differences in OTA-mediated toxicity may be related to variations in the expression levels of organic anion transporters.

Acute toxicity

The oral LD_{50}s ranged from 0.2 to 58 mg/kg bw. Dogs and pigs were the most sensitive species and rats and mice the least sensitive. Male rats given OTA at a single gavage dose of 0, 17 or 22 mg/kg bw showed multifocal hemorrhages in many organs and fibrin thrombi in the spleen, the choroid plexus of the brain, liver, kidney and heart, suggesting disseminated intravascular coagulation, probably due to activation of extrinsic and intrinsic systems of coagulation. Other changes were hepatic and lymphoid necrosis, enteritis with villous atrophy, affecting the jejunum most severely, and nephrosis. The myocardial changes were considered to be related to shock and subsequent ischaemic injuries (Albassam et al., 1987).

Subchronic and chronic toxicity

OTA had nephrotoxic effects in all monogastric mammalian species tested so far. Its main target site is specific, being the straight segment of the proximal tubule S3 in the outer stripe of the outer medulla, where it exerts cytotoxic and carcinogenic effects. Significant species differences in sensitivity to nephrotoxicity were evident, in the order pig > rat > mouse. Male rats were found to be more sensitive than females (Berndt and Hayes, 1979; Munro et al., 1974) and old female rats (aged 27 to 30 months) were more sensitive to OTA than young adult rats (aged 12 weeks).

In short- and long-term studies in rats, OTA induced increased relative kidney weight, urine volume, blood urea nitrogen, urinary glucose, proteinuria and impaired urinary transport of organic substances. Renal lesions were histologically characterised by karyomegaly (large kidney epithelial cells with giant

polyploid nuclei and prominent nucleoli), necrosis of tubular cells and thickening of tubular basement membranes.

In a 90-day study, rats treated at a dose of 370 µg/kg bw/d showed kidney lesions involving karyomegaly and increased eosinophilia in cells of the proximal convoluted tubules, which persisted for an additional 90 days during which the animals received a control diet. At lower doses the changes were mild and reversible. The LOAEL was 15 µg/kg bw/d. A NOAEL could not be derived.

In 2-year studies in rats kidney lesions consisted of contraction and disorganization of the normal linear pattern of the S3 tubules due to marked development of karyomegaly and cytomegaly. The overall NOAEL was 21 µg/kg bw/d for five days/week, equivalent to 15 µg/kg bw/d (US-NTP, 1989).

Administration of 120 µg OTA/kg bw/d to Wistar rats, for 10, 30 or 60 days, produced oxidative stress and dose/time related apoptosis in proximal and distal epithelial kidney cells. OTA concentrations in the kidneys were proportional to the time of exposure.

In young beagle dogs oral (capsule) doses of OTA (0, 0.1 or 0.2 mg/kg bw/d for 14 days) did not affect renal function, but tubular necrosis and ultrastructural changes in the proximal tubules were observed at all doses. Necrosis of lymphoid tissues of the thymus and tonsils was also seen at all doses (Kitchen et al., 1977a, b, c).

Pigs are generally considered as the animal species most sensitive to the nephrotoxicity of OTA. In a 3-month study, a dose-related decrease in the renal activity of phosphoenolpyruvate carboxykinase and *gamma*-glutamyl transpeptidase and a dose-related decrease in renal function was found. Progressive nephropathy but no renal failure was seen in female pigs given feed containing 1 mg OTA/kg (40 µg/kg bw/d) for two years whereas nephropathy was not observed after 0.2 mg OTA/kg (8 µg/kg bw/d) for two years (Elling, 1979a, b, 1983; Elling et al., 1985; FAO/WHO, 2001b; Krogh and Elling, 1977; Krogh et al., 1988; Meisner and Krogh, 1986). From these studies in pigs JECFA derived a LOEL of 8 µg/kg bw/d for effects on the kidneys (effects on enzymes and function) (FAO/WHO, 2001b).

In pigs exposed to 90 to 180 µg OTA/kg food, microscopic lesions, as well as changes in various hematological and biochemical parameters, were observed in all groups. Increasing OTA levels to 130, 305 or 790 µg OTA/kg food for an additional two months induced degenerative changes affecting the epithelial cells of the proximal tubules, which predominated at the initial stage and proliferative changes in the interstitium, which predominated at the later stage of the disease (Stoev et al., 2001).

Pigs given a diet containing OTA at 800 µg/kg showed degenerative changes affecting epithelial cells in some proximal tubules of pigs after six months and proliferative changes in the interstitium which predominated after one year.

Carcinogenicity

OTA produces renal tumours in rats and mice with marked differences in species and sex specificity (US-NTP, 1989). Male animals are more sensitive than females and rats are considerably more sensitive than mice. In addition, increased incidences of hepatic tumours have been found.

Male and female mice treated with OTA in the food at 5.6 mg/kg bw/d for 44 weeks or male mice treated with OTA at 3.5 mg/kg bw/d for 70 weeks showed renal cystic adenomas, solid renal-cell tumours and hepatic-cell tumours. No hepatic or renal tumours were observed in control mice (Kanisawa and Suzuki, 1978). Male mice exposed to 3.5 mg/kg bw/d showed renal cystic adenomas,

solid renal tumours and hepatic-cell tumours. One of the 17 control mice had a hepatic-cell tumour (Kanisawa, 1984).

In a dietary study in which mice were exposed to OTA at 7 mg/kg bw/d for five to 30 weeks, followed by control diet for 40 to 65 weeks, increased incidences of renal-cell tumours were found in animals treated for 15 weeks or longer. The incidence of liver tumours was increased in mice fed OTA for 25 or 30 weeks (Kanisawa, 1984).

Rats treated with OTA by gavage at a concentration of 0, 21, 70 or 210 µg/kg bw/d, five days/week for up to103 weeks showed a dose-dependent increase in renal adenomas and carcinomas. The incidences were higher in males than in females. Histological examination showed that the site of injury was the straight segment of proximal tubule S3 in the outer stripe of the outer medulla.

Genotoxicity

Gene mutations were induced in bacteria and mammalian cells in a few studies of genotoxicity, but not in most. OTA did, however, induce DNA damage, DNA repair and chromosomal aberrations in mammalian cells *in vitro*, and DNA damage and chromosomal aberrations in mice treated *in vivo*. Putative DNA adducts were found consistently with a ^{32}P-postlabelling method in the kidneys of mice and rats dosed with OTA, but none of these adducts has been demonstrated to contain fragments of OTA. It was therefore uncertain whether OTA interacts directly with DNA or whether it acts by generating reactive oxygen species. There was no indication that a reactive metabolite of OTA is generated *in vivo*. OTA is thus genotoxic both *in vitro* and *in vivo*, but the mechanism of genotoxicity is unclear and there was no evidence that it is mediated by direct interaction with DNA. The doses used in the studies of genetic toxicity were in the same range as those at which the incidence of renal tumours was increased in mice. In rats, however, the incidences of nephrotoxicity and renal tumours were increased at much lower doses; therefore the contribution of the genotoxicity of OTA to neoplasia in rats is unknown (FAO/WHO, 2001b).

Reproductive and developmental toxicity

No adequate studies on reproductive toxicity of OTA are available.

OTA can cross the placenta and it is embryotoxic and teratogenic in mice, rats and rabbits in the dose-range between 0.1 to 1 mg OTA/kg bw/d given orally during gestation. In mice, a single gavage dose of OTA 0, 1, 2 or 4 mg/kg bw on day eight or nine of gestation induced craniofacial anomalies (aplasia and dysplasia of the upper facial structures, such as exencephaly, microcephaly, blunt jaws, anophthalmia, microphthalmia, open eyelids, cleft lip, median cleft face, agenesis of external nares and malformed jaws or short maxilla with protruding tongue), which were considered to be the result of failure of closure of the neurocranium, resulting in abnormal configuration, position, and size of the bones of the base and lateral walls of the skull. The incidence, multiplicity and severity increased with increasing dose. The peak effect was seen on day nine (Arora and Frolen, 1981).

Pregnant Wistar rats were given OTA at a total dose of 5 mg/kg bw in 0.16 mol/L sodium bicarbonate by gavage, as follows: a single dose of 2.5 mg/kg bw on each of days eight and nine of gestation (vaginal plug considered to be day one), a dose of 1.2 mg/kg bw on each of days eight to 11 of gestation, a dose of 0.83 mg/kg bw on each of days eight to13 of gestation or a dose of 0.63 mg/kg bw on each of days eight to 15 of gestation. In a similar way, pregnant rats were given OTA at a single dose of 2.5 mg/kg bw on each of days eight and nine of gestation or a dose of 1.7 mg/kg bw on each of days eight to 10 of gestation. There was a dose-related increase in the number of resorptions per female and decreases in the mean number of fetuses per female, mean fetal weight and mean placental weight. A high dose-related incidence of fetal hemorrhages (seen at 2, 2.5 and 4 times the 1.2 mg/kg dose) and

coelosome with or without oedema were considered to be teratogenic responses. Females that had received the same total amount of OTA but divided into fewer single doses and early in gestation were most affected (Moré and Galtier, 1974).

In pregnant rats, gavage doses of 0.50 and 0.75 mg OTA/kg bw/d during days six to 15 of gestation caused a dose-dependent increase in various skeletal and visceral anomalies (skeletal defects in skull, ribs, sternebrae and vertebrae, exencephaly, incomplete closure of skull, micrognathia, micromelia, scoliosis, small hind portion, and soft tissue defects such as hydrocephalus, microphthalmia, dilated renal pelvis, hydronephrosis and cryptorchid testis) (Wangikar et al., 2004a, b). In addition, histological examination revealed an increased incidence of lesions in liver, kidney, brain and eyes as well as bile duct proliferation, new bile duct formation, defective ossification of cranial bones, hypoplasia of cerebellum and defects of the retina.

Oral administration of OTA to pregnant rats at 1 mg/kg bw/d on days six to 15 of gestation resulted in decreased fetal weight and increased numbers of resorptions but no overt adverse effects on the dams. Skeletal and/or lung malformations were reported in up to 20 % of the fetuses; the incidence of renal malformations was 40 %.

In rabbits on oral dose of 0.10 mg OTA/kg bw/d, given by gavage from days six to 18 of gestation (Wangikar et al., 2005) resulted in an increased incidence of malformed foetuses (skeletal and visceral anomalies, e.g., knuckling of fetlock, rudimentary tail or agenesis of tail, wavy ribs, hydrocephalus, microphthalmia and agenesis of kidney).

Neurotoxicity

OTA was shown to be neurotoxic in mice, rats and rabbits. In mice, OTA induced striatal dopamine depletion and oxidative stress, oxidative DNA damage and a transient inhibition of oxidative DNA repair in different brain regions after single i.p. doses of 3 mg/kg bw and higher. OTA administered in the diet induced cytotoxicity in the ventral mesencephalon, hippocampus, striatum and cerebellum of rat brain (Belmadani et al., 1998b).

In rats receiving OTA at 290 µg/kg bw orally every 48 hours for one to six weeks OTA accumulated in the brain in a linear time-dependent manner. OTA caused changes in the concentrations of the amino acids tyrosine and phenanthrene and damage tissues in the hippocampus (Belmadani et al., 1998a). Oral administration of 120 µg OTA/kg bw/d for up to 35 days increased gamma-glutamyl transferase activity in the brain (Zanic-Grubisic et al., 1996)

In oral teratogenicity studies, brain lesions were seen in foetuses of rats (Wangikar et al., 2004a) and rabbits (Wangikar et al., 2005), when dams were treated during gestation with 500 µg OTA/kg bw/d or more (rats) or 50 µg/kg bw/d (rabbits), respectively. In a 28-day study in rats, vacuolation of the white brain matter (cerebellar medulla and ventral parts of the brain stem) was significantly increased by 70 µg OTA/kg bw/d or more by gavage (Dortant et al., 2001). In a drinking water study, rats receiving 289 µg OTA/kg bw/d for four weeks showed reduced concentrations of hippocampal N-methyl-D-aspartate (NMDA) receptor subunits 2A and 2B (Delibas et al., 2003).

Immunotoxicity

In male rats receiving 0, 50, 150 or 450 µg OTA/kg bw/d by gavage for 28 days natural killer cell activity was strongly affected in all treatment groups. The bacteriolytic capacity of macrophages was significantly reduced at 50 and 450 µg/kg bw/d. In an oral 28-day study in female rats (Dortant et al., 2001), decreased IgG levels were seen at 340 and 1,680 µg/kg bw/d. In addition, OTA induced a dose-related reduction in the splenic T-cell fraction reaching statistical significance in the highest dose only.

In a study by Harvey et al. (1992), growing pigs, administered 2.5 mg OTA/kg feed (about 100 µg/kg bw/d for 35 days, showed a reduced immunologic response to phytohemagglutinin (PHA) and concanavalin.

Observations in humans
OTA has been found in human blood samples. However, no cases of acute intoxication in humans have been reported. OTA is found more frequently and at higher average concentrations in blood from people living in regions where a fatal human kidney disease (known as Balkan endemic nephropathy) occurs and is associated with an increased incidence of tumours of the upper urinary tract. However, similar average concentrations have been reported in several other European countries where this disease is not observed.

Patulin

Introduction/characterization of exposure
Patulin is a mycotoxin that is produced by certain species of the *Aspergillus, Penicillium and Byssochylamys* genera. These fungi are present in several foods such as fruits, grains and cheese. In the latter however, patulin is rendered inactive due to high cysteine contents. Patulin is mainly found in apples and pears with brownrot. In whole fruits, rotten portions are often removed prior to consumption, and patulin intake via these sources will therefore be limited. Also patulin is destroyed by fermentation and hence not present in alcoholic beverages or vinegars. However, heat treatment causes only a minor reduction in patulin level and patulin survives pasteurization processes. As a consequence patulin can be present in apple sauce, apple juices and potentially also in other fruit juices (FDA, 2001). Infection of rotten apples with above mentioned fungi is common. As apple juice and apple sauce are often regular constituents of a child's diet, this means that exposure to (low levels of) patulin will likely occur on a chronic basis. In addition, variations in harvest quality may influence the patulin content of apples significantly and as a result occasional high(er) intake levels of patulin may occur.

Possible exposure-reducing measures
The main sources of patulin intake are apple juice, apple sauce and apples. As mentioned above, consumers most likely remove any rotten or damaged parts of apples prior to consumption, which will limit patulin intake. In case of apple juice and apple sauce, a reduction of patulin content may be achieved similarly by treating fruit prior to production. The FDA indicates that such controls for patulin are needed to ensure optimal protection of consumers from potential adverse effects due to long-term exposure to patulin. Health effects from patulin are thought to be negligible if levels in apple juice would be controlled at 50 µg/kg or less. This can be achieved by trimming away the rotten portion of apples before further processing. In addition, it is indicated that water treatment may effectively reduce patulin levels. However certain varieties of apples may be more prone to patulin formation within the apple and hence the presence of patulin in apple juice and apple sauce cannot be avoided completely (FDA, 2001).

In EU regulation EC 1881/2006 (EC, 2006b) maximum levels of contaminants, among which patulin, in food groups were provided (see Table M-3). This regulation applies from March 2007 and prohibits using foods that do not comply with the maximum levels indicated. In 2004-2006, VWA determined patulin concentrations in several batches of juice, beers and wines. It is indicated that patulin concentrations rarely exceeded the maximum levels in more than 500 samples evaluated. However, in two cases the maximum levels were approximated, with 48 µg/kg found in apple juice and 57 µg/kg in a mixed apple-berry juice (VWA, 2007b).

Table M-3. Maximum levels of patulin allowed in food groups (EC, 2006b).

Food group	Maximum levels (µg/kg)
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	50
Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice	50
Solid apple products, including apple compote and apple puree intended for direct consumption	25
Apple juice and solid apple products, including apple compote and apple sauce for infants and young children, labelled and sold as such	10
Baby foods other than processed cereal-based foods for infants and young children	10

Toxicology

Adequacy toxicity database

The toxicity database of patulin is limited. A 2-year oral toxicity study in rats is available, which has been used to derive TDI values (critical effect reduced body weight gain) and adequate reproduction toxicity studies are present. Short-term immunotoxicity studies were available but information concerning potential neurotoxicity and endocrine toxicity of patulin after oral exposure is lacking. As the available data suggest that the main toxic effect of patulin is a local effect on the gastrointestinal tract with limited systemic availability of patulin, overall the information was nevertheless considered sufficient.

Toxicokinetics and toxicodynamics

The information on (toxico)kinetics of patulin is limited. A study in rats showed that 49 % of a single radioactively labelled oral dose of patulin was excreted via the faeces and 36 % via the urine within seven days after dosing, mostly within the first 24 hours. The radioactive label in the urine consisted of metabolites and/or conjugates of the original patulin (Dailey et al., 1977). A study in human volunteers showed that after consumption of apple juice patulin content of the blood was less than 200 ng/L. Also after consumption of apple juice containing the maximum tolerable amount of patulin no patulin was found in the serum when analyzed shortly after consumption. *In vitro* studies showed that patulin is rapidly degraded in whole blood, with only 6.1 % of patulin detected after two minutes of incubation. From this study it was concluded that patulin from food is likely degraded and will not reach other tissues than the gastrointestinal tract (Rychlik, 2003). A similar conclusion was drawn in a study on the absorption of patulin from perfused rat stomachs. This study showed that patulin passes through the gastric wall but is largely disintegrated (Rychlik et al., 2004). Patulin was shown to disrupt the epithelial barrier in cultures of human epithelial cell lines, without major signs of toxicity. It was suggested that this property of patulin may contribute to the development of gastrointestinal inflammation and ulcers that are observed in acute toxicity studies in experimental animals. It was mentioned that the concentrations of patulin tested in these *in vitro* experiments were likely higher than those that humans are naturally exposed to. It is unknown whether chronic low dose exposure to patulin might also result in injury to the epithelial barrier of the gastrointestinal tract (Mahfoud et al., 2002).

The signs of acute oral toxicity of patulin in experimental animals include toxicity to the gastrointestinal tract, agitation, dyspnea and lung toxicity. In short-term toxicity studies, the observed toxic effects are in general restricted to the gastrointestinal tract. Administration of high doses of patulin (> 16 mg/kg bw/d) to rats, mice and hamsters for two weeks resulted in lesions in the gastrointestinal tract, including epithelial degeneration, hemorrhage and ulceration of the mucosa. In

the only available 2-year toxicity study, the most sensitive toxic effect of patulin was a decreased body weight observed in male rats at oral doses above 0.1 mg/kg bw by oral gavage three times a week for two years. Oral dosing of 1.5 mg/kg bw/d resulted in increased mortality in both sexes. It is indicated that patulin induced mortality in (sub)chronic toxicity studies in experimental animals due to dilatation of the gut and/or pneumonia. The latter is thought to be secondary to the antibiotic effects of patulin on gut bacteria, which could give a selective advantage to pathogenic bacteria. This hypothesis is supported by a 13-week study in pathogen free rats in which no such mortality was observed at similar dose levels (FAO/WHO, 1996).

Carcinogenicity

The information on potential carcinogenic effects of patulin in experimental animals was considered inadequate for evaluation by IARC (1986). JECFA concluded that patulin is genotoxic but no adequate evidence exists for carcinogenicity in experimental animals (FAO/WHO, 1996). An oral study using the F1 generation from the previously mentioned reproduction toxicity study did not show a difference in tumour incidence in rats exposed to 0 to 1.5 mg patulin/kg bw/three times a week for two years. Subcutaneous injection of 0.2 mg patulin biweekly for 61 to 64 weeks resulted in the development of local sarcomas in rats (FAO/WHO, 1996). No information on the potential carcinogenicity of patulin in humans is available. The mycotoxin was classified category 3 ‘not classifiable as to its carcinogenicity to humans’ (IARC, 1986). There are no indications that patulin is carcinogenic in humans or experimental animals after oral intake.

Health based limit value

Two TDI values for patulin were derived. JECFA estimated a provisional maximum TDI for patulin of 0.4 µg/kg bw based on a NOEL for decreased weight gain of 0.1 mg/kg bw in the above mentioned 2-year toxicity study in rats. In this study, rats derived from the F1 generation of a reproductive toxicity study were exposed to 0, 0.1, 0.5 or 1.5 mg/kg bw of patulin in citrate buffer by gavage three times per week. Body weights of male but not female rats were reduced when dosed 0.5 mg/kg bw. The highest dose of patulin resulted in increased mortality in rats of both sexes. As patulin was administered only three times a week the NOEL of 0.1 mg/kg bw was recalculated to a daily intake of 43 µg/kg bw. A safety factor of 100 was applied (FAO/WHO, 1996). In 2000, SCF endorsed the provisional maximum TDI for patulin of 0.4 µg/kg bw/d that was allocated by JECFA (SCF, 2000a).

Relevance of the TDI values for young children

Decreased weight gain can be a critical toxic effect for children exposed to patulin however it should also be considered whether effects for which children are potentially more sensitive than adults may occur at similar dose levels. Specifically, information on potential reproductive toxicity, immune toxicity, neurotoxicity and endocrine toxicity was evaluated. The result of this evaluation is indicated below.

Reproduction toxicity

In an oral reproduction toxicity study, rats were administered 0 to 15 mg patulin/kg bw/d seven weeks prior to mating and during pregnancy. P and F1 generations were studied for toxicological effects. Animals from the low dose group were used to produce an F2 generation. No teratological effects of patulin treatment were observed, however, decreased pup growth and increased mortality of F2 females were observed at 1.5 mg/kg bw/d. Higher concentrations of patulin resulted in high mortality in both males and females (FAO/WHO, 1996). In another study, rats were exposed to 0, 0.1, 0.5 or 1.5 mg/kg bw/d of patulin by gavage four weeks prior to mating and during pregnancy and lactation. No effects on reproductive parameters such as mating success, litter size, fertility, gestation, viability, pup weight or histological parameters were observed (FAO/WHO, 1996). In contrast, an oral study in which rats aged 5 to 6 weeks were gavaged with 0.1 mg patulin/kg bw/d for 60 or 90 days showed a decrease in

sperm counts, a slight increase in the number of sperm abnormalities and some histopathological changes in epididymis and prostate in patulin treated rats, indicating a possible adverse effect on male fertility (Selmanoglu, 2006). In a similarly designed study, the same research group found increased serum testosterone levels and histological abnormalities in the testes of male rats treated with patulin (Selmanoglu and Kockaya, 2004). From the results of reproduction toxicity studies it was concluded that patulin is not a developmental or reproductive toxicant.

Immune toxicity

Potential immune toxicity of patulin was evaluated by the NTP in 28 days oral gavage studies in female mice and in rats. In mice, doses from 0.08 to 2.56 mg patulin/kg bw/d did not affect the body weight or the weight of spleen, liver, lungs, thymus and kidney. The only hematological parameters studied that were affected were the leukocyte and lymphocyte count, which were decreased after administration of 1.28 and 2.56 mg/kg bw/d of patulin. Besides, alterations in the absolute number and percentages of spleen cell types were observed. The NOEL was 0.64 mg/kg bw/d. However, no immunosuppressive effects of patulin were observed and it was concluded that patulin is not toxic to the immune system at the tested doses (HSDB, 2008; Llewellyn et al., 1998). In rats, patulin did not adversely affect the immune system after administration of doses up to and including 5.12 mg/kg bw/d (HSDB, 2008; Llewellyn et al., 1998). In a study in which sublethal oral doses were administered to mice and rabbits, reversible immunosuppressive effects of patulin were observed, involving both leucocytes and lymphocytes (Escoula et al., 1988). No long-term studies on the effect of patulin on the immune system are available.

Neurotoxicity

Rats treated with intraperitoneal injections of 0 to 100 µg patulin on alternate days for one month showed convulsions, tremors, impaired locomotion, stiffness of the hind limbs and wagging of the head. Patulin inhibited acetylcholinesterase in certain brain regions with concomitant increase of acetylcholine levels. The administered dose of patulin was calculated to be approximately 1.6 mg/kg bw/d. No further information on neurotoxic effects of patulin was found in the available sources (FAO/WHO, 1996).

rivm

Appendix N Toxicological profile of nitrate, nitrite and n-nitrosodimethylamine

Introduction
The toxicology of nitrate (and nitrite) was recently evaluated by the CONTAM (EFSA, 2008a). The following summarizes the main conclusions of this evaluation, with added information from recent research by Zeilmaker et al. (In preparation) on the formation of N-nitrosodimethylamine from nitrate and amines in food.

Nitrate (NO_3^-) is a naturally occurring compound that is part of the nitrogen cycle, as well as an approved food additive. It plays an important role in the nutrition and function of plants. Nitrate is formed naturally in living and decaying plants and animals, including humans. It is an important component of vegetables due to its potential for accumulation. Higher levels of nitrate tend to be found in leaves whereas lower levels occur in seeds or tubers. Thus leaf crops such as lettuce and spinach generally have higher nitrate concentrations. Despite being a major source of nitrate, increased consumption of vegetables is widely recommended because of their generally agreed beneficial effects for health.

Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent water and other foods. To a limited extent nitrate is also formed endogenously. Exposure to its metabolite nitrite (NO_2^-), however, is mainly from endogenous conversion of nitrate to nitrite.
Nitrate as such is relatively non-toxic, the main toxicological endpoints in laboratory animals result from the formation of nitrite and its ability to react to form N-nitroso compounds (*e.g.*, N-nitrosodimethylamine: O=N-N-$(CH_3)_2$). Among the adverse health effects observed are methemoglobin (metHb) formation, hyperplasia of the zona glomerulosa of the adrenal cortex and gastric neoplasia. On the other hand recent research indicates that nitrite participates in host defence having antimicrobial activity (*e.g.*, in the stomach), and other nitrate metabolites (*e.g.*, nitric oxide: NO), have important physiological and pharmacological roles such as vasoregulation.

Toxicology nitrate and nitrite

Adequacy of the toxicity database
The database of nitrate toxicity (including nitrite and NDMA) is substantial. However, some of the studies have been done quite some time ago, and do not meet modern standards. Next to this, nitrate has also a number of beneficial functions in mammalian physiology, and the health based limit values are not very different to the levels at which beneficial effects are observed. Secondly, subgroups within a population may be more susceptible than others to the adverse health effects of nitrate (and nitrite). Thirdly, individuals with an increased rate of endogenous formation of carcinogenic N-nitroso compounds are likely to be susceptible to the development of cancers in the digestive system (Powlson et al., 2008). Apparently there are thus a number of uncertainties with respect to the toxicity of nitrate, nitrite and the potential formation of N-nitroso compounds resulting from exposure to nitrate. These uncertainties are only partly due to the inadequacy of the database; the inherent complexity of the diversity in biological effects (adverse as well as beneficial) of nitrate is as much of importance.

Toxicokinetics

Nitrate

The toxicokinetics of nitrate are complex. In humans, dietary nitrate is rapidly and effectively absorbed via the stomach and the upper part of the small intestine into the plasma. No or very little nitrate or nitrite was found in ileostomic fluid from persons who had ingested 250 mg of nitrate, suggesting that nitrate does not reach the large intestine. In humans, an average 25-fold increase in plasma nitrate was found 10 min after ingestion of nitrate, and intake peaked in blood after 40 min. Nitrate can also be absorbed via inhalation, for example, from cigarette smoke and car exhausts, although in absolute terms the quantitative amount is of minor importance compared to the oral route via the diet. Absorbed nitrate is rapidly transported by the blood, and in humans (and most laboratory animals) plasma nitrate is selectively absorbed by the salivary glands. Via bioconcentration this results in a salivary nitrate concentration of approximately 10-fold that of plasma, and so the salivary secretion represents approximately 25 % of the ingested dose. In humans, the dose-dependent increase in salivary nitrate secretion, peaking one to three hours after oral ingestion, is mediated by an active transport system that is shared also by iodide and thiocyanate. On the surface of the tongue, commensal bacteria reduce approximately 20 % of the secreted nitrate into nitrite which is then swallowed along with the unconverted nitrate. Healthy adults have a salivary conversion of nitrate to nitrite of normally 5 to 7 % of the total nitrate intake, whereas infants and patients with gastroenteritis who have a higher gastric pH can have a considerably greater conversion rate.

Nitrite

In humans, gastrointestinal (GI) absorption of sodium nitrite is rapid, with maximum plasma nitrite concentrations observed 15 to 30 min after dosing. Moreover, nitrite disappeared rapidly from plasma, with an average elimination half-life of 30 min. It was concluded that under fasting conditions 90 to 95 % of orally administered sodium nitrite is absorbed from the GI tract. However, extensive pre-systemic metabolism in the GI tract, results in a considerable part of the nitrite that enters the GI tract potentially being transformed to other N-containing species before absorption takes place. Plasma nitrite levels are normally much lower than nitrate levels, firstly because of the lower exposure and secondly due to the rapid oxidation from nitrite to nitrate by oxygenated hemoglobin in the blood. Therefore, the sum of nitrate and nitrite in blood is almost identical to the nitrate levels. This is also seen in body fluids and tissues of laboratory animals, where nitrite in the normal situation is practically absent, except in saliva where it increases as nitrate levels decrease. In mice and rabbits, intravenous injection of labelled nitrite resulted in a homogenous distribution of radioactivity to a number of organs, including liver, kidneys and urinary bladder.

Metabolism

In humans, dogs and mini-pigs nitrate is concentrated from the plasma to the saliva and partly reduced to nitrite, which is then swallowed into the stomach. Nitrate is also secreted in the gut. In the rat, however, oral reduction of nitrate to nitrite in the saliva is limited and nitrate is mainly secreted in the gastric and intestinal fluid by active transport involving entero-systemic recirculation as observed in man.

In humans, about 25 % of ingested nitrate is secreted in the saliva and approximately 20 % of the secreted salivary nitrate is then converted to nitrite by commensal bacteria present on the tongue, and thus for normal individuals about 5 to 7 % of ingested nitrate can be detected as salivary nitrite. However, for individuals with a high rate of conversion this figure may be up to 20 %. The major site for nitrate reduction is at the base of the tongue where a stable, nitrate-reducing microflora is present. The concentration of salivary nitrite is directly related to orally ingested nitrate, but the conversion may become saturated at high nitrate intakes.

Oral reduction of nitrate is the most important source of nitrite for humans, and will account for approximately 70 to 80 % of the human total nitrite exposure. Factors that may influence the oral microbial flora are, for example, nutritional status, infection, environmental temperature and age. Salivary nitrite levels were generally higher in older age groups, although considerable variation between individuals was noted. Other factors such as antibacterial mouth wash may markedly lower the transformation of nitrate to nitrite.

After transport to the stomach, the acidic conditions will rapidly transform nitrite to nitrous acid, which in turn will spontaneously decompose to nitrogen oxides including nitric oxide. Compared to the enzymatically produced nitric oxide in mammalian cells (from L-arginine by nitric oxide synthases, see below), the concentration of nitric oxide in the upper intestine is up to 10,000 times higher. A low pH in the fasting stomach (pH 1-2) is considered too low for microbial growth and, as a consequence, for bacterial nitrate reduction. However, in normal healthy adults a significant proportion (30 to 40 %) of the population was found to have a fasting pH over 5, which results in increased bacterial activity and hence increased nitrite levels.

Infants younger than 3 months are highly susceptible to gastric bacterial nitrate reduction to nitrite because they have very little production of gastric acid. Gastrointestinal infections in infants may produce an additional increase in the reduction of nitrate to nitrite.

Nitrate undergoes active secretion in humans not only in the salivary duct cells but also in the gastric parietal cells and occurs at a number of other sites leading to enterosystemic cycling of nitrate and nitrite. Additionally nitrate biotransformation is complex and involves nitrate reduction, nitrite formation, nitrite reoxidation to nitrate, and resulting metHb in a dynamic equilibrium. Nitrite appears to have a transient role with nitrate being the normal state.

Excretion
In humans about 2 % of an oral nitrate dose was secreted in the saliva, but there were marked inter-individual and diurnal variations in this secretion. In the minipig, an appropriate model for humans in terms of salivary secretion, bilateral removal of the parotid glands led to a significant decrease of nitrate secretion from blood to saliva, and thus low nitrite levels. The study suggests that the parotid salivary glands play an important role in the balance of nitrate and nitrite levels in the body. Single oral gavage of varying doses of potassium nitrate gave a urinary nitrate excretion of 65 to 70 % irrespective of dose. Excretion was maximal five hours after ingestion and returned to baseline levels within 18 hours, which in fasting subjects were 10 to 20 mg/L. Results indicate a predominantly tubular excretion of nitrate.

In a study on healthy infants, the urinary excretion of nitrate (average 8.7 mg nitrate/d) was as high as, or even higher, than a low (average) intake of 2 to 7 mg nitrate plus nitrite per day. It was concluded that excretion probably included endogenously formed nitrate. In the anaesthetized dog, urinary excretion rates of nitrate increase progressively in response to increases in the circulating levels without exhibiting a maximum; however, there was a progressive decrease in fractional reabsorption with increasing. It should be noted that a major part of the primary urinary nitrate (ca 80 %) is pumped back to the blood by an active transport mechanism. This salvaging of nitrate from the urine, in addition to the known recycling of nitrate from saliva and also from the intestines (after biliary excretion) strongly suggests that the body is acting to conserve a substance of physiological importance. In faeces, low levels of nitrate and nitrite are present. However, the observed conversion of nitrate to nitrite by the faecal microflora suggests that biliary excretion of nitrate may be higher than the amount detected in the faeces. The bacteria of the large intestine were suggested to be responsible for about half of the extrarenal removal of nitrate from the body. Levels up to 5 mg nitrate/kg breast milk have been

reported. Nitrate levels in milk from lactating women after a normal meal did not exceed the simultaneously measured maternal plasma nitrate levels.

Acute toxicity

Nitrate
The acute oral toxicity of nitrate in animals is generally low with LD_{50} values of approximately 2,500 to 6,250 mg/kg bw/d in mice, 3,300 to 9,000 mg/kg bw/d in rats, 1,900 to 2,680 mg/kg bw in rabbits and 300 mg/kg bw in pigs. It has been observed that the oral lethal dose of nitrate in humans is around 330 mg/kg bw.

Nitrite
Sodium nitrite is approximately 10-fold more toxic than sodium nitrate with LD_{50} values of 214 mg/kg bw in mice, 180 mg/kg bw in rats and 186 mg/kg bw in rabbits.

Subchronic toxicity

Nitrate
No adverse effects were observed in two dogs after dosing sodium nitrate in the diet at a level of 2 % for 105 and 125 days, equivalent to 370 mg/kg bw nitrate. Short-term studies in rats dosed up to 10 % sodium nitrate in drinking water over six weeks showed slight elevation of metHb.

Nitrite
A 14-week study in mice with sodium nitrite in drinking water resulted in lower body weight, spleen weight and sperm counts in males. In females, absolute and relative organ weights (heart, kidney, liver and spleen), together with the length of estrous cycle, were impaired. Histopathological examination showed that squamous cell hyperplasia of the forestomach and extramedullary hematopoiesis were more frequent at the higher dose levels in both sexes. Degeneration of the testis was seen in males at 750 mg/kg bw and above. The NOAEL was concluded to be 190 mg/kg bw/d. A 14-week study in rats with sodium nitrite in drinking water resulted in elevated metHb at all dose levels. Sperm motility was the endpoint related to a no observed effect level (NOEL) of 37 mg/kg for nitrite.

Hypertrophy of the adrenal zona glomerulosa has been investigated in a 13-week study with rats. A NOAEL of 5.4 mg/kg bw/d (nitrite) was found. The mechanism is considered to involve nitrite-induced vasodilatation via nitric oxide production and a reduction in blood pressure activating the reninangiotensin system in the kidney. Consequential mechanisms to restore the physiological blood pressure result in the production of the vasoconstrictor angiotensin II and release of aldosterone from the adrenal zona glomerulosa, resulting in hypertrophy of the zona glomerulosa.

Chronic toxicity/carcinogenicity

Nitrate
A number of long-term toxicity/carcinogenicity studies with nitrate have been performed. Firstly, rats were given sodium nitrate in the diet for two years. A NOEL of 370 mg/kg bw/d was established for nitrate (ion) based on a slight depression in growth rate and inanition at higher doses. No adverse histological changes or increase in tumour frequency were found. Secondly, rats were dosed with sodium nitrate in drinking water over 84 weeks. No histopathological effects of treatment were observed. Thirdly, in a more recent 2-year study rats were given sodium nitrate in drinking water. At the highest dose slight to moderate reduced body weight gain was observed. From this study a NOAEL

of 1,350 mg/kg/bw/d for nitrate (ion) was derived. Overall, these studies demonstrate a low chronic toxicity of nitrate.

Nitrite
Also with nitrite several long-term toxicity/carcinogenicity studies have been done. In a 2-year chronic toxicity study in rats given nitrite in the drinking water, no significant differences between control and treated groups were shown for growth, mortality and total hemoglobin levels. At the highest three doses, metHb increased to 5, 12 and 22 %, and lung toxicity was observed with dilatation of the bronchi with infiltration of lymphocytes and emphysema. At the highest dose, focal degeneration and fibrosis of the heart muscle as well as dilatation of coronary arteries were also observed. Based on heart and lung toxicity the NOAEL for nitrite (ion) was 6.7 mg/kg bw/d. In a 2-year carcinogenicity study with rats exposed to nitrite in drinking water, the survival of treated groups was similar to that of controls. The mean body weights of males and females at the highest dose were lower than those of controls. Males and females at this dose drank less water than controls throughout the study, and the water consumption of the other treated group was generally lower only after week 14. The incidences of hyperplasia of the forestomach epithelium in males and females at the highest dose were significantly higher than in controls. The incidence of fibroadenoma of the mammary gland was significantly increased in females at the intermediate dose, and the incidences of multiple fibroadenoma were increased in females at the two lower doses; however, these neoplasms occur at a high background incidence, and no increase was seen at the highest dose. The incidences of mononuclear-cell leukaemia were significantly decreased in males and females at 80 or 150 mg/kg bw per day. There was no evidence of carcinogenicity. In a 2-year carcinogenicity study with mice exposed to nitrite in drinking water, there was also no difference in survival between exposed groups compared to controls, although mean body weights were lower in females treated with the highest dose. Exposed groups generally consumed less water than the control groups. The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a positive dose-related trend, but not statistically significant. The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in males treated at the highest dose. In females, there was equivocal evidence for carcinogenic activity based on the trend in the combined incidence of squamous cell papilloma and carcinoma of the forestomach.

Reproductive toxicity

Nitrate
Studies in experimental animals have shown reproductive toxicity associated with intake of high levels of nitrate and nitrite, which are not likely to be encountered in drinking-water. Feeder bulls given 100 to 150 g nitrate (ion) per day (approximately 150 to 250 mg/kg bw/d) during 30 days showed increased levels of metHb and serum bile acids, prolonged biological half life of progesterone, increased cortisol concentration and depressed thyroid gland activity. Intake of nitrate also reduced sperm motility. Histological examination revealed degenerative lesions in cells of the spermiocyte and spermatid layers. In a study with sheep, potassium nitrate concentrations in the diet resulting in doses of 0.27 to 5.4 mg/kg bw as nitrate ion did not affect cyclic sexual behaviour, but the fertility and gestation rates of sheep fed nitrate were considerably lower (36 % at 2.7 and 33 % at 5.4 mg/kg bw/d, respectively) than in the control group.No teratogenic effects were observed in rats, mice, rabbits or hamsters. Several cases of metHb were reported among infants in the USA who drank water containing nitrate at concentrations higher than 45 mg/L. There are, however, considerable uncertainties in this database.

Nitrite
In a study with mice, sodium nitrite was given in drinking water (resulting in doses of 120 to 420 mg/kg bw/d) for the continuous cohabitation phase. Although water consumption was reduced at the high dose, nitrite had no adverse effect on reproduction or reproductive performance.

Endocrine toxicity
Nitrate intake could have the potential to adversely affect thyroid function as nitrate shares the same transport mechanism as iodide. This inhibition could lead to a decrease in circulating thyroid hormone levels with feedback resulting in compensatory thyroid gland enlargement (goitre). To investigate this, a 4-week oral study performed in human volunteers showed that sodium nitrate exposure (15 mg/kg bw/d in water) did not cause changes in the thyroid gland function.

Genotoxicity
From a number of *in vitro* and *in vivo* studies (including humans) it was concluded that there was no evidence for the classification of either nitrate or nitrite as genotoxic compounds.

Health based limit value for nitrate and nitrite

Nitrate
A NOEL of 500 mg/kg bw/d sodium nitrate corresponding to 370 mg/kg bw nitrate ion was derived from long-term studies in rats and the subchronic toxicity study in dogs. Applying an uncertainty factor of 100 resulted in an ADI of 3.7 mg/kg bw/d for nitrate (ion). It has been argued that the rat may not be a good model for humans due to its low conversion of nitrate into nitrite in the saliva. However, because of the importance of the chronic toxicology, the rodent toxicokinetics and similar NOAELs found in the dog (a relevant model for humans) these studies continue to be considered to be relevant for risk assessment.

Nitrite
For nitrite the recent 2-year chronic toxicity/carcinogenicity study in rats was considered the pivotal study for deriving a health based intake limit value. An ADI of 0.07 mg/kg bw was established, expressed as nitrite ion, on the basis of the NOEL of 6.7 mg/kg bw per day for effects on the heart and lung, with an uncertainty factor of 100.

Nitrate-nitrite conversion
Also the conversion of nitrate to nitrite in the saliva was considered in order to derive a ‘transposed’ NOAEL for nitrate based on the NOAEL for nitrite. These ‘transposed’ NOAELs then were compared to the current ADI of nitrate. This derivation resulted in a ‘transposed’ ADI of 3.2 mg/kg bw day. Because this was in the same range as the originally derived ADI for nitrate (3.7 mg/kg bw/d based on nitrate toxicity), there was no justification to amend this.

Toxicology n-nitrosodimethylamine

N-Nitrosodimethylamine (NDMA: O=N-N-$(CH_3)_2$) is carcinogenic in all animal species tested: mice, rats, Syrian golden, Chinese and European hamsters, guinea-pigs, rabbits, ducks, mastomys, various fish, newts and frogs. It induces benign and malignant tumours following its administration by various routes, including ingestion and inhalation, in various organs in various species. It produces tumours, mainly of the liver, kidney and respiratory tract. It is also carcinogenic following its administration prenatally and in single doses. In several studies, dose-response relationships were established. Human case reports or epidemiological studies are not available.

According to IARC (1987), there is *sufficient evidence* of a carcinogenic effect of NDMA in many experimental animal species. Similarities in its metabolism by human and rodent tissues have been demonstrated. Although no epidemiological data are available, NDMA should be regarded for practical purposes as if it were carcinogenic to humans. IARC (1987) classified NDMA in group 2A (probably carcinogenic to humans).

NDMA is generally considered to be a genotoxic carcinogen, and for this type of carcinogens the risk assessment is usually based on the so-called *non-threshold* approach. In this approach, the exposure level which leads to one additional case of cancer in one million lifelong exposed persons is taken as acceptable human exposure level (VSD[17]). In the absence of human data, the HBLV is extrapolated from animal carcinogenicity experiments.

A number of chronic animal studies with NDMA have been published, the most extensive and reliable of which is the study by Peto et al. (1991a). In this study, 4,080 rats (males and females) were exposed to 16 dosages of NDMA in their drinking water (males: 0.001 to 0.697, females: 0.002 to 1.224 mg/kg bw/d), starting at the age of 6 weeks for a period of just over three years. NDMA induced various types of liver cancer: hepatocellular carcinomas, and mesenchymal and Kupfer cell tumours, and bile duct cancer. Also some lung tumours were observed (Peto et al., 1991a, b).

The study lends itself quite well for dose-response modelling, which was done by Zeilmaker et al. (In preparation). Using the PROAST software (Slob, 2002; Slob and Pieters, 1998), they fitted the experimental data for several carcinogenic endpoints, and used the obtained dose-response relationships to derive a human HBLV. The dose-response relationship with the best fit was used to find the BMD dose associated with a 10 % increase in tumor response above background incidence (BMD_{10}). The lower bound 2.5 % confidence limit of this ($BMDL_{10}$) was taken as the starting point for extrapolation to the human HBLV, *i.e.*, the VSD. With a $BMDL_{10}$ of 29 µg/kg bw/d this resulted in a VSD of 0.4 ng/kg bw/d for chronic exposure of humans.

NDMA was also proven to be carcinogenic in rats after one single dose. In the study of Driver et al. (1987), rats were exposed, after weaning, to single i.p. doses of 2 to 50 mg NDMA per kg bw. Already one week after the treatment pre-neoplastistic proliferation was found in the kidneys of the treated animals. Using the PROAST software (Slob, 2002; Slob and Pieters, 1998), Zeilmaker et al. (In preparation) fitted the dose-response relationship for the resulting mesenchymal kidney tumours, from which they derived a $BMDL_{10}$ of 11 mg/kg bw. From this value they calculated the acute human VSD (the VSD for single or short-term exposure of humans) at 110 ng/kg bw.

Endogenous formation of nitrate, nitrite, nitrosoamines and methemoglobin

Nitrate and nitrite
There are many reports of an excess of urinary nitrate excretion compared to that ingested at low nitrate intakes. It was suggested that a part of this excess excretion could originate from the inhalation of nitrate and nitrite from indoor and outdoor air and cigarette smoke, although the main part most probably originates from endogenous synthesis.

The main source of endogenous nitrate in mammals is the L-arginine-NO synthase pathway, which is constitutively active in numerous cell types throughout the body. Nitric oxide is produced from the amino acid L-arginine and molecular oxygen by nitric oxide synthetase. Under basal conditions, the

[17] The amount of a chemical that, if ingested daily over a lifetime, will result in one additional case of cancer in one million exposed individuals.

metabolites of endogenous nitric oxide in plasma are mainly derived from the L-arginine-NO pathway in the endothelium of blood vessels and possibly neuronal tissue. However, during systemic inflammatory reactions or infections, white blood cells express an inducible NOS, which produces large amounts of nitric oxide and ultimately, by the binding to oxidised hemoglobin (Hb), results in metHb and a considerable increase in the concentrations of nitrate in plasma. In tissues other than the blood, nitrite is formed by reductive pathways and further oxidation produces nitrate.

In recent years, the function of nitric oxide in vascular physiology has become better understood and nitrite is now considered to be a nitric oxide donor under physiological conditions. Thus, low oxygen pressure, low pH and high nitrite concentration favour nitric oxide formation from nitrite, and in mammalian red blood cells nitrite is thus reduced to nitric oxide by deoxyhemoglobin. On the other hand, oxidized hemoglobin will react with nitrite to form nitrate and metHb. The balance between the two different hemoglobin reactions produces nitric oxide at low oxygen pressure, and the vasodilation induced by nitric oxide will increase blood flow to reverse the situation.

Nitrosamines
In healthy human volunteers, N-nitrosomorpholine was detected in stomach samples, and the level increased after ingestion of nitrate. Radioactive labelled nitrogen confirmed that the nitrosamine-nitrogen originated from nitrate, demonstrating *in situ* formation of N-nitrosamine from dietary nitrate via nitric oxide. Nitrosamines were formed in the gastrointestinal tract of Sprague Dawley rats after feeding a normal rat chow, and also in rats fed semipurified diets mixed with meat. In the latter case, the nitrosamine levels increased two to three times above control. In a dynamic *in vitro* gastrointestinal model, the formation of N-nitrosodimethylamine (NDMA) was observed after gradually adding nitrite to food samples (cod fish). The model produced NDMA levels proportional to the amounts of added nitrite and fish. The addition of orange juice or tea (antioxidants) generally decreased the NDMA formation. Thus overall, when nitrate is consumed as part of a normal diet containing vegetables, amines in concomitantly consumed food contribute to the formation of nitrosamines.

Methemoglobin
Methemoglobin (MetHb) results from the reaction of nitric oxide with oxyhemoglobin at the same time forming nitrate. A number of factors are critical to metHb formation including the presence of increased nitrite, intestinal infection together with inflammation of the stomach lining and NADH-cytochrome b5 metHb reductase (which converts metHb back to hemoglobin). MetHb is produced normally with background levels of 1 to 3 %. Levels of 10 % or more have been shown clinically to reduce oxygen transport. At levels above 20 %, cyanosis and hypoxia can occur and an increase to 50 % metHb can prove fatal. Infants younger than 3 months of age are more susceptible to methemoglobinaemia than adults due to a 40 to 50 % lower activity of NADH-cytochrome b5 metHb reductase and their increased risk for intestinal infections.

Appendix O Toxicological profile of acephate

Introduction
Acephate (O,S-dimethyl acetylphosphoramidothioate), is an organophosphorous cholinesterase inhibitor. Its toxicology has recently been evaluated by JMPR (FAO/WHO, 2002; FAO/WHO, 2005). The following summarizes the main conclusions of the JMPR evaluations. Because in the present report only the risk of acute cumulative exposure of children to OPs is assessed, we will restrict the toxicological profile to acute toxicity.

Toxicokinetics
After oral administration acephate is rapidly absorbed (peak plasma concentration half to one hour after dosing) and uniformly distributed. Excretion is rapid (terminal half life ± 1.4h) and mainly through urine, with minor quantities of radiolabel excreted in faeces (1 to 2 %) and expired air (1 to 9 %). Less than 1 % was found as a residue in tissues and organs 72 hours after the last dose. Unchanged acephate (73 to 77 %), O,S-dimethyl phosphorothioate (3 to 6 %) and S-methyl acetylphosphoramidothioate (3 to 4 %) were identified in urine. Small amounts of O-desmethyl acephate, O-desmethyl methamidophos have also been identified in the urine. Observed methamidophos concentrations in urine ranged from 0 to 5 %.

After oral administration of acephate at a dose of 100 mg/kg bw per day for four days, rats converted a portion to methamidophos. Both acephate and methamidophos are highly water-soluble and are rapidly metabolized and excreted. There was no tendency for acephate or methamidophos to accumulate. Three hours after the last dose, the carcass contained 0.6 to 1.6 % and the excreta (chiefly urine) 1.1 to 1.5 % of the final dose of acephate as methamidophos. In pregnant and lactating rats treated with ^{14}C-acephate, radiolabel is recovered from the placenta, foetuses and suckling pups.

In human studies, the time to T_{max} in plasma was one to four hours for both acephate and methamidophos. The half-life was between 3.5 and 6.6 hours for acephate and between 3.5 and 12 hours for methamidophos. Methamidophos accounted for about 1.3 % of the amount recovered in the urine, independently of the dose administered. The kinetics of acephate were similar in men and women given a single oral dose.

There were no relevant differences with respect to dose administered and C_{max} between humans and rats, considering the different methods used.

Toxicodynamics
The most prominent and sensisitive effect of acephate (and other OPs) is the inhibition of acetylcholinesterase (AChE) activity. In accordance with JMPR, inhibition of erythrocyte or brain cholinesterase ≥ 20 % is considered toxicologically relevant.

Acute toxicity
The oral LD_{50} values in rats were 1000 to 1400 mg/kg bw. The dermal LD_{50} value in rabbits was > 10,000 mg/kg bw. The LC50 value was > 15 mg/L of air (four h, nose-only) in rats. Clinical signs of toxicity were typical of cholinergic poisoning. Acephate was not irritating to the eyes or skin of rabbits, and was not a dermal sensitizer in the maximization test in guinea-pigs. WHO has classified acephate as ‘slightly hazardous’ (WHO, 2005).

The NOAEL for acephate, given as a single dose by gavage to rats, was 2.5 mg/kg bw on the basis of a 30 to 34 % reduction in brain cholinesterase activity in females at 5 mg/kg bw. JMPR 2002 considered

that the 13 to 22 % reduction in cholinesterase activity at 2.5 mg/kg bw in various regions of the brain not toxicologically significant. No treatment-related clinical signs were observed at doses up to 5 mg/kg bw. Behavioural effects and decreased erythrocyte cholinesterase activity were found at doses of 10 mg/kg bw and above.

Human studies
Volunteers receiving single oral doses of acephate showed no inhibition of erythrocyte AChE nor any clinical signs, electrocardiography, hematology, clinical chemistry, urine analysis or physical examination at dose up to and including 1.2 mg/kg bw (men) or 1.0 mg/kg bw (women).

Special studies
In *in vitro* studies, acephate was a slightly more effective inhibitor of brain and erythrocyte acetylcholinesterase activities in rats (IC_{50} = 1.6 and 1.3 mmol/l, respectively) than in cynomolgus monkeys (IC_{50} = 3.4 and 2.7 mmol/l, respectively) or humans (IC_{50} = 5.4 and 2.7 mmol/l, respectively).

Similarly, the 1984 Meeting reported that in monkeys receiving acephate at a dose of 2.5 mg/kg bw/d by gavage for 33 to 34 days, the mean inhibition (relative to mean pre-treatment values) of AChE activity was 50 % in erythrocytes and 47 % in brain.

The inhibitory effects of acephate and its main metabolite on cholinesterase activity have been investigated extensively both *in vivo* and *in vitro* in several species, including humans. It is likely that the inhibitory effect of acephate is due to its conversion to methamidophos. No significant sex or species difference in cholinesterase inhibition was observed *in vivo*.

Health based limit value
To establish the ARfD, JMPR 2005 considered the following elements derived from available information:
- The critical toxicological effect of acephate is the inhibition of AChE activity in the nervous system.
- Data on inhibition *in vitro* indicate that human brain AChE are slightly less sensitive to inhibition by acephate than is rat brain AChE.
- Well conducted toxicokinetics studies, available for both rats and humans, show that there is no significant difference between the two species.
- Data for rats *in vivo* indicate that inhibition of brain AChE activity occurs at lower doses than those required for similar inhibition of erythrocyte AChE activity.
- Data for dogs and monkeys *in vivo* indicate that brain and erythrocyte AChE activities are nearly equally inhibited at any given dose, and do not show the difference seen in rats, which might thus be rat-specific.
- Well-conducted single- and repeated-dose studies in humans clearly show a NOAEL for inhibition of erythrocyte AChE activity.
- Data from animals *in vivo* do not show sex differences in inhibition of AChE activity or clinical signs.

JMPR established an ARfD of 0.1 mg/kg bw/d on the basis of the NOAEL of 1.2 mg/kg bw/d from the study of single doses in humans and an overall safety factor of 10. The overall safety factor of 10 was derived by dividing the default value of 10 by two (because inhibition of AChE activity depends on the C_{max}) and by multiplying by two (because some uncertainty remains with respect to the *in vivo* sensitivity to inhibition of human brain AChE activity relative to that of erythrocyte AChE activity, since brain AChE activity may be more sensitive than erythrocyte AChE activity).

RIVM

National Institute
fot Public Health
and the Environment

Centre for
Nutrition and Food

PO Box 1
3720 BA Bilthoven
The Netherlands

www.rivm.nl