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Cumulative exposure to acetylcholinesterase inhibiting compounds in the Dutch population and young children

Toxic equivalency approach with acephate and phosmet as index compounds

P.E. Boon, J.D. van Klaveren

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RIKILT - Institute of Food Safety
Bornsesteeg 45, 6708 PD Wageningen, The Netherlands
P.O. Box 230, 6700 AE Wageningen, The Netherlands
Telephone +31 (0)317-475400
Telefax +31 (0)317-417717
Internet: www.rikilt.wur.nl

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Dutch Health Inspectorate, Amsterdam (Drs. H. van der Schee)

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NEDERLANDSE SAMENVATTING

Risicobeoordeling van de blootstelling aan residuen van o.a. bestrijdingsmiddelen via de voeding richt zich voornamelijk op risico's gerelateerd aan één residu via de consumptie van één product. In de praktijk is de blootstelling aan residuen via de voeding veel complexer, maar het ontbreekt aan modellen om de risico's van complexe mengsels in the schatten. Om tegemoet te komen aan dit probleem zijn enerzijds de toxic equivalency benadering en anderzijds probabilistische technieken ontwikkeld. Deze technieken kunnen de inname van residuen van verschillende producten combineren, alsmede de gelijktijdige inname van verschillende residuen. Zij houden ook rekening met de dagelijkse variatie in voedselopname en de variatie in residu concentraties in voedingsmiddelen.

Met behulp van de probabilistische methode is de cumulatieve blootstelling aan 40 acetylcholine-esterase (AChE) remmende stoffen via de voeding berekend in zowel de totale Nederlandse bevolking (1-97 jaar) als bij jonge kinderen (1-6 jaar). De residu gegevens van deze bestrijdingsmiddelen zijn afkomstig van het monitoringprogramma van de Keuringsdienst van Waren uitgevoerd in 2000 en 2001. De 'relative potency factor' (RPF) benadering is gebruikt om de toxische effecten van de 40 stoffen te cumuleren. Hiervoor zijn twee index stoffen gebruikt: acefaat en fosmet. Eerst is de mate berekend waarin elke stof in staat is AChE te remmen ten opzichte van acefaat en fosmet, uitgedrukt in RPFs. Hiervoor zijn acute 'no-observed-adverse-effect levels' (NOAELs; 22 stoffen) en 'benchmark' doses (18 stoffen) gebruikt. Met behulp van de berekende RPFs is de cumulatieve residu concentratie voor elk monster berekend, uitgedrukt in acefaat- en fosmetequivalenten. Deze cumulatieve concentraties per geanalyseerd monster zijn gecombineerd met voedselconsumptie data om de blootstelling aan AChE remmers via de voeding vast te stellen. Het effect van bewerking and variabiliteit tussen units binnen één monster is meegenomen in de analyses.

In ongeveer 6% van de samengestelde monsters werd een combinatie van AChE remmende stoffen aangetroffen (exclusief producten die niet worden geconsumeerd in de Nederlandse voedselconsumptie peiling). De P99.9 van de blootstellingsdistributie aan AChE remmende stoffen in de totale populatie was gelijk aan 13.4 ± 2.8 $\mu g \cdot kg^{-1} \cdot d^{-1}$ met acefaat als index stof en 27.6 ± 0.9 $\mu g \cdot kg^{-1} \cdot d^{-1}$ met fosmet als index stof. Overeenkomstige getallen voor jonge kinderen waren respectievelijk 35.7 ± 6.8 $\mu g \cdot kg^{-1} \cdot d^{-1}$ en 70 ± 11.0 $\mu g \cdot kg^{-1} \cdot d^{-1}$. Druiven droegen het meeste bij aan de blootstelling in beide populaties. De resultaten gaven aan dat zeer waarschijnlijk parathion en monocrotophos de twee stoffen zijn die het meeste bijdragen aan de blootstelling aan AChE remmende stoffen via de voeding.

Geconcludeerd kan worden dat de probabilistische methode de mogelijkheid biedt om de gelijktijdige blootstelling aan verschillende bestrijdingsmiddelen via de consumptie van verschillende voedingsmiddelen te berekenen. In de discussie wordt ingegaan op verschillende onzekerheden die gerelateerd zijn aan de berekening van de (cumulatieve) blootstelling aan bestrijdingsmiddelen via de voeding met de probabilistische methode. Een belangrijk item bij de cumulatieve blootstelling is het gebruik van een gestandaardiseerd kritisch eindpunt (bijvoorbeeld de 'benchmark' dosis zoals toegepast door de US EPA) voor de afleiding van RPFs.

SUMMARY

Dietary risk assessment usually focuses on the risk associated with exposure to a single toxic e.g. pesticide residue via the consumption of a single product. However, in practice dietary risk assessment is far more complex than exposure to one residue through one product. To address this issue the toxic equivalence approach and probabilistic techniques have been developed. These techniques can combine the intake of residues from different food sources, as well as intakes of different residues simultaneously. They also take into account the variability in daily food consumption and in residue concentrations in food.

With the use of the probabilistic approach, we calculated the distribution of the cumulative dietary exposure to 40 acetylcholinesterase (AChE) inhibiting compounds in the total Dutch population (1-97 years) and in young children (1-6 years). Pesticide data were derived from monitoring programmes of the Dutch Health Inspectorate carried out in 2000 and 2001. We used the relative potency factor (RPF) approach to accumulate the toxic effects of the 40 compounds, with the use of two index compounds: acephate and phosmet. For this we first calculated the toxicological potency of each compound to inhibit AChE relative to acephate or phosmet, expressed as RPFs. For this either acute no-observed-adverse-effect levels (NOAELs; 22 compounds) or benchmark doses (18 compounds) were used. With these RPFs the cumulative residue level on each sample was calculated, expressed in acephate- and phosmet-equivalents. These cumulative concentrations were combined with food consumption data to determine the dietary exposure to AChE inhibiting compounds. Processing effects and unit variability were included in the analyses.

Results of the analyses showed that about 6% of the composite samples analysed in 2000 and 2001 by the Dutch Health Inspectorate contained a combination of AChE inhibiting compounds (excluding food products not consumed in the Dutch food consumption survey). The P99.9 of the exposure distribution to AChE inhibiting compounds in the total population equalled 13.4 ± 2.8 $\mu g \cdot kg^{-1} \cdot d^{-1}$ with acephate as index compound and 27.6 ± 0.9 $\mu g \cdot kg^{-1} \cdot d^{-1}$ with phosmet as index compound. Corresponding numbers for children were 35.7 ± 6.8 $\mu g \cdot kg^{-1} \cdot d^{-1}$ and 70.0 ± 11.0 $\mu g \cdot kg^{-1} \cdot d^{-1}$, respectively. In all cases, exposure was unaffected by including unit variability in the analyses. Grape contributed most to the intake of AChE inhibiting compounds in both the total population and children. Results indicated that parathion and monocrotophos were most likely the main risk-drivers for the exposure to AChE inhibiting compounds.

We concluded that with the use of the probabilistic approach it is possible to calculate the simultaneous exposure to different pesticide residues through the consumption of different foods. In the discussion different uncertainties related to the calculation of (cumulative) dietary exposure to pesticide residues using the probabilistic approach are discussed. An important item in cumulative dietary exposure is the use of a standardised critical endpoint (e.g. benchmark dose as used by the US EPA) for the derivation of RPFs to describe the relative toxic potency between different compounds with the same mode of action.

1 INTRODUCTION

Dietary risk assessment usually focuses on the risk associated with exposure to a single toxic compound via a single crop. However in real life humans are exposed daily to many different chemicals via the diet (and other routes; ILSI 1999). If these compounds by one way or another interact, the conventional way of assessing the dietary risk of pesticides separately may lead to an underestimation of the health risk humans are exposed to. The exposure assessment is normally focussed on a single toxic compound, because it is mainly used for legislation purposes (admittance of the use of a certain pesticide on a certain crop; (WHO 1997)). However, it is evident that dietary risk assessment is far more complex than exposure to one compound through one crop, but in practice it is difficult to estimate the risk involved in exposure to complex mixtures of compounds For compounds that share a common mechanism of action the toxic equivalency approach has been introduced. Furthermore, to improve the exposure assessment probabilistic approaches have been developed. These approaches take into account the variability in daily food consumption and in residue concentrations in food, and can combine the intake of residues from different food sources, as well as intakes of different pesticides simultaneously.

In 2000 the Dutch consumer's organisation together with the Dutch environmental group ('Stichting Natuur en Milieu') published a first report on the cumulative exposure to acetylcholinesterase (AChE) inhibiting (organophosphorus and carbamate) pesticides in the Netherlands (Luijk et al 2000). In this study the authors concluded that, especially, children are exposed to high levels of neurotoxic compounds that may have detrimental effects for their health and development. However, several comments can be made concerning this study that may put this conclusion in another perspective:

- The toxicity equivalence factor' (TEF) approach was used to accumulate the toxic effects of the different pesticides. This approach has been and is successfully applied in assessing the toxicity of mixtures of PCDD's and PCDF's (Berg et al 1998, 2000; Safe 1998). It was also used by the US Environmental Working Group (EWG) to estimate the exposure to organophosphorus pesticides in children's food (EWG 1998, 1999). Chlorpyrifos was used as the index compound as by the US EWG. This compound is one of the organophosphorus chemicals for which, in the US, an extra 10-fold safety factor was introduced by the Food Quality Protection Act of 1996 when converting a no-observed-adverse-effect level (NOAEL) to an acute reference dose (US EPA 1996). This factor was introduced to protect infants and children from exposure to pesticides, and maintained in 1998 by the Food Safety Factor Committee because chlorpyrifos was one of the few organophosphates for which neonates (rats) may be more sensitive than adults (US EPA 1998). When using chlorpyrifos as the 'index-compound' all AChE inhibiting compounds will be compared with the strict norm of chlorpyrifos. This may not be correct, because for most of these compounds the extra FQPA factor was withdrawn during the 1998 meeting (US EPA 1998).
- Only limited information was available on NOAELs (used to calculate TEFs) derived from experiments studying acute effects of AChE inhibiting compounds. Of the 40 compounds considered in Luijk et al (2000) more than 50% (n=24) was without an acute NOAEL. They therefore assumed for those compounds that the acute NOAEL equalled 10 times the (semi-)chronic NOAEL of this pesticide. This factor was based on the ratio between the acute reference dose (ARfD) and the acceptable daily intake (ADI) for chlorpyrifos according to the Joint Meeting on Pesticide Residues (JMPR; (Luijk et al 2000)). This is an estimate and may

not have been correct. Because of limited information on acute NOAELs, Luijk et al (2000) were not able to exclude species and kinetic differences when calculating TEFs. For example, data of AChE inhibition in red blood cells in dogs were related to inhibition levels in brain of rat.

- Pesticide levels used originated from monitoring programmes performed during 1997 till 1999. It is however well known that pesticide levels in fruits and vegetables fluctuate yearly (Klaveren 1999).

To address these different points we recalculated the cumulative distribution of dietary exposure to AChE inhibiting compounds in the total Dutch population (1-97 years) and in young children (1-6 years), using pesticide data of more recent years, namely 2000 and 2001. We chose two different index compounds for which the FQPA factor had been withdrawn (US EPA 1998) and for which accurate and well-performed acute toxicology studies were available. In this way the influence of the choice of the 'index-compound' on the outcome of the exposure assessment could be evaluated. We also filled in more information about acute NOAELs established since the publication of Luijk et al (2000).

At the end of 2001 the US Environmental Protection Agency (EPA) published an assessment of the aggregate exposure to organophosphate pesticides via food, water and residential areas (US EPA 2001). In this document they published relative potency factors (RPFs) of 24 organophosphate compounds with methamidophos as index compound. These factors were based on benchmark doses at which AChE activity in the brain of female rats was reduced by 10% compared to background activity (BMD_{10}). The BMD_{10} was derived from modelling dose-response curves of each compound. This was done for 18 of the 40 compounds addressed in our report. For these 18 compounds we used the BMD_{10} data to calculate TEFs. Because the TEF approach as used by Luijk et al (2000) is comparable with the RPF approach of the US EPA (US EPA 2002), we will henceforth replace the term 'TEF' with the term 'RPF'.

2 METHODS

2.1 Pesticide residue data

The different pesticides addressed for the intake calculations are listed in appendix 1. In total 40 pesticides were addressed in this study as in Luijk et al (2000). Residue data originated from the monitoring programme of the Dutch Health Inspectorate, which are based on the analysis of composite samples. These data are stored in the Quality Agricultural Products Database (KAP; (Klaveren 1999)). Residue data of 2000 and 2001 were used in the analyses.

2.2 Food consumption data

Food consumption data of the Dutch National Food Consumption Survey (DNFCS) of 1997/1998 were used to calculate the dietary intake of acetylcholinesterase (AChE) inhibiting compounds (Kistemaker et al 1998), as by Luijk et a (2000). In this survey 6,250 respondents aged 1 to 97 years (of which 530 young children, aged 1 to 6 years) recorded their food intake over two consecutive days. The amount eaten was weighed accurately. The unit of intake for the calculations is 24 h in order to obtain random daily consumption patterns. In this way 12,500 eating 'moments' were available for the total Dutch population and 1,060 moments for young children. With the use of the conversion model Primary Agricultural Products (CPAP), developed at the RIKILT - Institute of Food Safety, the consumption of food products, as recorded in the DNFCS, was translated to the consumption of raw agricultural commodities (Dooren et al 1995). In this way the residue concentrations analysed in raw agricultural commodities could be linked directly to consumption. Only vegetables and fresh fruits, and some processed foods frequently consumed by young children (fruit juices and applesauce) were included in the analysis as in Luijk et al (2000; appendix 2, 3).

2.3 Index compounds and acute NOAELs

A thorough literature search was conducted to establish the acute NOAELs for the 22 AChE inhibiting compounds not addressed by the US EPA (US EPA 2001). The species we focussed on was the rat, because for this species frequent data are available. We were able to identify two index compounds for which the FQPA factor had been withdrawn in 1998 and for which toxicology data were available on the inhibition of AChE in brain and red blood cells (RBC), known to lead to cholinergic toxicity (Mileson et al 1998). These compounds were acephate (US EPA 2000b) and phosmet (US EPA 2000c). We did not select the same index compound as applied by the US EPA when establishing the RPFs based on BMD_{10} (US EPA 2001), because methamidophos is a compound with an extra FQPA uncertainty factor of three for the protection of infants and children. As reported in Luijk et al (2000) we experienced that the information available on acute effects on AChE in brain and RBC is still far from complete (appendix 1). Because of this we included three acute NOAELs based on human studies (appendix 1). For the pesticides for which we were unable to locate an acute NOAEL and which were not addressed by the US EPA, we assumed the acute NOAEL to be 10 times the (semi-)chronic NOAEL as in Luijk et al (2000). We did this for 13 compounds (appendix 1). The ARfD of acephate is 5 $\mu g \cdot kg^{-1} \cdot d^{-1}$ (US EPA 2000b) and of phosmet 45 $\mu g \cdot kg^{-1} \cdot d^{-1}$ (US EPA 2000c).

2.4 Calculation of the relative potency factors

For the 22 compounds not addressed by US EPA, relative potency factors (RPFs) were calculated as the ratio of acute no-observed-adverse-effect levels (NOAEL) for AChE inhibition using either acephate or phosmet as index compound (appendix 1). In this way, the toxicological potency of each compound to inhibit AChE was expressed relatively to acephate or phosmet (acephate- or phosmet-equivalents). To exclude kinetic differences as much as possible, RPFs were calculated using the same endpoint (appendix 1). When both brain- and RBC-inhibition comparisons were available, brain derived values prevailed. For example, the calculation of the RPFs for ethion based on either the index compound acephate or phosmet was as follows (appendix 1). The available acute NOAEL of ethion for AChE inhibition is that in RBC of dogs, and was calculated as 10 times the (semi-)chronic NOAEL of 0.05 mg.kg.bw^{-1}, resulting in 0.5 mg·kg bw^{-1}. The corresponding value for acephate is 2.5 mg·kg bw^{-1} (RBC in rats; footnote 3 in appendix 1), resulting in a RPF for ethion of 5. The corresponding factor based on the acute NOAEL of phosmet in RBC equals 9.

For 18 compounds we used the BMD_{10} as determined by the US EPA and calculated RPFs using acephate and phosmet as index compound (appendix 1). For example, the calculation of the RPFs for methamidophos based on either the index compound acephate or phosmet was as follows (appendix 1). The BMD_{10} of this compound for AChE inhibition in female rat brain equals 0.08 mg·kg bw^{-1} (US EPA 2001). The corresponding value for acephate is 1 mg·kg bw^{-1}, resulting in a RPF for methamidophos of 12.5. The corresponding factor based on the BMD_{10} of phosmet (4 mg·kg bw^{-1}) equals 50 (appendix 1).

2.5 Calculation of residue concentrations

The concentration of pesticides on a commodity was expressed as acephate- and phosmet-equivalents by multiplying the level (mg·kg^{-1}) of each compound by its RPF and adding up the different equivalents to one concentration per sample. For both fruit juices and applesauce no data were available and therefore one default concentration of AChE inhibiting pesticides was calculated. For this, we calculated the mean concentration of each pesticide separately on the primary product from which the juice or sauce originated. Each average concentration was multiplied by the corresponding RPF and added up to one concentration in acephate- and phosmet-equivalents for each juice and applesauce.

2.6 Processing factors

Concentrations of pesticides found on raw agricultural commodities (RAC) were corrected for processing effects, such as washing, peeling and heating. The processing factors applied were derived from Luijk et al (2000; table 1). The processing factors were multiplied with consumption data of RAC. In the Dutch consumption database foods are coded in such a way that information can more or less be obtained about different processing practices, such as cooking and canning. However, no information is available on washing practices and for peeling information is only available for apples. We therefore assumed washing or peeling of fruits and vegetables when likely, e.g. peeling when an orange or banana was consumed and washing when endive or lettuce was consumed raw.

Table 1. Processing factors used for the cumulative exposure to acetylcholinesterase inhibiting pesticides.

type of processing		processing factor
washing / pickled[1]		
	fruit	0.75
	vegetables	0.75
peeling		
	fruit (citrus / exotic)	0.05
	other fruits	0.75
washing / cooking / canning[2]		
	vegetables	0.27
juices		
	citrus fruits	0.07
	other fruits	0.40
sauces		
	apple	0.27

[1] except pickled beetroot, which was considered as cooked (factor = 0.27).
[2] except canned sauerkraut, which was considered as washed (factor = 0.75).

2.7 Monte Carlo technique

Data of residue concentrations in and consumption of raw agricultural commodities (RACs) were linked using the Monte Carlo technique. This method was developed to simulate real life dietary exposure to pesticides and other possible compounds in the best way possible. At the RIKILT, the programme 'Monte Carlo Risk Analysis' (MCRA) has been developed to assess the acute exposure to pesticides through the diet using the probabilistic approach (Boer et al 2002, Voet et al 2002). This programme is written in the statistical package GenStat (GenStat 2000) and was applied here for the calculation of the acute dietary exposure to AChE inhibiting compounds. The programme operates as follows. First it selects randomly a consumer out of the food consumption database. The consumption of every single RAC (that could contain one or more of the pesticides of interest) for this person on one day is multiplied with a randomly selected cumulative residue concentration out of the residue database for that particular RAC. For example, a person consumed apple. Out of the residue database a cumulative residue concentration for apple was selected which was either equal to 0 $mg \cdot kg^{-1}$ (66% of all samples; appendix 3) or higher than 0 $mg \cdot kg^{-1}$. When a positive apple residue concentration was selected, this concentration, when applying acephate as index compound, ranged from 0.004 $mg \cdot kg^{-1}$ till 0.331 $mg \cdot kg^{-1}$, with a mean concentration of 0.047 $mg \cdot kg^{-1}$ (appendix 3). After each RAC consumed by the selected person is multiplied with a selected residue concentration, the total cumulative residue intake of this consumer is added over products and stored in the output programme. By repeating this procedure many times a probability distribution for the cumulative intake of pesticides is generated. To estimate the median and the upper percentiles of the distribution of dietary exposure to AChE inhibiting compounds, the Monte Carlo analysis was repeated five times with 25,000 iterations for the total Dutch population and 10,000 iterations for children (1-6 years) each. The number of iterations for children was lower, because only 1,060 eating 'days' were available for this group compared to 12,500 in the total population. All estimates of possible intakes were adjusted for the individual's self-reported body weight, and all respondents were included (both consumers and non-consumers).

2.8 Variability within composite samples

In this report we included calculations in which variability was incorporated. Variability (ratio of a high residue level (e.g. maximum, 97.5th percentile) in an individual commodity item to mean (or median) composite sample residue level) was introduced in acute exposure assessment to account for the fact that most analyses are performed on composite samples (FAO/WHO 1997; PSD 1998a). Theoretically it is possible that the residue concentration found in a mixed sample originates from one individual commodity. The application of variability is well defined in the classical approach of acute risk assessment ('point estimate'; (PSD 1998b)), but no guidelines exist on how and which distribution of variability to apply in a probabilistic approach. Furthermore, hardly any data are available on variability within composite samples (Hamey et al 1999). Because of this, default variability values have been proposed depending on the unit weight of the product. In this study we included variability into the probabilistic approach using default factors as defined by the Joint Meeting on Pesticide Residues (JMPR; (FAO/WHO 2001)), and used the unit weights as listed in appendix 4. Variability was not included for processed fruit juices and applesauce. Due to lack of guidelines, we incorporated variability in the analyses by calculating for a selected respondent the amount of units consumed by dividing the amount consumed during one day by the unit weight of the product. The number of units consumed determined the number of residue levels to be selected from the cumulative residue database (e.g. consumption of two apple units resulted in the selection of two levels). For each 'unit' residue level a lognormal distribution was assumed characterised by μ and σ, the mean and standard deviation of the log-transformed concentrations. The variability factor v was converted into the standard deviation according to $\sigma = 1/2\ln(v)$, the sampled residue level c was converted to the mean according to $\mu = \ln(c) - 1/2\sigma^2$. For each consumed unit a residue level was drawn from the lognormal and back transformed to normality. Applying variability factors that are not based on empirical data, as done here, is a worst-case approach, at least for the high upper tail percentiles of the lognormal distribution.

For example, a respondent with a body weight of 80 kg consumed 0.48 kg of pear. The amount of units consumed equals 3.2, based on a unit weight of 0.15 kg (appendix 4). The default variability factor for pear is 7 ((FAO/WHO 2001), appendix 4) which is converted to a standard deviation of 0.973. From the cumulative residue database four levels (3×1 unit and 1×0.2 unit) are drawn, e.g. 1.60, 11.9, 7.2 and 238.2 $mg \cdot kg^{-1}$. The means of the lognormal are 0.0, 2.0, 1.5 and 5.0 $mg \cdot kg^{-1}$, respectively. For each unit consumed a residue level is drawn from each lognormal and back transformed to normality, e.g. 1.1, 12.5, 10.3 and 210.2 $mg \cdot kg^{-1}$. The exposure, disregarding processing effects, will then equal

$$\frac{0.15 \times 1.1 + 0.15 \times 12.5 + 0.15 \times 10.3 + 0.03 \times 210.2}{80} = 0.12 \text{ mg} \cdot \text{kg bw}^{-1}$$

3 RESULTS

3.1 Samples with multiple residues

In total 4283 fruit and vegetable composite samples were analysed for AChE inhibiting compounds by the Dutch Health Inspectorate in 2000 and 2001 (excluding products not consumed during the Dutch food consumption survey). Of these 896 (21%) contained a positive level for at least one AChE inhibiting compound and 267 (6%) contained a combination of AChE inhibiting compounds (excluding the omethoate - dimethoate combination since omethoate is a metabolite of dimethoate). These ranged from two up to five pesticides with a combination of two residues occurring most frequently (70%). The products with the highest amount of samples containing a combination of AChE inhibiting compounds were the citrus fruits mandarin (53%) and orange (27%).

3.2 Exposure to AChE inhibiting compounds

Table 2 shows the percentiles of the distribution of dietary exposure to AChE inhibiting compounds in the total population and young children for both index compounds. In the total population the P99.9 equalled 13.4 ± 2.83 $\mu g \cdot kg^{-1} \cdot d^{-1}$ using acephate as index compound and 27.6 ± 0.91 $\mu g \cdot kg^{-1} \cdot d^{-1}$ using phosmet as index compound. The corresponding numbers for children were 35.7 ± 6.80 $\mu g \cdot kg^{-1} \cdot d^{-1}$ and 70.0 ± 11.0 $\mu g \cdot kg^{-1} \cdot d^{-1}$, respectively. Including variability

Table 2. Percentiles of the distribution of dietary exposure ($\mu g \cdot kg^{-1} \cdot d^{-1}$) to AChE[1] inhibiting pesticides in the total Dutch population and children, simulated with cumulative residue concentrations derived from RPFs[2] based on acephate or phosmet as index compound. Simulations were performed either with or without variability included. Values are means (± SD) of five simulations with 25,000 (total population) or 10,000 (children) iterations each.

	index compound			
percentile	acephate		phosmet	
	no variability	with variability	no variability	with variability
total population				
P50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
P95	0.39 ± 0.01	0.37 ± 0.00	1.52 ± 0.02	1.45 ± 0.01
P98	0.86 ± 0.03	0.80 ± 0.05	3.11 ± 0.08	2.98 ± 0.03
P99	1.53 ± 0.11	1.45 ± 0.11	5.35 ± 0.12	5.21 ± 0.24
P99.5	3.05 ± 0.38	2.75 ± 0.15	9.56 ± 0.39	9.15 ± 0.67
P99.9	13.4 ± 2.83	13.7 ± 1.78	27.6 ± 0.91	26.5 ± 4.27
children 1 - 6 years				
P50	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.01	0.07 ± 0.01
P95	0.86 ± 0.01	0.83 ± 0.03	3.38 ± 0.17	3.35 ± 0.16
P98	2.09 ± 0.13	1.97 ± 0.28	7.89 ± 0.48	7.72 ± 0.47
P99	4.01 ± 0.37	3.99 ± 0.42	13.9 ± 1.20	14.1 ± 0.54
P99.5	8.57 ± 0.71	8.34 ± 0.87	23.2 ± 2.94	23.6 ± 2.25
P99.9	35.7 ± 6.80	31.9 ± 2.68	70.0 ± 11.0	60.1 ± 5.26

[1] AChE = acetylcholinesterase
[2] RPF = relative potency factor

Table 3. Top 5 of raw agricultural commodities (RAC; %) that contribute most to the distribution of dietary exposure to AChE[1] inhibiting pesticides in the total population and children, simulated with residue concentrations derived from RPFs[2] based on acephate or phosmet as index compound; A. the total distribution and B. highest 5% of the distribution. Values are means (± SD) of five simulations with 25,000 (total population) or 10,000 (children) iterations each.

	index compound	
RAC	acephate	phosmet
A. total distribution		
total population		
grape	28.3 ± 2.1	20.0 ± 1.3
orange juice	17.6 ± 1.0	19.7 ± 0.8
bean, snap	6.3 ± 1.9	
apple	6.2 ± 0.5	8.3 ± 0.3
mandarin, tangerine	5.8 ± 0.6	6.6 ± 0.5
carrot		6.0 ± 0.3
children 1 - 6 years		
grape	37.9 ± 5.8	27.0 ± 2.7 (1)[3]
orange juice	13.3 ± 1.1	15.0 ± 0.9 (2)
grape juice	7.9 ± 1.1	
mandarin, tangerine	6.9 ± 0.7	7.3 ± 0.4 (5)
apple	6.8 ± 0.8	9.4 ± 0.7 (4)
apple juice		10.9 ± 0.5 (3)
B. highest 5% of the distribution		
total population		
grape	39.7 ± 2.5	30.9 ± 1.8 (1)
bean, snap	8.6 ± 2.6	6.5 ± 1.5 (5)
grape juice	6.9 ± 1.2	
orange juice	6.7 ± 0.7	7.4 ± 0.5 (2)
pepper, sweet	5.8 ± 0.7	7.1 ± 1.7 (3)
apple		6.5 ± 0.6 (4)
children 1 - 6 years		
grape	52.7 ± 7.0	43.2 ± 3.8 (1)
grape juice	11.4 ± 1.8	10.1 ± 1.3 (2)
bean, snap	7.0 ± 3.5	5.9 ± 1.2 (4)
pear	5.5 ± 2.0	
mandarin, tangerine	4.4 ± 0.7	4.6 ± 0.8 (5)
apple		7.0 ± 0.9 (3)

[1] AChE = acetylcholinesterase
[2] RPF = relative potency factor
[3] Numbers in brackets indicate order of ranking.

into the analyses did not affect the results significantly (table 2). For a better understanding of the distribution of possible exposures we plotted a graph of the exposure distribution for that part of the total population (± 50%) with a positive intake of AChE inhibiting compounds (figure 1). Acephate was used as index compound.

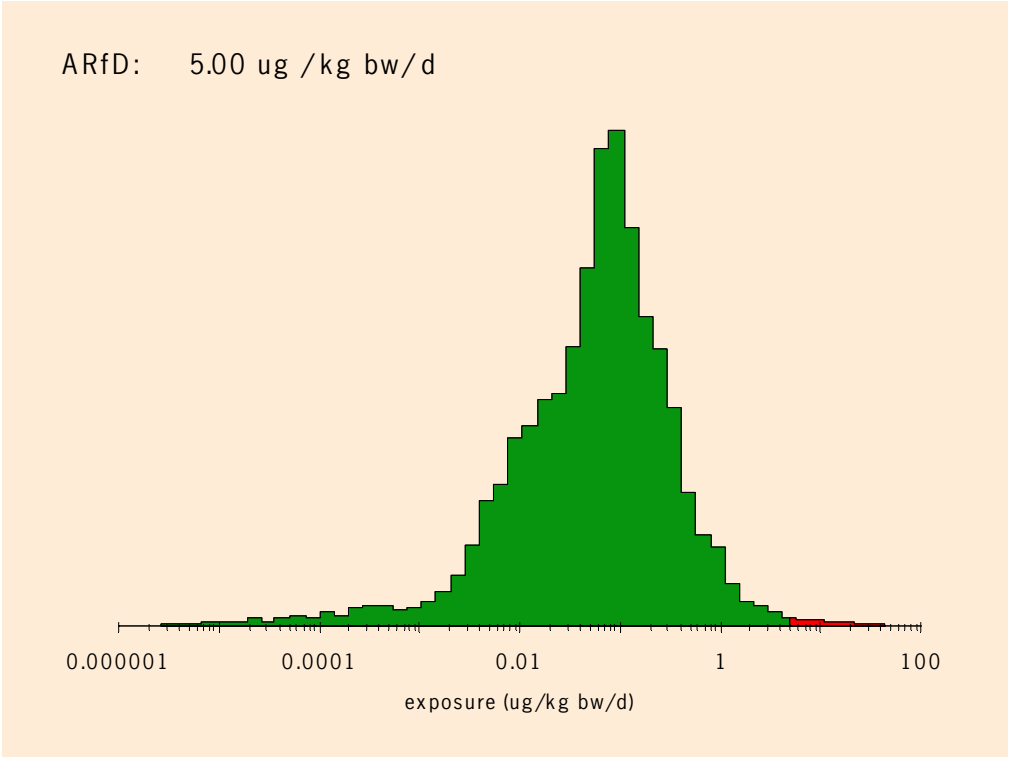

Figure 1. Distribution of the cumulative dietary exposure ($\mu g \cdot kg^{-1} \cdot d^{-1}$) to acetylcholinesterase inhibiting compounds in the total Dutch population. Only exposures above zero are plotted. Results shown are derived from one Monte Carlo simulation with 20,000 iterations and calculated with acephate as index compound.

3.3 Contribution of RAC to the intake of AChE inhibiting pesticides

Table 3 lists the top 5 of products that contributed most to the distribution of exposure to AChE inhibiting pesticides in the total population and children using RPFs based on acephate or phosmet as index compound. The contribution (%) was calculated for the total and for the highest 5% of the distribution. Considering both the total distribution and the highest 5%, grape contributed most to the intake of AChE inhibiting compounds in both the total population and children, and irrespective of the index compound used (table 3). The top 5 of products was hardly influenced by the index compound used. Minimally one product in the top 5 calculated with acephate as index compound was not present with phosmet as index compound. For example, when studying the highest 5% of the distribution for children pear was only present in the top 5 of acephate and apple in the top 5 of phosmet. The ranking of the products within the top 5 differed between the two index compounds. The difference was least for the total distribution of the total population. Including variability in either of the analyses did not influence the top 5 of products significantly and is therefore not shown.

Table 4. Mean concentration (± SD) of samples with level above limit of reporting (LOR) and percentage above LOR (%) of three pesticides present in the highest concentration in three raw agricultural commodities (RAC). Concentrations were translated in acephate- and phosmet-equivalents ($mg \cdot kg^{-1}$).

RAC and pesticide	concentration ($mg \cdot kg^{-1}$; equivalents)		samples > LOR (%)
	acephate	phosmet	
grape			
parathion	7.467 ± 5.370	13.440 ± 9.666	4.9
monocrotophos	3.897 ± 3.857	7.000 ± 6.928	1.0
fenitrothion	1.488 ± 1.429		2.3
azinphos–methyl		3.025 ± 2.581	0.7
orange			
parathion	5.333 ± 4.163	9.600 ± 7.494	1.4
monocrotophos	5.010	9.000	0.5
methidathion	0.980 ± 1.258	3.919 ± 5.031	27
bean, snap			
monocrotophos	15.030	27.000	1.1
methamidophos	1.063 ± 0.619	4.250 ± 2.475	2.2
acephate	0.410		1.1
carbaryl		2.363 ± 3.023	4.9

3.4 Contribution of individual pesticides to the intake of AChE inhibiting pesticides

As in Luijk et al (2000) we identified those pesticides that contributed most to the dietary exposure to AChE inhibiting compounds. In an early stage the concentrations of the different pesticides for one commodity are added up to one concentration for AChE inhibiting compounds. Consequently, it is not possible to distil from the results which pesticide contributed most to the exposure. For an indication of the pesticides that may be the main risk-drivers, we selected the three products that contributed most to the exposure to AChE inhibiting compounds (table 3) and selected those pesticides present in the highest concentrations expressed as acephate- and phosmet-equivalents. The products selected and the three pesticides found at the highest levels in these products are shown in table 4. The pesticides most likely to be the main risk-drivers for the exposure to AChE inhibiting compounds, based on the products selected, were parathion and monocrotophos. Parathion is found most frequently on grape and is present at high concentrations in both grape and orange (table 4). Monocrotophos is present in all three products and at a high level in span bean.

4 DISCUSSION

In this report we showed that about 6% of the composite samples analysed in 2000 and 2001 by the Dutch Health Inspectorate contained a combination of AChE inhibiting compounds (excluding those products not consumed in the Dutch food consumption survey). The P99.9 of the exposure to acetylcholinesterase (AChE) inhibiting compounds in the total population equalled 13.4 ± 2.8 $\mu g \cdot kg^{-1} \cdot d^{-1}$ with acephate as index compound and 27.6 ± 0.9 $\mu g \cdot kg^{-1} \cdot d^{-1}$ with phosmet as index compound. Corresponding numbers for children were 35.7 ± 6.8 $\mu g \cdot kg^{-1} \cdot d^{-1}$ and 70.0 ± 11.0 $\mu g \cdot kg^{-1} \cdot d^{-1}$, respectively. In all cases, exposure was unaffected by including unit variability in the analyses (table 2). Grape contributed most to the intake of AChE inhibiting compounds in both the total population and children (table 3). Results indicated that parathion and monocrotophos were most likely the main risk-drivers for the exposure to AChE inhibiting compounds (table 4).

We demonstrated that with the use of the probabilistic approach it is possible to calculate the simultaneous exposure to different pesticides through consumption of different foods. In risk assessment to toxic compounds it is important to consider all possible sources of variation that may influence the outcome of such estimation. Factors recognised to influence exposure to pesticides are the ratio of consumption of imported to domestically grown products, representativeness and completeness of the residue concentration database, and the concentration applied to samples below limit of reporting (LOR). These different factors are not always taken into account because of very limited information available on these variables. These factors will not be discussed further in this report. Other important factors that need to be considered are variability within composite samples and processing factors. When addressing cumulative exposure to pesticides also the calculation of the relative potency factors (RPFs) and the selection of the index compound need attention.

Below we discuss different items that need to be considered when calculating the cumulative exposure to pesticides.

4.1 Calculation of relative potency factors

To calculate the cumulative risk to AChE inhibiting compounds the RPF approach was used, one of different methods to add up the exposure to different pesticides (ILSI 1999; Seed et al 1995; Wilkinson et al 2000). We used no-observed-adverse-effect levels (NOAELs) to derive RPFs for 22 compounds addressed in this study. It is important, when applying this approach, to critically select the toxicology studies from which NOAELs are derived, and to choose as much as possible the same endpoint and species for each compound relative to the index compound. In this way the RPF reflects best the relative toxic potency of a pesticide to its index compound. When deriving these RPFs the following limitations were identified which led to uncertainties in the calculations presented here:

1. Absence of acute NOAELs for inhibition of AChE in brain or red blood cells for 13 compounds. For these compounds we assumed, following Luijk et al (2000), that the 'acute' NOAEL equalled 10 times the '(semi-)chronic' NOAEL of this pesticide. The factor 10 was based on the ratio of the ARfD and ADI of chlorpyrifos (see also chapter 1). In our study we used two other index compounds. It may therefore be worthwhile to repeat the calculations with a factor that is based on the ratio of the ARfD and ADI of acephate and phosmet.

2. Animal - animal agreement: NOAELs of the index compound were derived from toxicity studies available of rat. However not for all pesticides data were available of this specie. Sometimes data of other species (dog, human) had to be used and were related to the NOAEL of acephate and phosmet in rat (appendix 1).
3. Reports of the US Environmental Protection Agency (EPA) are not always clear about the extent of AChE inhibition and the tissue involved. JMPR applies a minimal inhibition level of 20% before speaking of a toxicological relevant inhibition (Raaij 2001). US EPA speaks of a 'significant' inhibition that is not necessarily at least 20% (US EPA 2000d).

The limitations mentioned above are not complete. These (and possible others) should be considered when interpreting the results presented here (e.g. the conclusion that parathion and monocrotophos were the main risk-drivers of exposure). The uncertainty is mainly due to the defective descriptions in the open literature about the magnitude of the critical effect (AChE inhibition) and the tissue (brain and RBC) involved. Due to concise reporting and the lack of a clear comparable toxicological endpoint (10% or 20% inhibition in relevant tissue) the relative toxicity of the pesticide compared to the index compound can be higher or lower. Some of problems mentioned we tried to solve by incorporating RPFs as established by the US EPA (US EPA 2001). These factors are based on dose-response relationships from which BMD_{10} (benchmark dose associated with a 10% response adjusted for background) of AChE inhibition in female rat brain were derived to describe the toxic potency of each chemical, as opposed to the NOAEL. However, the US EPA only established RPFs for 18 of the 40 compounds addressed in this study (appendix 1), e.g. not including parathion and monocrotophos. For the remaining compounds we applied the RPFs based on NOAELs, as described above. We are also aware that the NOAEL is not an optimal approach to calculate RPFs because they do not necessarily reflect the relationship between dose and response. The 'real' NOAEL may be lower or higher than the observed level due to dosing levels or insensitivity of the study.

Apart from the availability of accurate and well-performed toxicology studies, other factors need to be kept in mind regarding the use of RPFs in cumulative risk assessment. RPFs, for example, address incompletely the temporal issues when half-lives of the compounds in question vary, and when there is a large degree of variability in the time interval between the exposure to the various compounds, or between ingestion of the compounds and their effect on the target-organ (ILSI 1999). Another disadvantage is the dependence of the RPF approach on the quality of the toxicology database of the index compound (ILSI 1999; Wilkinson et al 2000). This shows that although the RPF approach is accepted in addressing cumulative risk, the outcome of the simulation will be a simplification of the real risk people run when consuming more than one compound over a certain period, and results must be interpreted with caution.

One of the aims of this study was to gather more information on acute NOAELs to account for differences in specie and/or endpoint when calculating RPFs. This was not possible in the study of Luijk et al (2000) because of limited information. Due to new toxicological evaluations performed since then and the use of benchmark doses as established by the US EPA we were better able to account for a difference in specie and/or endpoint when calculating RPFs for acute exposure. For 10 compounds we were not able to hold on to the animal - animal agreement. However, all RPFs were calculated following the tissue - tissue demand. We also reduced the number of compounds

without an acute NOAEL by almost 50% compared to Luijk et al (2000). Further evaluations performed in the future may fill in the gaps still there and improve the cumulative dietary exposure assessment.

4.2 Selection of index compounds

In this study we choose two index compounds of which the FQPA factor had been withdrawn (US EPA 1998) and for which accurate and well-performed acute toxicology studies for the inhibition of AChE in both brain and RBC of rat were available (US EPA 2000b; US EPA 2000c). The US EPA identified methamidophos as index-compound, because this compound has a high quality database for the common mechanism endpoint of inhibition of AChE for the oral, dermal, and inhalation routes of exposures (aggregate exposure; (US EPA 2001)). We decided not to use this compound as index compound, because methamidophos still has an extra FQPA uncertainty factor of three. As explained in the introduction, methamidophos as index compound may thus have led to the comparison of all AChE inhibiting compounds with the strict norm for methamidophos, which may not have been correct (chapter 1).

Although the contribution of the different food products to the exposure to AChE inhibiting compounds was not affected significantly by the choice of index compound (table 3), it did influence the percentage of the distribution exceeding the acute reference dose (ARfD). When acephate was used as index compound the ARfD of 5 $\mu g \cdot kg^{-1} \cdot d^{-1}$ was exceeded at the P99.9 level of the exposure distribution in the total population. With phosmet as index compound, less than 0.1% of the distribution exceeded the ARfD (45 $\mu g \cdot kg^{-1} \cdot d^{-1}$; table 5). For children the ARfD was exceeded at the P99.5 and P99.9 level, respectively. This difference is explained by the fact that the ARfDs of both compounds are based on AChE inhibition in the brain, while most RPFs (83%) were calculated using the NOAEL of AChE inhibition in RBC (15 compounds) or the BMD_{10} (18 compounds; appendix 1). The ratio of the ARfDs is 9 (45/5) while the ratio for the NOAEL of AChE inhibition in RBC of the two compounds equals 1.8 (4.5/2.5), and that for the BMD_{10} 4 (4/1). This lower RPF ratio for the majority of compounds compared to the ratio of the ARfDs resulted in a lower exposure level when applying phosmet as index compound compared to acephate. The percentiles of exposure exceeding a certain reference point would have been similar if the ratio of the reference point had equalled the ratios of all RPFs.

Comparing these percentages of the distribution exceeding the ARfD with those reported in Luijk et al (2000) demonstrated that the percentage of the population exceeding the norm was reduced independent of the index compound used. Luijk et al (2000) concluded that 0.5% of the total population and 2% of the children exceeded the ARfD (from the US EPA) of 1.7 $\mu g \cdot kg\ bw^{-1}$ for chlorpyrifos. In the present study comparable percentages were about five times lower in the total population and four times in children with acephate. This reduction was even more pronounced with phosmet (table 2). These results should be interpreted with caution, bearing in mind the uncertainties mentioned in chapter 1 and §4.1.

4.3 Samples with multiple residues

A question within cumulative exposure assessment is whether the exposure to AChE inhibiting compounds is mainly due to the presence of multiple compounds on one sample or of one compound per sample. We showed that of all fruit and vegetable samples in which AChE inhibiting compounds were analysed during 2000 and 2001 by the Dutch Health Inspectorate and which were consumed in the Dutch food consumption survey 6% contained a combination of AChE inhibiting compounds. A combination of two residues occurred most frequently (70%). Some examples of these combinations were chlorpyrifos with methidathion or malathion on orange. Of the three products contributing most to the dietary exposure to AChE inhibiting compounds (table 3) also a minority of the samples contained multiple residues. For grape 6% of the samples contained multiple residues, for orange 27% and for span bean 1%. Furthermore, samples with multiple residues did not occur more frequently in the upper percentiles of the cumulative residue level distribution than samples with just one residue. This was mainly due to monocrotophos and parathion, which were frequently present as individual pesticides. Due to their high RPFs they contributed highly to the upper part of the cumulative residue level distribution. As described in §4.1 the RPFs used in this report should be considered with caution. It must also be kept in mind that the residue data used in this report are derived from composite samples in which several units of a product have been combined. It is very likely that the possibility of co-occurrence of pesticide residues in these samples is higher than when single serving units are analysed. This is especially relevant for those fruits and vegetables consumed as individual units (e.g. apple, tomato).

Although it seems that samples with multiple residues are not those mainly determining the exposure to AChE inhibiting compounds, this does not mean that the exposure to AChE inhibiting compounds is more a problem of single than multiple compound exposure. Even though the majority of the samples containing AChE inhibiting compounds at levels above reporting limit contain just one compound, people consume daily different products that very likely contain different AChE inhibiting compounds. In that way people are exposed to different compounds with the same mode of action via the daily consumption of different food products.

Samples with residue levels at or above reporting limit do not necessarily exceed the maximum residue limit. In a smaller study (not published) we showed for grapes that when samples with at least one pesticide exceeding the norm were not included in the analyses, cumulative exposures were reduced but still well above the ARfD. This indicated that the problem with multiple exposure is not a norm enforcement problem, but that the practice of establishing product norms may be inadequate when studying multiple exposure. If this also holds when all food products are considered as done in the present study needs further investigation.

4.4 Individual pesticides contributing most to the dietary exposure

We identified parathion and monocrotophos as possible candidates that contributed most to the dietary exposure to AChE inhibiting compounds. Luijk et al (2000) also identified parathion as a risk-driver, but also mentioned dimethoate as a possible risk-driver. We were not able to confirm this, likely due to the use of different index compounds and / or different residue levels. The conclusion that parathion and monocrotophos are the main risk-drivers should be considered with

caution, because the RPFs of both compounds are subject to the uncertainties described in §4.1. Both these compounds are no longer allowed for use in agriculture in The Netherlands.

4.5 Variability within composite samples

Variability was not considered in Luijk et al (2000). In the present study we experimented with the incorporation of variability into the probabilistic approach using default values and showed that the exposure was not substantially affected (table 2). This absence of an effect can be explained by the fact that for each consumed unit an equal chance exists of selecting a higher 'unit' residue level from the lognormal than the 'composite' residue level as a lower level. The way variability was incorporated in this report needs further investigation. We introduced variability assuming that the units that comprise a daily portion are derived from different lots: for every separate unit a new composite residue level was selected from the 'cumulative' residue database. It could be that this approach might not reflect the actual situation, as was concluded by the JMPR (FAO/WHO 2001). They stated that it is more likely that the supply available for consumption during one day is derived from a single lot. In other words, units of a daily portion have a 'shared history'. In that case, not different composite residue levels should be selected for each unit consumed, but only one composite residue level. Depending on the variability factor a lognormal distribution should be assumed and from this one distribution a number of 'unit' residue levels need to be selected depending on the amount of units consumed. In this report variability was applied to the total cumulative residue level, and not to the separate levels of the chemicals found on a product. The effect of this on the outcome is not clear and needs further consideration.

Variability was introduced assuming that it represents a measure of the variation in pesticide levels within a composite sample. In that case, as was done here, the variability factor together with the pesticide level analysed in a composite sample can be used to parameterise a lognormal distribution. From this distribution individual residue levels can be selected for the units consumed (§ 2.8). Because of the assumption of a lognormal distribution residue levels selected have no upper limit and the exposures will become extreme when the number of simulations is large enough (see upper tail of the exposure distribution in figure 1). A cautionary remark here is that we have no empirical evidence on maximum consumption portions and/or concentrations, and can thus not know whether the extreme exposures found using the lognormal approach are or are not according reality. In general it is advisable to study percentiles of the exposure distribution that have some empirical basis. Applying variability factors that are not based on empirical data, as done here, is a worst-case approach, at least for the high upper tail percentiles of the lognormal distribution. Other approaches to incorporate variability in the analyses are being developed. These new approaches employ distributions (beta or Bernoulli distributions) that maximise the levels that can be selected. For example, as in the definition of the conservative default variability factors of the JMPR (based on the assumption that residues in a composite sample are all present in a single unit; (FAO/WHO 2001)), the maximum residue level selected can never be higher than the monitoring measurement multiplied by the number of units in a composite sample. In this way, individual exposures will never be based on concentrations multiplied by more than the default variability factors from the JMPR (FAO/WHO 2001).

4.6 Concentrations used for juices and sauces

No information was available on concentrations of AChE inhibiting compounds in fruit juices and applesauce, products frequently consumed by children. Because of this we assumed one default concentration of AChE inhibiting compounds for these processed foods. This implied that whenever a person consumed for example apple juice a default concentration of AChE inhibiting compounds was ingested. This is however very unlikely and may have led to an overestimation of AChE inhibiting compounds exposure through juices. This could explain the presence of different fruit juices (grape, orange, apple) in the top 5 of products that contributed most to the exposure (table 3). The US Environmental Working Group (EWG) used measured concentrations of organophosphorus compounds in juices in their cumulative risk assessment and demonstrated that juices were great risk-drivers in children's food (EWG 1999). Actual measurements of pesticides in these processed foods may therefore be essential for an accurate assessment of the exposure to AChE inhibiting compounds.

Other processed foods were not considered in our study as in Luijk et al (2000). With the inclusion of processed foods, such as tomato ketchup and pizza, many uncertainties would have risen concerning the fate of pesticides during different processing procedures. This may have led to an underestimation of the exposure to AChE inhibiting compounds in the Netherlands. However, it is not expected that these foods will contribute substantially to the exposure. Due to industrial processing these foods will, very likely, not contain high pesticide levels.

4.7 Reference point of the exposure distribution

Another question within cumulative exposure assessment is the relevant cut-off point of the exposure distribution used as a reference point above which health effects occur. This point can be compared with toxicological endpoints (such as ARfD). In the US the P99.9 level of the exposure distribution is an acceptable reference point when considering the exposure to a single toxic compound (US EPA 2000a). In this document, we address the intake of 40 different compounds simultaneously. This raises the question if the demarcation point used in single compound exposure should also be applied for the evaluation of the exposure to multiple compounds. It could be that the reference point decided on in single compound exposure may be too conservative when estimating the exposure to multiple compounds. Regardless of this issue, it is advisable, when, for example, the P99.9 is close to the ARfD, to perform a qualitative judgement of the certainties and uncertainties involved in the higher exposure levels (e.g. reporting errors in food consumption, extreme high residue levels).

4.8 Conclusions

We demonstrated that with the use of the probabilistic approach it is possible to calculate the simultaneous exposure to different pesticides through consumption of different foods. The different issues raised above need to be addressed in improving the methodologies for cumulative risk assessment before a valid estimate of real cumulative exposure will be possible. An important item is the use of a standardised critical endpoint (e.g. benchmark dose as used by the US EPA) for the derivation of RPFs to describe the relative toxic potency between different compounds with the same mode of action. Also the selection of the index compound when applying the RPF approach needs further attention.

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APPENDIX 1 RPFs OF ACETYLCHOLINESTERASE INHIBITING COMPOUNDS

Acetylcholinesterase inhibiting pesticides addressed in this report, including their acute no-observed-adverse-effect level (NOAEL; mg·kg bw^{-1}), the benchmark dose (BMD_{10} mg·kg bw^{-1}), the effect on which the acute NOAEL and BMD_{10} were based, the source of the acute NOAEL and BMD_{10}, and the relative potency factor (RPF).

						RPF[3] based on	
compound	type[1]	NOAEL	BMD_{10}	effect[2]	source	acephate	phosmet
acephate	ofos		1	rat/brain	EPA	1	4
aldicarb	carb	0.025		human/RBC	JMPR95	100	180
azinphos-methyl	ofos		0.80	rat/brain	EPA	1.25	5
bromophos-ethyl	ofos	2.6[4]		dog/RBC	JMPR75	1	1.73
carbaryl	carb	1		rat/brain	RIVM	0.5	4.5
carbofuran	carb	2.2[4]		dog/RBC	JMPR96	1.1	2
chlorfenvinphos	ofos	0.5[4]		rat/brain	JMPR94	1	9
chlorpyrifos	ofos		1.33	rat/brain	EPA	0.75	3.0
chlorpyrifos-methyl	ofos		160	rat/RBC	EPA	0.006	0.025
diazinon	ofos		8	rat/brain	EPA	0.125	0.50
dichlorvos	ofos		2.67	rat/brain	EPA	0.375	1.5
dimethoate	ofos		0.25	rat/brain	EPA	4.0	16
ethiofencarb	carb	100[4]		rat/RBC	JMPR82	0.03	0.05
ethion	ofos	0.5[4]		dog/RBC	EPA	5	9
fenitrothion	ofos	0.36		human/RBC	JMPR00	6.9	12.5
fenthion	ofos		0.24	rat/brain	EPA	4.125	16.5
heptenophos	ofos	0.5		rat/RBC	RIVM	5	9
malathion	ofos		266.67	rat/brain	EPA	0.004	0.015
mecarbam	ofos	2.1[4]		rat/RBC	JMPR86	1.2	2.14
methamidophos	ofos		0.08	rat/brain	EPA	12.5	50
methidathion	ofos		0.25	rat/brain	EPA	4.0	16
methiocarb	carb	3		rat/RBC	JMPR98	0.8	1.5
methomyl	carb	40		rat/brain	RIVM	0.013	0.11
mevinphos	ofos		0.11	rat/brain	EPA	9.5	38
monocrotophos	ofos	0.015		human/RBC	JMPR95	167	300
omethoate	ofos		0.09	rat/brain	EPA	11.625	46.5
oxamyl	carb	14.6[4]		dog/RBC	JMPR85	0.17	0.31
parathion	ofos	0.025		rat/RBC	EPA	100	180
parathion-methyl	ofos		0.67	rat/brain	EPA	1.5	6.0
phosalone	ofos		8	rat/brain	EPA	0.125	0.5
phosmet	ofos		4	rat/brain/RBC	EPA	0.25	1
pirimicarb	carb	18[4]		dog/RBC	Tomlin97	0.14	0.25
pirimiphos-ethyl	ofos	0.8[4]		rat/brain	Tomlin97	0.6	5.63
pirimiphos-methyl	ofos		2	rat/brain	EPA	0.5	2.0
profenophos	ofos		20	rat/brain	EPA	0.05	0.2
propoxur	carb	5		rat/brain	RIVM	0.1	0.9
pyrazophos	ofos	0.9[4]		dog/brain	JMPR92	0.56	5
quinalphos	ofos	30[4]		rat/RBC	Tomlin97	0.083	0.15
toclophos-methyl	ofos	65[4]		rat/brain	JMPR94	0.008	0.07
triazophos	ofos	1.2[4]		dog/RBC	JMPR93	2.1	3.75

[1] ofos = organophosphorus pesticide, carb = carbamate pesticide

[2] RBC = red blood cells

[3] RPFs were either based on NOAELs for which the following NOAELs for acephate and phosmet were used: acephate rat/brain = 0.5 mg·kg bw^{-1} and rat/RBC = 2.5 mg·kg bw^{-1}; phosmet rat/brain/RBC = 4.5 mg·kg bw^{-1}, or they were derived from BMD_{10} (US EPA 2001).

[4] NOAELs were assumed to be 10 × NOAEL for (semi-)chronic effects, due to absence of acute NOAELs.

APPENDIX 2 CUMULATIVE RESIDUE LEVELS IN VEGETABLES

Cumulative concentrations of acetylcholinesterase inhibiting pesticides (acephate- and phosmet-equivalents) in vegetables with a positive consumption and a concentration above the limit of reporting (LOR): mean (± SD), minimum and maximum concentration, and the number of samples with a concentration either below (n=0) or equal/above (n≥0) LOR.

			acephate			phosmet		
product	n=0	n≥0	mean ± SD	min	max	mean ± SD	min	max
artichoke	2	1	0.006	-	-	0.011	-	-
asparagus	24	1	0.375	-	-	1.500	-	-
aubergine	54	4	0.020 ± 0.024	0.003	0.055	0.048 ± 0.046	0.006	0.100
bean	72	7	0.163 ± 0.186	0.002	0.400	0.389 ± 0.459	0.003	1.120
bean, span	79	11	1.848 ± 4.405	0.001	15.030	4.575 ± 7.910	0.006	27.0
bean, string	3	3	27.5 ± 32.0	2.250	63.46	51.0 ± 55.6	9.000	114
broccoli	41	2	0.584 ± 0.419	0.287	0.880	2.334 ± 1.677	1.148	3.520
cabbage, Chinese	35	1	0.007	-	-	0.013	-	-
cabbage, conical	21	2	0.176 ± 0.244	0.004	0.349	0.701 ± 0.981	0.008	1.395
carrot	65	39	0.118 ± 0.128	0.020	0.530	1.062 ± 1.155	0.180	4.770
celery, bleach	21	6	0.109 ± 0.232	0.000	0.581	0.210 ± 0.034	0.002	0.072
cucumber	134	8	0.299 ± 0.476	0.000	1.375	1.142 ± 1.926	0.003	5.500
endive	94	31	0.136 ± 0.287	0.003	1.250	0.459 ± 1.138	0.008	5.000
legume	25	11	0.432 ± 0.295	0.020	0.909	1.738 ± 1.165	0.180	3.635
lettuce, cabbage	73	64	0.182 ± 0.738	0.000	5.000	0.543 ± 1.971	0.001	11.78
lettuce, iceberg	63	6	0.160 ± 0.210	0.000	0.552	0.457 ± 0.422	0.001	1.000
lettuce, lamb's	12	4	0.178 ± 0.287	0.000	0.601	0.338 ± 0.541	0.003	1.138
mushroom	22	1	0.038	-	-	0.150	-	-
pepper, sweet	144	36	1.873 ± 3.153	0.000	13.986	7.134 ± 11.51	0.001	44.9
radish	37	1	0.000	-	-	0.004	-	-
spinach	55	5	0.141 ± 0.207	0.004	0.480	0.470 ± 0.824	0.013	1.920
tomato	232	3	0.017 ± 0.021	0.005	0.041	0.073 ± 0.081	0.010	0.165

APPENDIX 3 CUMULATIVE RESIDUE LEVELS IN FRUITS

Cumulative concentrations of acetylcholinesterase inhibiting pesticides ($mg \cdot kg^{-1}$ in acephate- and phosmet-equivalents) in fruits with a positive consumption and concentration above the limit of reporting (LOR): mean (± SD), minimum and maximum concentration, and number of samples with a concentration either below (n=0) or equal/above (n≥0) LOR.

			acephate			phosmet		
product	n=0	n≥0	mean ± SD	min	max	mean ± SD	min	max
fresh fruit								
apple	151	78	0.047 ± 0.061	0.004	0.331	0.207 ± 0.333	0.008	2.250
apricot	11	6	0.714 ± 0.666	0.075	1.800	5.397 ± 6.337	0.300	16.2
banana	90	1	0.003	-	-	0.048 ± 0.059	0.006	0.090
berry, black-	21	1	0.380	-	-	1.520	-	-
berry, rasp-	31	3	0.008 ± 0.006	0.003	0.014	0.014	-	-
berry, straw-	211	38	0.087 ± 0.195	0.000	1.045	0.254 ± 0.718	0.001	4.180
cherry	23	8	0.993 ± 2.310	0.004	6.680	2.373 ± 4.115	0.008	12.0
currant	30	6	0.010 ± 0.008	0.004	0.025	0.017 ± 0.015	0.008	0.045
grape	204	106	1.474 ± 3.312	0.000	24.1	3.290 ± 6.003	0.000	43.4
grapefruit	20	42	0.780 ± 1.302	0.000	7.085	2.614 ± 4.884	0.000	28.3
kiwi	50	3	0.008 ± 0.006	0.002	0.015	0.030 ± 0.028	0.005	0.060
lemon	15	22	1.466 ± 2.193	0.000	9.070	4.307 ± 5.242	0.002	17.9
lime	12	1	0.000	-	-	0.001	-	-
litchi	3	1	100	-	-	180	-	-
mandarin, tangerine	23	108	1.123 ± 1.513	0.000	7.240	3.980 ± 4.948	0.001	25.6
melon	109	12	0.779 ± 1.069	0.000	2.766	3.116 ± 4.305	0.001	11.1
melon, water	14	1	0.000	-	-	0.001	-	-
nectarine	32	18	0.555 ± 0.817	0.013	2.750	2.217 ± 3.272	0.050	11.0
orange	86	135	0.791 ± 1.467	0.000	10.0	2.518 ± 4.440	0.001	28.9
passion fruit	8	5	3.440 ± 3.072	0.465	6.680	7.872 ± 5.022	1.860	12.0
peach	41	10	0.421 ± 0.478	0.050	1.380	1.232 ± 1.261	0.200	4.125
pear	141	20	0.480 ± 1.927	0.004	8.665	1.008 ± 3.499	0.008	15.9
pineapple	36	3	0.004 ± 0.000	0.003	0.004	0.012 ± 0.005	0.006	0.015
plume	55	13	0.124 ± 0.178	0.000	0.581	0.492 ± 0.714	0.001	2.325
Sharon fruit	6	2	0.291 ± 0.298	0.009	0.430	0.877 ± 1.193	0.033	1.720
processed fruits								
apple, juice/sauce	1	1	0.019	-	-	0.098	-	-
grape juice	1	1	0.550	-	-	1.469	-	-
grapefruit juice	1	1	0.595	-	-	2.372	-	-
orange juice	1	1	0.441	-	-	1.550	-	-
pear juice	1	1	0.062	-	-	0.145	-	-
pineapple juice	1	1	0.001	-	-	0.001	-	-

APPENDIX 4. UNIT WEIGHTS AND VARIABILITY FACTORS

Unit weights (kg) and default variability factors of all fruits and vegetables with a positive consumption and concentration above the limit of reporting.

vegetables			**fruits**		
product	unit weight (kg)	variability[3]	product	unit weight (kg)	variability
artichoke[1]	0.103	7	apple[2]	0.112	7
asparagus[1]	< 0.025	-	apricot[2]	0.039	7
aubergine/eggplant[1]	0.444	5	banana[2]	0.100	7
bean	< 0.025	-	berry, black-	< 0.025	-
bean, French	< 0.025	-	berry, rasp-	< 0.025	-
bean, string	< 0.025	-	berry, straw-[1]	< 0.025	-
broccoli[2]	0.074	7	cherry[1]	< 0.025	-
cabbage, Chinese[1]	> 0.500	5	currant	< 0.025	-
cabbage, conical[1]	> 0.500	5	grape[1]	0.118	7
carrot[2]	0.080	7	grapefruit[2]	0.160	7
celery, bleach[2]	0.030	7	kiwi[1]	0.074	7
cucumber[2]	0.060	7	lemon[1]	0.072	7
endive (see lettuce)[2]	> 0.500	10	lime[1]	0.056	7
legume	< 0.025	-	litchi	< 0.025	-
lettuce, cabbage[2]	> 0.500	10	mandarin, tangerine[2]	0.100	7
lettuce, iceberg[2]	> 0.500	10	melon[1]	> 0.500	5
lettuce, lamb's	< 0.025	-	melon, water[1]	> 0.500	5
mushroom[2]	< 0.025	-	nectarine[2]	0.149	7
pepper, sweet[2]	0.160	7	orange[2]	0.160	7
radish[1]	< 0.025	-	passion fruit	0.045	7
spinach, bunch[1]	0.081	7	peach[2]	0.110	7
tomato[2]	0.085	7	pear[2]	0.150	7
			pineapple[1]	0.420	5
			plume[2]	0.055	7
			Sharon fruit	0.150	7
			processed fruits		
			apple, juice/sauce	-	-
			grape juice	-	-
			grapefruit juice	-	-
			orange juice	-	-
			pear juice	-	-

[1] GEMS/FOOD, 15 April 2000; worst case of all countries except UK.
[2] U.K. unit weights (PSD 1998b).
[3] JMPR (FAO/WHO 2001)