Cumulative exposure to cholinesterase inhibiting compounds: a review of the current issues and implications for policy

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Rapport in het kort

Cumulatieve blootstelling aan cholinesterase remmende bestrijdingsmiddelen: een overzicht van de huidige kennis en implicaties voor het beleid.

Blootstelling aan meerdere bestrijdingsmiddelen tegelijk in de dagelijkse voeding is een potentieel probleem. Dit probleem kan zich met name voordoen bij bestrijdingsmiddelen met een zelfde werkingsmechanisme (zoals de z.g. organofosfaten). Maatschappelijke organisaties dringen aan op het meewegen van dergelijke gecumuleerde blootstelling in de risicobeoordeling. Het RIVM zet in dit rapport op een rij wat er bekend is over dit onderwerp en op welke manier zo'n cumulatieve blootstelling kan worden bepaald. Er zijn op dit moment methoden beschikbaar maar een wetenschappelijke onderbouwing voor de optelling van effecten ontbreekt nog deels. Het is niet duidelijk of de effecten van de organofosfaten wel additief zijn en volgens het principe van Relatieve Potentie Factoren (RPF) kunnen worden opgeteld. De informatie over residuen van bestrijdingsmiddelen en de methoden voor innameberekeningen zullen ook verbeterd moeten worden. Het meewegen van cumulatieve blootstelling heeft ook consequenties voor het risicomanagement en de besluitvorming bij handhaving en toelating; hiervoor zullen door het beleid keuzes moeten worden gemaakt.

Trefwoorden: bestrijdingsmiddelen, pesticiden, organofosfaten, carbamaten, cumulatieve blootstelling, relatieve potentie

Abstract

Cumulative exposure to cholinesterase inhibiting compounds: a review of the current issues and implications for policy

Cumulative exposure to various residues of pesticides in food is a potential area of concern. This issue is especially relevant for pesticides with a common mechanism of toxicity (e.g. organophosphates). Non-governmental organisations emphasise the need for inclusion of cumulative exposure in the risk assessment procedures for pesticides. In this report, RIVM evaluates the available information on cumulative exposure to pesticides and by what methods a cumulative risk assessment can be performed. Although methods are currently available the scientific basis for the summation of the effects is partly lacking. It is not clear whether the effects of all organophosphate combinations are truly additive and whether the approach with Relative Potency Factors (RPF) is valid. In addition the available residue data of pesticides and the available probabilistic tools for intake assessment should be improved. The inclusion of cumulative exposure to pesticides also has an impact on risk management decisions for authorisation and inspection procedures; policy makers will have to make choices in this area.

Contents

1. Introduction

In the last decade, cumulative exposure to pesticides has become an issue of concern. Although in the scientific area increased interest was present for some time, the introduction of the Food Quality Protection Act (FQPA) in the USA (in 1996) moved cumulative exposure to pesticides into the regulatory frameworks. Several non-governmental organisations have published reports on the potential public health risks of cumulative exposure to pesticides (e.g. EWG, 1999; Luijk et al., 2000). Publications on this issue were focussed on the potential problems with cumulative exposure to cholinesterase (ChE) inhibiting compounds (carbamates and organophosphates (OPs)) because these chemicals all have a similar mechanism of action. These non-governmental bodies claim that some fraction of the population (especially children) are subject to a cumulative intake of cholinesterase inhibiting compounds exceeding the health based limit values for chronic or acute exposure. In addition, the effects of exposure to cholinesterase inhibiting compounds was linked to many other neural effects such as learning and behaviour (EWG, 1999; Luijk et al., 2000).

In 2003, Boon and Van Klaveren analysed the cumulative exposure to cholinesterase inhibiting compounds in The Netherlands. They concluded that about 0.1% of the calculated person-day exposures to ChE-inhibiting compounds would exceed the acute Reference Dose (ARfD). In response to this analysis, the Dutch Food and Non-Food Authority (VWA) requested RIVM to review the report of RIKILT and to provide an opinion on the current issues on cumulative exposure to ChE-inhibiting compounds. In the present report the most important issues on risk assessment of cumulative exposure to ChE-inhibiting compounds (and pesticides in general) will be discussed. In addition, the main comments on the analysis of Boon and Van Klaveren (2003) will be presented. Finally, a general discussion is provided focussing on the potential consequences for risk assessment and pesticide regulatory policy.

2. Cumulative exposure to multiple compounds with a common mechanism of action

Although in the public discussion potential adverse effects of cumulative exposure to multiple pesticides is placed in a very wide scope, in the scientific area emphasis is placed on chemicals with a common mechanism of action. Before starting the discussion on current issues of cumulative exposure it is important to clarify what cumulative exposure is within the scope of pesticides.

Cumulative exposure: The total concurrent exposure to multiple chemicals with a common mechanism of action via one specific route of exposure (e.g. food).

In addition, the term aggregate exposure is also frequently used.

Aggregate exposure: The total exposure to a single (or multiple) chemical(s) from multiple sources (all routes of exposure), e.g. food, air, indoor use etc…

In this report we will only focus on cumulative exposure to chemicals with a common mechanism of action being present in food. This limitation is used because the current interest of the Food and Non-Food Authority and the Ministry of Health in the Netherlands (with regard to cumulative exposure) is focussed on dietary exposure.

In 1996, passage of the Food Quality Protection Act (FQPA) in the USA imposed the requirement upon the US-EPA to consider potential human health risks from all pathways of dietary and non-dietary exposures to more than one pesticide acting through a common mechanism of toxicity. In order to do such evaluations, identification of groups of compounds with a common mechanism of toxicity is needed followed by guidance and criteria. In 1999 US-EPA published a Guidance for Identifying Pesticide Chemicals and other Substances that have a Common Mechanism of Toxicity (EPA, 1999). The ChE-inhibiting compounds were a group of compounds identified for priority re-evaluation. In addition to this publication, EPA asked the International Life Science Institute (ILSI) to convene an expert panel to address the question whether OPs act by a common mechanism of toxicity (Mileson et al., 1998). This panel applied several criteria: 1) do the compounds cause the same critical effect ?, 2) do the compounds act on the same molecular target at the same target tissue ?, and 3) do the compounds act by the same biochemical mechanism of action or do they share a common intermediate ? This panel concluded that OPs should be considered as a group of compounds acting

through a common mechanism of toxicity (Mileson et al., 1998). Carbamates, another group of ChE inhibiting compounds, were not included in this statement.

3. Mechanism of action for Cholinesterase inhibiting compounds

3.1. General

Organophosporus esters (OPs) and carbamates are widely used insecticides. One of their major effects is inhibition of the enzyme acetyl cholinesterase (AChE; EC 3.1.1.7). This enzyme is primarily located in the synapses of the somatic, autonomous, and central nervous systems, but also in erythrocytes and blood plasma. AChE is involved in the breakdown of the neurotransmitter acetylcholine, which diminishes or terminates the activation of postsynaptic cholinergic receptors. Inhibition of AChE leads to acetylcholine-induced overstimulation of the postsynaptic receptors, which in its turn results in so-called cholinergic toxicity or "cholinergic crisis" (Silver, 1974; Richardson,1995; Ray, 1998). Besides AChE inhibition in the central (CNS) or peripheral nervous system (PNS), OPs and carbamates can also inhibit AChE in the blood. We will discuss the mechanisms in more detail in the following paragraph; for more information see Luttik and Van Raaij (2001).

3.2. Mechanism of action

Although they both induce AChE inhibition, OPs and carbamates do not have quite the same mechanisms of action. OPs are analogues of the normal biological substrates of AChE. The various steps of the interaction between an OP and AChE are shown in Figure 1.

An OP reversibly binds to the hydroxyl group of a serine residue in the enzyme, resulting in a Michaelis-Menten complex (step 1). After separation of the residual group XH (step 2), the OP and AChE form a covalent bond (for acetylcholine the residual group is choline). The enzyme may be reactivated (step 3). For the original substrate acetylcholine, step 3 will last a few microseconds only. However, for OPs the half-life times can be very long (Ray, 1998), resulting in long-term AChE inhibition. Some OP-enzyme-complexes, however, do not seem to undergo any reactivation (Ray, 1998). An additional complicating factor is "ageing". This phenomenon refers to separation of one of the residual groups that are linked to the phosphorus through an oxygen atom (step 4). This irreversible reaction will inactivate the enzyme. The half-life times of ageing are 4 hours for dimethoxy-OPs, and about 10 hours for diethoxy-OPs. This implies that the rate of ageing (step 4) could far exceed that of reactivation (step 3), which makes the inhibition of AChE by OPs *virtually irreversible* (Richardson, 1995; Ray, 1998; Marrs and Moretto, 1998). The AChE activity is then only restored by de-novo synthesis of the enzyme. Ageing is probably also an essential step in the induction of OP-induced delayed neurotoxicity (OPIDN). This subject is only relevant for some OPs and is therefore not taken into account in this report.

The behaviour of carbamates, however, is different from that of OPs. They are hydrolyzed by AChE and the enzyme-carbamate-complex has a relatively long half-life time (compared to the very short half-life of the physiological substrate acetylcholine). The enzyme-carbamate-complex, unlike that of OPs, does *not* undergo ageing however, and AChE is thus reactivated according to step 3. Consequently, the AChE inhibition of carbamates is *reversible* (within a period lasting from minutes to hours).

For further information on the mechanisms of action see Richardson (1995) and Ray (1998).
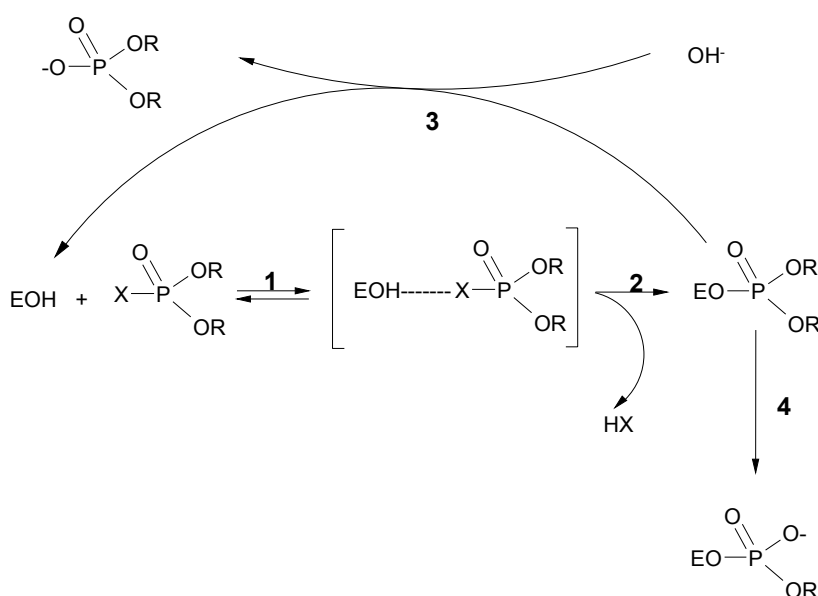
Figure 1. Schematic representation of the interaction and enzymatic steps in the actions between an OP and AChE. (the various steps are explained in the main body of the text).

As mentioned earlier, AChE is found particularly in the *CNS* and the *PNS*. The CNS is protected by the so-called 'blood-brain-barrier', hampering a number of AChE-inhibiting substances to reach their target site (the synapses) in the CNS. The PNS is surrounded by a perineurium, but this is less effective than the blood-brain-barrier, especially at the peripheral ganglia. Hence, exposure of peripheral nerves and ganglia may be occasionally higher than that of the CNS (Marrs and Moretto, 1998). However, many organophosphorus esters pass the blood-brain-barrier relatively easily, with little or no difference in exposure between the CNS and the PNS [A. Moretto, personal communication].

Both the erythrocytes and the blood plasma contain cholinesterases, which, in contrast to neural AChE, are not involved in cholinergic transmission. The enzyme

in the *erythrocytes* is biochemically identical to that in the PNS, viz. <u>acetyl</u>cholinesterase. The rate at which AChE is being resynthesized, is however much higher in the PNS than it is in erythrocytes (Chen et al., 1999; JMPR, 1998). In contrast, cholinesterase activity in *plasma* does not only comprise acetyl cholinesterase but also <u>butyryl</u>cholinesterase (BuChE). In rat plasma the proportion of AChE and BuChE is 1:1, but in humans it is about 1:100 (Ray, 1998; Padilla et al., 1994; Chen et al., 1999). This means that plasma cholinesterase activity in humans almost exclusively relates to BuChE. This is a so-called 'pseudo-cholinesterase', which is also used as a biomarker for exposure. Most analytical methods determine total cholinesterase activity (i.e. the sum of AChE and BChE activity) by measuring the conversion of acetylthiocholine in a colorimetric assay in which no actual differences between AChE and BuChE are established.

The physiological role of cholinesterase activity in erythrocytes and blood plasma is not known. From the available literature it appears that selective inhibition of erythrocyte or plasma ChE itself does not induce harmful effects (Padilla et al., 1992). Nevertheless, BuChE might be involved in the detoxification of other xenobiotics (e.g. cocaine), which suggests that inhibition of plasma ChE activity might have physiological consequences (Richardson, 1995).

Essentially, carbamates and OPs inhibit the same enzymes and can principally provide a cumulative effect when present at the same time. However, because the ChE-inhibition by carbamates is relatively rapidly reversible, time spacing in cumulative exposure may be an issue of concern. In addition, from the description above it can be concluded that comparisons between different tissues / sites where ChE-inhibiting takes place (CNS, PNS, whole blood, erythrocytes, plasma) might be problematic because of the different extent of effect and the kinetic aspects involved. These issues are further discussed in chapter 4.

4. Current issues in toxicology

4.1. Use of Relative Potency Factors and drawbacks

When cumulative exposure to a certain group of compounds needs to be performed there must be some method for cumulation of different substances. Wilkinson et al. (2000) provide an overview of current methods that can be used for cumulative risk assessment, including e.g. the Hazard Index method and the use of Relative Potency Factors (RPFs). All methods currently available have limitations and all are associated with some level of uncertainty. Without going into detail, the basic differences between the methods available are associated with two aspects: a) are the potencies of the compounds related to a health based limit value (ADI, RfD) or to each chemical's toxicity profile (NOAEL or BMD) and b) is the level of uncertainty accounted for at the level of individual substances or once only at the end of the cumulative procedure. (Wilkinson et al., 2000).

In the analysis by RIKILT (Boon and Van Klaveren, 2003) an approach based on Relative Potency Factors (RPFs) was used. Relative potency factors are also called Toxicity Equivalence Factors (TEFs). The TEF or RPF approach has also been used previously for 'dioxin-like' substances and has been internationally accepted for this class of chemicals. In this approach the potency of an individual compound is expressed as the potency of a single index compound by using a constant factor, given that both compounds induce the same extent of effect. This approach was also used by the National Research Council (1993) and by US-EPA (EPA, 2001) for their cumulative exposure analyses for organophosphates. In the UK, the Pesticide Safety Directive (PSD) aims to use the RPF approach for cumulative risk assessment of ChE-inhibiting compounds in the future (I. Dewhurst, pers. comm.). The RPF approach is a theoretically sound procedure given that a number of assumptions are fulfilled.

First of all, the RPF approach can only be used if the effects of the individual substances are dose-additive. The mathematical definition of dose-addition requires a constant proportionality among the effectiveness of the chemicals (EPA, 2001). Dose-additivity is further explained in section 4.3 along with a discussion on whether or not this is true for ChE-inhibiting compounds. The point raised here is that the RPF approach makes no sense if the cumulative effects of OPs do not follow dose-additivity. In that case total cumulative exposure calculations based on the RPF approach are not valid.

In the RPF approach the total exposure of all substances involved is expressed in terms of dose equivalents of a single index compound. The choice of the index compound therefore may have consequences for the outcome of the risk assessment. Important criteria for selecting an index compound are the availability

and quality of toxicological data. The reliability of RPFs is much lower if expressed as equivalents of a substance with a limited data quality. Thus, there are statistic reasons for selecting an index compound with a high data quality. Another aspect for selecting an index compound may be the basis of the health-based limit value for that compound because the total cumulative exposure will be often compared to the health-based limit value of the index compound.

Because Boon and Van Klaveren (2003) included a total of 40 substances in their analysis, data on the same single endpoint were not available for all these individual compounds. The RPFs were based on several different endpoints (including brain AChE inhibition and blood AChE inhibition). Therefore the RIKILT analysis needed index compounds for which various types of data (e.g. brain and blood ChE inhibition) were available (Boon and Van Klaveren, 2003) in order to be able to establish an RPF for all of the 40 compounds. Availability of data was thus an important issue for selecting acephate and phosmet as index compounds. In contrast, US EPA selected methamidophos as index compound because EPA required one single index compound having data for all routes of exposure (including dermal and inhalation) and little variation in the toxicity between the various routes. Methamidophos fulfilled these criteria (EPA, 2001). Within the UK, the PSD is working on their internal strategy for the choice of an index compound. So, depending on the type of analysis performed and the availability of data, different index compounds can be selected.

After expressing all individual compounds in equivalents of a single index compound, the total exposure is compared to the toxicological database or the health based limit value (ADI, RfD, ARfD) for that specific index compound. Wilkinson et al. (2000) pointed out that in most cases the index compound is the best studied compound with the largest and most reliable database. These authors questioned whether it is valid to compare the total exposure (including that of less-well studied compounds with all uncertainties attached) with the 'high quality' database of an index compound. However, RIVM feels that using an index compound with a well established toxicological database and a well founded limit value has an advantage in preventing erroneous conclusions on human health risks. When the total cumulative exposure is compared to a limit value (ADI, RfD or ARfD) of a single index compound, much emphasis is placed on that single limit value. This includes the selected toxicological endpoint, animal species, selected doses used in the critical study, choice of assessment factors, choice of modifying factors, and other regulatory input. For example, in the analysis done by Luijk et al. (2000) the total exposure was compared to the RfD of chlorpyrifos set by US-EPA. However, this RfD was established with an additional safety factor of 10 for potential enhanced sensitivity of children. The total OP exposure is in that case compared to a very strict limit value of a single compound whereas the toxicity

profile of many other OP's do not indicate the necessity of additional safety factors. Comparison of the total exposure to the limit value of such an index compound may also lead to erroneous conclusions on human health. Scientifically, such additional safety factors can be omitted in the risk assessment if good argumentation is provided although such an approach will be more difficult to explain to people with less toxicological background knowledge.

4.2. Long-term and acute effects

Because of differences in pharmacokinetics and pharmacodynamics, inhibition of ChE by different OPs and carbamates can result in very different patterns of inhibition over time. Variations in the rate of absorption, distribution, metabolism, excretion and variations in duration of the ChE inhibition (reversible, non-reversible, half-time etc) determine the final extent of ChE-inhibition. Because of these differences, US-EPA has selected the inhibition of RBC-AChE after several weeks of exposure, in order to compare the *steady state inhibition* of the compounds during repeated daily exposure (EPA, 2001). However, the relative potency's of ChE-inhibiting compounds after repeated exposure are not necessarily similar to the relative potency's after acute exposure. In the report by Luijk et al. (2000), it was tried to establish RPFs for both chronic and acute exposure. Although many data were lacking (especially for acute exposure), this approach showed that the RPFs for acute exposure may be highly different from the RPFs obtained after chronic exposure. As pointed out also by Wilkinson et al. (2000), cumulative risk assessment should (just as in normal chemical risk assessment) utilize the toxicological data most appropriate to the exposure scenario under interest. Thus, acute exposure should be compared primarily to acute toxicological endpoints and chronic exposure should be compared to chronic endpoints. This means also that RPFs should be defined for the appropriate period of exposure (acute RPFs versus chronic RPFs). The RIKILT analysis used the RPF factors established by US-EPA (which were (sub)chronic RPFs) next to RPFs based on acute NOAELs, and compared the total cumulative exposure to the ARfD (an acute endpoint). This is in principle not valid. On the other hand, it should be acknowledged that there is still a considerable data gap for acute neurotoxicity effects of ChE-inhibiting compounds although with time this gap will be filled. This makes it very difficult to reliably establish acute RPFs at this moment.

When acute toxicological data were not available for a substance, the RIKILT report uses the assumptions that the acute NOAEL for that substance would be 10x the chronic NOAEL. This factor was based on a factor of 10 between the acute and chronic NOAELs of chlorpyrifos. For some OPs, there may be indeed a factor of 10 between the acute and chronic NOAELs but certainly not for all. In addition, for most carbamates the acute and chronic NOAELs are usually quite similar or

different by much less then a factor of 10. The relative short and reversible inhibition of ChE by carbamates means that a chronic exposure is more or less equivalent to a successive daily exposure of single acute exposures. Often, the chronic NOAELs of carbamates are identical to the acute NOAELs and sometimes even higher (JMPR 2000; JMPR 2001).

4.3. Assumption of additivity

In chemical risk assessment of cumulative exposure two concepts exist on additive effects. The concept of *dose*-additivity is generally used for chemicals with the same mechanism of action. Because such compounds act through the same biochemical pathway one can cumulate the doses of individual compounds (e.g. by using RPFs) in order to estimate the final effect. The concept of *effect*-additivity is generally used for chemicals that do not act by the same mechanism but may finally lead to similar effects which can be additive to each other. An illustrative example of the latter may be the concurrent exposure to a liver enzyme inducer (e.g. a barbiturate) and an overload of glucose. Both will result in an increase in liver weight: the barbiturate by inducing liver metabolic capacity and cell volume and glucose by a rise in liver glycogen deposition. Such an effect can be additive but not at the level of the doses of the individual compounds.
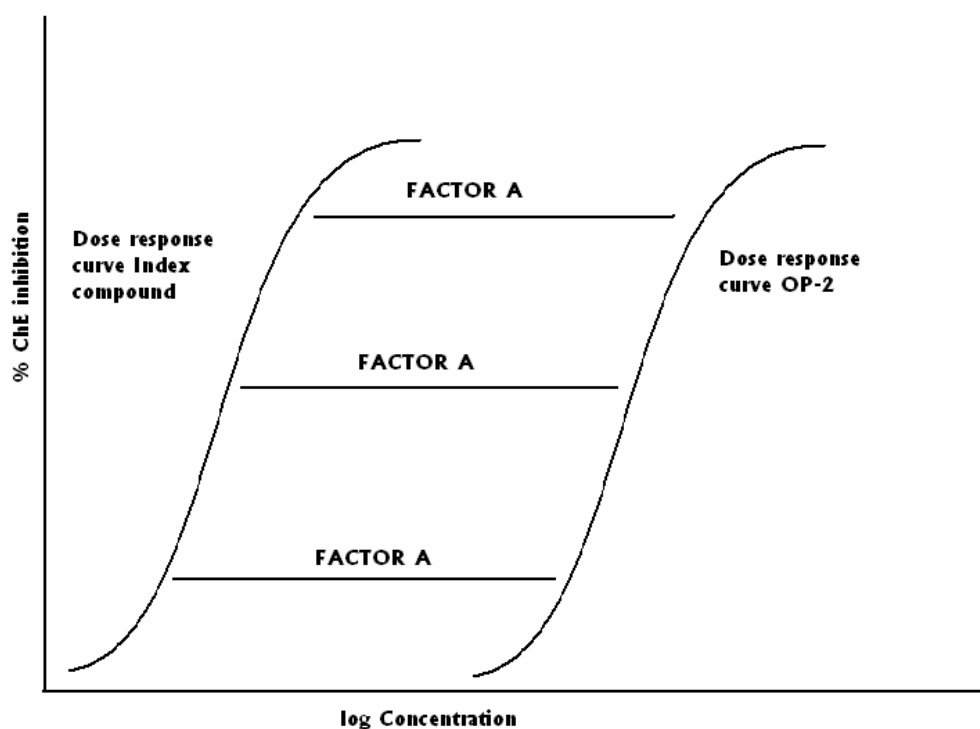

Figure 2. Illustration of RPFs (factor A) of two dose response curves

It is generally assumed that inhibition of ChE by various OPs will result in dose-additive effects. In fact, the RPF-approach is only valid when dose-additivity is true. In this approach it is a prerequisite that each point on the dose response curve of a certain pesticide (OP-2 in figure 2) can be expressed as a point on the dose response curve of an index compound (giving the same effect) by multiplication with a constant factor (factor A in figure 2). This factor is in fact the RPF. When the two dose response relations for two compounds can be translated into each other by a single factor, than it follows that the dose response curves are parallel to each other on the log-dose scale (see figure 2). When the RPF concept is not true, the dose response curves are not parallel and it is impossible to express each point on the dose response curve of a certain pesticide (OP-3 in figure 3) as a point on the dose response curve of an index compound by a single factor (factors A, B and C in figure 3 are different). In such a case, the outcome of a combined exposure can still result in dose-additive effects but the concept of the RPF is invalid for expressing the all substance in equivalents of a single index compound.
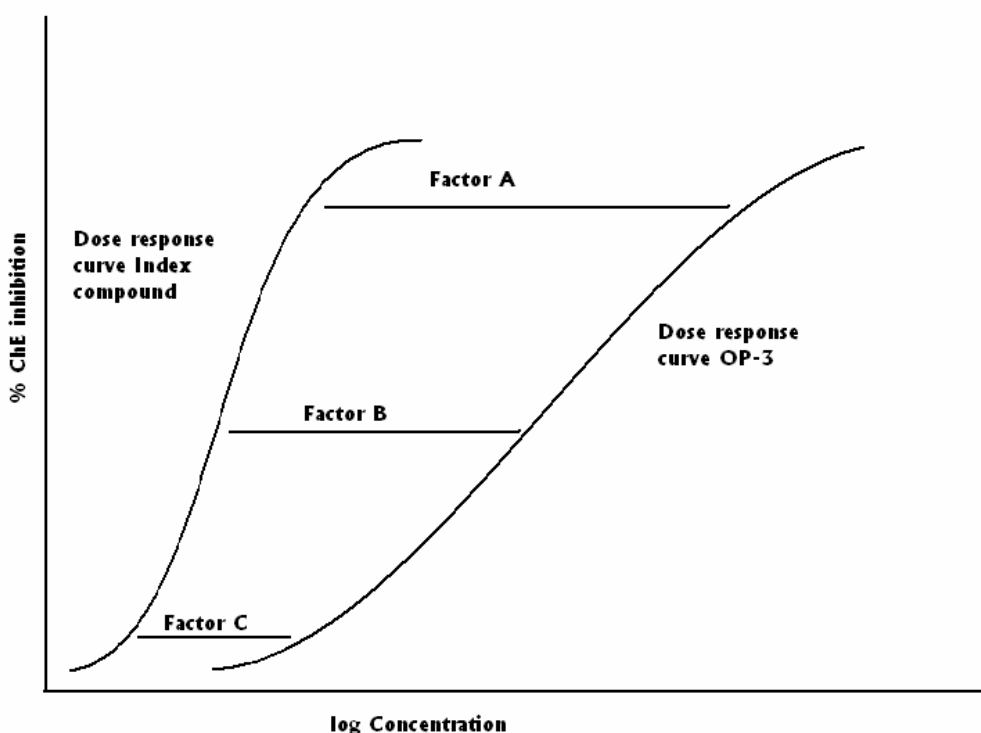

Figure 3. Illustration of two dose response curves for which the RPF concept is not true.

The use of dose additivity requires the assumption of no interactions among the chemicals other than the additivity on the common mechanism of action. In reality many OPs behave differently in the body (variation in pharmacokinetics and

biotransformation). Some OPs require metabolic conversion before ChE can take place. On the other hand, metabolic pathways operate to detoxify the various OPs. Upon combined exposure, also interactions may occur among the kinetic pathways of OPs. OPs may also bind to other neural target sites such as nicotinic- or muscarinergic choline receptors, all providing additional targets for interaction.

There is very little information in the scientific literature about dose additivity of OPs. Most of the studies available on dose additivity used high doses and irrelevant endpoints such as mortality. A paper by Singh (1986) reports that AChE inhibition by methamidophos (both in-vivo and in-vitro) was reduced by co-exposure to acephate (another OP pesticide and parent compound of methamidophos[1]). When acephate was given some time after methamidophos, however, the inhibition of AChE by methamidophos was normal. This paper clearly demonstrates that in some cases of co-exposure to OP-pesticides, dose additivity is not a valid assumption and an increase of the effects does not occur.
Other types of esterase inhibition (also non-competitive inhibition) can occur. The effects of the OP mipafox on NeuroToxic Esterase (NTE-inhibition) and the subsequent development of clinical symptoms of delayed neurotoxicity (OPIDN)[2], were highly changed by the concurrent presence of another NTE-inhibitor PMSF (Pope and Padilla, 1990). When PMSF was dosed before mipafox was added, the effects of mipafox were largely abolished. However, when PMSF was added after mipafox, a marked increase in the severity of clinical signs was observed indicating additive effects. According to the authors this may be explained by the presence of different biochemical binding sites with interacting consequences. Such mechanisms may also play a role with AChE-inhibition, questioning the validity of the concept of dose additivity in situations of co-exposure.
Richardson et al. (2001) reported in vitro studies on additivity of chlorpyrifos-oxon (C=O) and azinophos-methyl-oxon (AZM=O). It was shown that the dose response curves of these two substances were not parallel. In brain homogenates, C=O was about 9.6 times more potent than AZM=O at a level of 10% ChE inhibition whereas it was 15.2 times more potent at a level of 80% ChE inhibition. In serum the differences were even more pronounced. First, AZM=O was about twice as potent (0.45) as C=O at the 10% inhibition level whereas AZM=O was 4.6 times less potent than C=O at the 80% inhibition level (a tenfold difference in potency). Mileson et al. (1998) state that the overt toxicity of some OPs is not directly related to the extent of AChE-inhibition indicating that other pathways may modulate the

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[1] In this respect it is noteworthy to know that methamidophos is the predominant metabolite of acephate. In an in-vivo situation it is expected that additional acephate exposure next to methamidophos generates an additional source of methamidophos.

[2] See RIVM factsheet "Delayed Neurotoxicity/NTE-inhibition" for more information on this effect of OPs. In: RIVM report 601516 007, April 2001. Eds. Luttik, R and Van Raaij, M.T.M. RIVM, The Netherlands.

neurotoxicity by the compound or that other additional biochemical pathways than AChE inhibiting are involved, e.g. binding directly to muscarinergic- and nicotinergic acetylcholine receptors (see also Smulders, 2004).

On the other hand, analyses of the dose response curves for several OPs indicates that the slopes of the dose response curves are similar on the log-dose scale (EPA 2001; Wilkinson et al., 2000), which can be considered indicative for the concept of dose-additivity (see above). According to US-EPA (2001), dose additivity is a reasonable and appropriate approach for estimating the cumulative risk associated with joint exposure to OPs although firm proof of dose-additivity is lacking. The papers of Singh (1986) and partly also Pope and Padilla (1990) show that co-exposure to OPs does not necessarily result in simple additivity. Nevertheless, US EPA concluded that there was not sufficient evidence to deviate from the default assumption of dose-additivity when cumulating OPs.
From a pragmatic point of view dose-additivity could be assumed in handling the risks of cumulative exposure to OPs. From a more scientific point of view, more data are needed before it can be decided what is really happening when dealing with concurrent exposure of ChE-inhibiting pesticides.

4.4. Carbamates, OPs and time spacing

Carbamates and OPs share a common effect: they both inhibit ChE. In this respect one could state that the effects of OP's and carbamates are additive (in a general sense) when concurrent exposure occurs. However, although the ultimate endpoint is similar, the inhibition of ChE by carbamates in a biochemical sense is different from that of OPs (see section 3.2 above). Since this is the case, one may question whether it is valid to assume dose-addition when cumulating intakes of both carbamates and OPs. Because of these reasons the NRC in 1993 focussed on the combination of OPs only when looking at cumulative pesticide exposure of children (NRC, 1993). Also the US-EPA protocol for cumulative exposure focussed on OPs only, leaving carbamates out of the assessment (EPA, 2001). Furthermore, scientific advisory panels in the UK have proposed not to cumulate carbamates and OPs (I. Dewhurst, pers. comm.). In the RIKILT analysis of 2003, carbamates and OPs were all cumulated (Boon and Van Klaveren, 2003) just as was done by Jensen et al. (2003) and analyses of non-governmental bodies (Luijk et al., 2000; EWG, 1998).

Cumulation of carbamates and OPs also involves the aspect of time spacing. Most OPs inhibit ChE for a considerable period of time (because of the aging etc.., see section 3). Therefore, it may be valid to assume that the total exposure to OPs on a

single day (even when not present in the same meal) will lead to dose-addition. I.e., the exposure can be considered to be concurrent since it leads to concurrent effects. In the case of carbamates, the inhibition is reversible and can recover very rapidly. Exposure to carbamates in the morning may only lead to a temporary ChE-inhibition during the day with full recovery at the end of the day. Intakes of subsequent OPs may in that case not result in any additive effect because the effect of the carbamates has already resolved. On the other hand, when carbamate intake follows the intake of OPs than the ChE-inhibition induced by carbamates will still result in an additive effect.

To summarise, both biochemical (mechanism of action) and exposure (timing) aspects raise some questions on the validity of cumulating carbamates and OPs. In any way, cumulating daily exposure to both carbamates and OPs will probably lead to an overestimation of the risk associated with true cumulative exposure of ChE-inhibiting compounds.

5. Current issues with regard to residue data

5.1. Use of residue concentrations from monitoring programs

Inspection services follow strict procedures when sampling fruits and vegetables for monitoring or enforcement purposes. For each commodity, the amount of individual units needed to make up a representative analytical sample is clearly defined. For example, to monitor residues on apples a number of apples obtained from a shop or from an auction are homogenised, extracted and measured for residues of pesticides. This approach of so-called composite sampling has some consequences. Firstly, residues present on some apples can be 'diluted' by the presence of apples without any residue. For the risk assessment of chronic exposure this is not a major problem because in that case one is interested in the mean exposure. However, for risk assessment of acute exposure, one would like to know what the level of the residue is on a single unit (one apple or one bunch of grapes). Secondly, when the analysis shows more than one pesticide in the composite sample, this does not necessarily mean that more than one pesticide was present on a single unit (e.g., apple). It is possible that different units in the composite sample contain only one residue of a single pesticide instead of a mixture of pesticides. In the intake calculations such as performed by Boon and Van Klaveren (2003), but also e.g. by Jensen et al. (2003), it is implicitly assumed that all residues of mixtures of pesticides are present on a single unit (when more than one pesticide is found in the composite sample) leading to concurrent exposure. In many cases this will not be correct. When various pesticides found in a composite sample are in reality present in different units, this will not lead to a concurrent acute exposure of these pesticides but to a successive exposure pattern over time at most (see also section 4.4 for a discussion on time spacing). Implicitly assuming that concurrent exposure occurs can thus be regarded as a worst case assumption and may be used as a first step risk evaluation.

Essentially, the Inspection Service is bound to European regulation prescribing the use of composite samples representative for the whole set of the product. Such a set can originate from one source but may also come from different sources. Presently, this cannot be traced but from 2005 onwards, the EU General Food Law requires the possibility of tracing the source of the products (Article 18 of Regulation (EC) No 178/2002).

In conclusion, no other or more reliable data are available in the Netherlands to be used in a cumulative exposure analysis. However, the drawbacks (i.e. the bias of non-random sampling) of using such data should be clearly specified and kept in mind when drawing any conclusions on the risks of cumulative exposure to pesticides. The approach presently followed is thus a worst case estimate of the cumulative exposure.

5.2. Juices and sauces

In the exposure analysis of Boon and Van Klaveren (2003), fruit juices and apple sauce have been included in the intake calculations. For juices and (apple) sauces a mean residue level was assumed based on the pesticide measurements available. For a chronic exposure scenario, where the mean exposure levels are important this can be a valid (worst case) approach but for acute exposure calculations it can be questioned whether such products should be included and how these should be handled. Within the FAO/WHO Joint Meeting on Pesticide Residues, a discussion on this subject revealed that this is a very complicated subject. Some experts state that there is no use of including fruit juices and apple sauce in acute intake calculations because the residues will probably be highly reduced due to the processing procedure (manufacturing of the sauces) and the dilution of residues due to the use of large volumes of fruits from different sources. A recent evaluation for phosmet shows that in orange juice almost no pesticide residue is recovered compared to the residues in orange fruit (JMPR, 2000). On the other hand, data for acephate show that residues in apple juice can still be 50% of the levels found in fruits (JMPR, 2001) although in this case all apples were obtained from a field trail study and thus all contained residues (this is a worst case condition). As already pointed out by Boon and Van Klaveren (2003) more data is needed on the issue of processed products such as juices and sauces to be able to correctly include these products in the exposure assessment.

5.3. How to handle 'zero's'

Because only a certain part of all food products have been treated with pesticides, monitoring data on pesticide residues contain a lot of 'zero's', i.e. samples in which no pesticide residue was found. Such samples are also called 'non-detects' because a pesticide was not detected with the analytical method used. These samples are often expressed with <LOQ[3], indicating that no pesticide residue was present up to the limit of quantification for that pesticide. In those cases, the true residue level is not known. The residue could have been really zero because many products will not have been treated with the specific pesticide. On the other hand, the true residue may have been somewhere between zero and the LOQ, since such a level cannot be identified analytically.

In a probabilistic risk assessment, the handling of these non-detects can be crucial, certainly when most of the samples are 'non-detects'. There are several ways how to include non-detect samples. First, one could treat these samples as being zero

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[3] LOQ = Limit of Quantification (the lowest value in which an amount of acompound can be validly quantified). Also the term LOD is used but this term has two meanings: a) limit of detection (lowest level that can be seen above background) or b) limit of determination (which is similar to LOQ). For reasons of clarity we will only use the term of LOQ in this report.

(containing no residue at all). In reality one does not know what fraction of the samples represent true zero's and what fraction represents residues < LOQ. One way to handle this is to assume that the fraction of non-detects being a true zero is similar to the percentage of crops not treated. This is frequently done in the USA (e.g. ChemSAC,1998) but not in Europe. In fact, it is questionable whether in Europe such statistics are available (probably only to a limited extent). In addition, the high turnover of imported and exported foodstuff makes it very difficult to attach any percentage of the crops (un)treated to monitoring data. If one would have data on the percentage of crops treated in the Netherlands for example, this would not be representative for the Dutch food on the market because of the high contribution of imported food. Even at local regional scales very high differences can occur. Dutch policy is not to include 'data on the percentage crops treated' in intake calculations for pesticides.

Another approach to handle 'non-detects' is to treat all these samples as having a residue at the level of the LOQ. A third – more often used – approach is to treat all these samples as having a residue at ½ LOQ. It must be clear that the most conservative approach would be to set all non-detects at the level of the LOQ because this will lead to the highest exposure. However, this will lead to a consistent overestimation of the intake since many products will truly have no pesticide residues at all (e.g. ChemSAC, 1998). On the other hand, treating all non-detects as being zero will probably lead to some underestimation of the intake since some products will in reality have a residue level somewhere between 0 and the LOQ. A solution for this condition may be statistical: if one fits the distribution of the residues above the LOQ one can estimate the shape of this residue distribution down to zero. Such an additional distribution might be used in a Monte Carlo sampling.
Taken together, the way non-detects are treated in a probabilistic intake calculation may have a large influence on the final outcome, especially when a large number of the samples are below the LOQ.
In the case of a cumulative risk assessment, an additional problem can be identified. Normally one only has to decide whether the non-detects should be treated as true zero, ½ LOQ, 1x LOQ, or follow a distribution for a single substance. In a cumulative assessment one also should decide whether this assumption holds true for one pesticide at a time or for multiple pesticides on a single product. For example, a sample of a certain food product can be analysed at once for 30 different pesticides. If for all these pesticides a non-detect is found, in principle one could discus whether or not true zero, ½LOQ or LOQ should be used for each of those pesticides. In reality, probably 28 non-detects will be true zeros and maybe for 2 pesticides a level between zero en LOQ might be present. This is a complex problem which is mostly left out of the calculation when assumed that

non-detects are true zeros. Assuming that non detects are true zeros is probably closer to reality than setting all non-detects at ½LOQ or LOQ.

In principle, when only a small number of samples contain a residue and there are many non-detects, the total intake can be largely dependent on the assumptions made for the non-detects. Taken together, all these assumptions in the handling of the non-detects can create a large uncertainty in the final outcome of the risk assessment.

6. Methodological considerations

The use of probabilistic approaches for assessing the risk of chemicals becomes more and more general. Probabilistic approaches are able to include the variation and uncertainties of all aspects into the risk analysis providing a distribution at the end of the process. Such approaches can be used for both the toxicological effect side and limit setting (e.g. Slob and Pieters, 1998; Slob, 1999) as well as for the intake assessment (Kroes et al., 2002). In most cases probabilistic approaches are used only for the exposure side of a risk assessment and not (yet) for the toxicological effect side. In the present discussion on cumulative risk assessment the focus is on the probabilistic dietary intake assessment (see Boon and Van Klaveren, 2003).

As stated above, probabilistic approaches have the advantage that all kinds of variabilities can be included in the analysis. However, one should clearly identify the difference between uncertainty (i.e. the probability of being wrong) and true variability. Variability in dietary intake calculations has different levels: daily consumption rates vary between persons, between consecutive days, between seasons, between days of the week etc. Which levels of variability are relevant is determined by the question at stake. Pieters et al. (2005) have prepared a (draft) report on the use of probabilistic modelling for dietary intakes of chemicals. This report is commissioned by the Ministry of Health, Sports and Welfare (VWS). In this report it is recommended that the type of probabilistic approach used should be dependent on the exposure conditions and the question to be answered. Pieters et al. (2005) identify four exposure conditions dealing with one substance in one product at a time:

    A. Long term exposure of frequently consumed products
    B. Long term exposure of incidentally consumed products
    C. Short term exposure of frequently consumed products
    D. Short term exposure of incidentally consumed products

Each of these domains has different characteristics which require different types of information. For example, for long term exposure the mean residue concentrations are relevant while for short term exposure the distribution (variability) of individual residue concentrations are relevant.

It is concluded by Pieters et al. (2005) that for long term exposures (domains A and B) appropriate probabilistic exposure tools are available or have been developed recently. For the short term exposure, the currently available probabilistic methods provide only an estimation of the fraction of *person-days* that exceed a certain limit value (e.g. the ARfD). This is e.g. the case with the Monte Carlo technique used by

RIKILT. The outcome of the acute exposure calculation provides the probability that **a** certain consumption pattern of an unknown individual on **a** certain day will lead to exceeding the ARfD value due to contamination of the food product.

Table 1. Scheme of the various domains in dietary intake calculations with indications of their availability.

Domain	Relevant exposure and limit value	Intake	Relevant data on residue conc.	Outcome	Method available if one food product contaminated	Method for calculation with more food products contaminated
A	Long term ADI	Frequent	Means	X > ADI	Available	Available
B	Long term ADI	Incidental	Means	X > ADI	Recently available	Not available
C	Short term ARfD	Frequent	Distribution	Z > ARfD	Available but problematic interpretation	
				X with Y > ARfD	Available	Not available
D	Short term ARfD	Incidental	Distribution	Z > ARfD	Available but problematic interpretation	
				X with Y > ARfD	Recently available	Not available

X = fraction of the population; Y = frequency of exceeding the limit; Z = person-days.

The main problem with such outcomes is that the probability of exceeding the ARfD may differ between individuals. It might be that most individuals in the population have a similar but low probability of exceeding the ARfD but it might also be that some individuals in the population exceed the ARfD with a large frequency (high probability). In other words, in the short term exposure domains, one would like to know *how many people* will exceed the ARfD *how often*. The present methods do not provide such answers.

However, methods that do allow to provide such answers have been recently developed at RIVM in which the inter-individual variability in consumption frequency of products is accounted for (Slob and Bakker, 2004). In this method the VCP data are used to obtain information on the variation between days and the variation between persons. In the situation of only one contaminated food product, this method provides outcomes that tell the risk assessor what fraction of the population has a certain probability of exceeding the acute limit value (Slob and Bakker, 2004). For example, the outcome may be that 7% of the individuals in the population have a probability of 5% of exceeding the ARfD on any single day.

In the case of cumulative exposure of pesticides, not only one compound in one type of products should be covered but the whole range of compounds as well as the fact that they can occur in different food products. This should include all differences in residue levels and consumption frequencies of different products. In addition, correlations between the consumption frequencies of different food items on the same day should be taken into account. For instance, an individual that has eaten one or more apples on a particular day may be less likely to consume other fruits on the same day compared to days where he/she did not eat any apples. It appears problematic to estimate this type of correlation from food consumption survey data.

To summarise, the available probabilistic methods available until recently (using straight forward Monte Carlo sampling from consumption and residue data) for assessing the cumulative exposure to multiple chemicals have some conceptual problems:
-   They do not (yet) take into account differences in consumption frequencies
-   The acute exposure calculations provide only a distribution of person-day exposure without providing an answer to the question: what fraction of the population has what probability of exceeding an (acute) limit value.

Further developments in the area of probabilistic intake calculations are scheduled for 2005. RIVM and RIKILT together will try to develop new approaches in this area.

7. Summary of the main comments on the RIKILT 2003 analysis

In the previous chapters the scientific issues concerning cumulative exposure and risk assessment of pesticides have been discussed. Within these chapters the approach followed by Boon and Van Klaveren (2003) has been reviewed. In this section we will provide a brief summary of the comments of RIVM on the report of Boon and Van Klaveren (2003).

In general, it should be stated that the report of Boon and Van Klaveren is clearly written and a number of the problems associated with the approach used have been pointed out quite well in the discussion of this publication. Nevertheless, some comments can be made to this report but they should be placed in a wider perspective of current available knowledge and approaches for cumulative risk assessment.

♦ Boon and Van Klaveren used data for de determination of RPFs from different sources (different toxicological endpoints, different species and different time scales). Part of this was avoided by using RPFs from a benchmark approach performed by US EPA. However, in addition to those RPFs data from other sources were introduced. This introduces a substantial amount of uncertainty in the analysis

♦ Most of the RPFs used by Boon and Van Klaveren (2003) were obtained from US EPA. These RPFs are relevant for (sub)chronic exposure since they were explicitly set at the steady state inhibition of OPs after at least 3 weeks of exposure. However, the exposure distributions were compared with the ARfD (an acute toxicological limit) of the index compounds. This is in principle not valid.

♦ When acute NOAELs were not available, they were estimated to be 10 times higher than the chronic NOAEL. For carbamates this is an unacceptable approach. This means that the 'acute RPFs' of the carbamates carbofuran, ethiofencarb, oxamyl, and pirimicarb as used by Boon and Van Klaveren (2003) have little reliability.

♦ There is no scientific consensus on the validity of cumulating OPs and carbamates together as done by Boon and Van Klaveren (2003) and others. The NRC (1993), US-EPA (2001), and UK PSD (I. Dewhurst, pers. communication) all excluded carmabates from their cumulative exposure assessments. Cumulation of carbamates and OPs is at present problematic en will probably result in an overestimation of the actual risk.

♦ The basis for all the cumulative risk assessments of OPs done so far is based on the assumption of dose-additivity. Dose-additivity for OPs is not proven and

there are indications that the interaction of different OPs may not follow simple dose-additivity. When dose-additivity would not be valid, the total RPF approach – and therefore the total intake calculations – is/are invalid. More research is needed on this aspect (see also chapter 8)

♦ The residue data presently available from inspection monitoring programmes (having a bias from non-random sampling) have some consequences for the interpretation of the outcomes of a cumulative risk assessment. These limitations could have been discussed more clearly. The present approach is a worst case approach. However, other residue data with more relevance for cumulative risk assessment are not available.

♦ The report does not describe how non-detects were handled in the exposure analysis even though this may have a large consequence on the final outcome of the analysis. Probably the non detects were treated as zeros which seems most realisitic in this case.

♦ The Monte Carlo approach for probabilistic exposure assessment has limitations in itself. It provides only a estimation of the fraction of person-days. I.e., the calculations provides the probability that *a* certain consumption pattern of an unknown individual on *a* certain day will lead to exceeding the (acute) limit value used. Is does not provide what fraction of the population exceeds the limit value nor does it provide any frequency of this occurring in individuals. In other words it does not provide an answer to the question *how many people* will exceed the limit value *how often*. The output of the probabilistic assessment by Boon and Van Klaveren (2003) results in a distribution of person-days, and cannot be translated in a fraction of the population exceeding the exposure limit.

8. Impact and consequences for risk assessment and policy

Cumulative exposure has received increased attention over the last decade. However, at this point in time it is necessary to make up the balance. What is known about cumulative exposure and what is not ? What type of information is needed to clarify the issue? Does the present knowledge (with or without additional investigations) require a change in regulations and policy ? And if so, how should cumulative exposure be handled and included ?
With respect to cumulative exposure to pesticides two main questions should be handled. Firstly, is there an actual health risk from cumulative exposure to pesticides ? Secondly, should cumulative exposure become an integral part of the authorisation and other risk assessment procedures for pesticides. Even when the answer to the first question is negative, still the second question needs to be answered.

8.1. Is there a real health risk ?

Whether or not current cumulative exposure to ChE-inhibiting compounds provides a real health risk is difficult to answer. First of all, we have explained various problems associated with the present available cumulative risk assessments which introduce a lot of uncertainties in their outcomes. The main problems are:

1. Lack of adequate representative residue data for cumulative risk assessment
2. Methodological problems associated with the probabilistic concept; the calculations provide only 'person-days' and no population or frequency outcomes as needed for an adequate risk assessment.
3. The validity of the dose-additivity concept for the OPs (by themselves or together with carbamates ?) - which forms the fundamental concept of the total cumulative approach – may be questioned.
4. Combining carbamates and OPs reveal several toxicological problems with respect to the mechanism of action and consequences of time spacing.
5. Many uncertainties still exist in the calculated Relative Potency Factors (especially for acute exposures) due to limited toxicity data.

Some of these problems are further discussed in section 8.3.

Despite these fundamental problems, several risk assessments of cumulative exposure to ChE-inhibiting pesticides have been performed. Different investigations have however revealed different opinions. In 1993, the NRC performed a pilot calculation on a select group of OPs revealing that 1.3% of the person-day calculations of US children were above the chronic RfD of the index compound. However, as the NRC pointed out, this does not necessarily mean that

1.3% of the population exceeds the (chronic) RfD. NRC states that there is too much uncertainty in the validity of the primary data to form an opinion on the actual health risk question, but that the calculations indicate a potential concern (NRC, 1993). In publications from non-governmental organisations (e.g. EWG, 1998; Luijk et al., 2000) however, alarming conclusions were drawn about the health risks of cumulative exposure to ChE-inhibiting pesticides, especially for children. A problem with these publications is (amongst others) that a number of 'person-days' above a certain limit value were interpreted from the calculations as fraction of the population at risk. This is incorrect, as discussed above.

In the report by Boon and Van Klaveren (2003) it is stated that only 6% of the composite samples analysed in 2000 and 2001 in the Netherlands contained a combination of different ChE-inhibiting compounds (more than one pesticide). This might indicate that the problem of cumulative exposure through the diet is relatively small. On the other hand, for certain individual food products, simultaneous contamination by ChE-inhibiting compounds may be more frequent, e.g. in grapes (Pieters et al., 2002). In Boon and Van Klaveren (2003) it can be seen that about 0.1% of the calculated person-days was higher than the ARfD. A recent publication of Jensen et al. (2003) shows that cumulative intakes to OPs and carbamates in Denmark were maximally 11% of the ADI and maximally 27% of the ARfD (depending on the index compound used) indicating no health risk at all. Because of all the problems still attached to these kind of cumulative calculations (see also section 8.3) and methodological considerations in the probabilistic approaches, it cannot be concluded based on these calculations that there is a health risk or not but the issues requires further attention.

> *Similar to the conclusion of the NRC in 1993 and a recent report of the Dutch Health Council (2004), RIVM feels that the various investigations can be taken as an indication for a potential area of concern.*

8.2. Should cumulative exposure be an integral part of pesticide risk assessment ?

The results of the cumulative risk assessment for OPs performed so far, might question the need for cumulative exposure calculations in the authorisation procedure (see above). On the other hand, cumulative exposure to pesticides is a fact of life. People are not exposed to individual compounds only but rather to a mixture of compounds. Concurrent exposure to compounds with a common mechanism of toxicity, is also a likely condition although the frequency at which this occurs and whether there is true dose- or effect-addition is not clear at present. For reasons of realism and (scientific) prudence, it can be proposed that cumulative exposure to chemicals with a common mechanism of toxicity should be an integral

part of the authorisation procedure (see also recommendations made by the Dutch Health Council, 2004).

In addition, there is a non-governmental and political movement within the EU to include cumulative exposure assessment for pesticides from a sense of high consumer protection levels and the precautionary principle. Even within the European Parliament recent initiatives have been undertaken to enforce the inclusion of cumulative exposure in pesticide regulations; although these preliminary proposals showed a lack of scientific validity (see Appendix). At his moment, various regulations exist in the EU concerning pesticides. During 2004 an update of the EU pesticide regulation(s) is taking place and member states are negotiating to replace the whole set of individual regulations with a new integral pesticide regulation (European Council, 2004). Although cumulative exposure is not yet an issue taken up in the new regulation, there are amendments within the EU to add cumulative exposure to the new regulation. The position of the Dutch policy (Ministry of VWS) is to support the inclusion of cumulative exposure but not aggregate exposure (see section 2 for definitions). From a pragmatic point of view this can be defended although an adequate discussion on the methodological approaches to use has not taken place within the EU.
If this situation becomes real (which seems likely) then there is no need for a scientific discussion about the necessity of integrating cumulative exposure in the authorisation procedure since it will be a political reality to do so.

When cumulative exposure becomes an integral part of pesticide risk assessment, the main question will then be, how to handle the issue in a practical sense (methods and approaches) and what information should become available to improve knowledge and procedures.

8.3. Gaps of knowledge

In the following paragraphs, we will briefly describe the gaps of knowledge and the need for further research.

8.3.1.   Criteria for a common mechanism of action

Cumulative exposure to pesticides is a very broad issue. According to the Food Quality Protection Act (FQPA), cumulative exposure to pesticides would only be relevant for chemicals with a common mechanism of toxicity. In Mileson et al. (1998), criteria have been formulated when pesticides should be considered to share a common mechanism of action (focused on OPs). EPA has proposed also other groups of pesticides for cumulative risk assessment (e.g. triazine pesticides and chloroacetanilides). A special group of pesticides that might share common effects are the pyrethroids. However, for this group of compounds it was found to

be very difficult to perform a cumulative risk assessment related to a common mechanism of action (Koers, 2001). Recently, Soderlund et al. (2002) argued that pyrethroids have various biochemical and pharmacological targets which would suggest that a simple additive model based on combined actions on a single target would not be appropriate. This illustrates the need for well supported criteria.

It is recommended that criteria should be developed to determine for which groups of compounds a cumulative risk assessment should be performed based on a common mechanism of toxicity. Such criteria should be developed preferentially at the EU level, taking into account the work already done by US EPA. Because of the knowledge available, RIVM –in close cooperation with CTB and RIKILT - can initiate a Dutch proposal for such criteria.

8.3.2. *Assumption of additivity*

In all of the cumulative risk assessment reports of ChE-inhibiting compounds published until now, the explicit or implicit assumption is made that the cumulative (concurrent) exposure to ChE-inhibiting compounds will follow dose-additivity. This is a very fundamental concept within the total area of cumulative exposure to ChE-inhibiting compounds. This concept also forms the basis for the view that OPs act by a common mechanism of action and in connection with this, the use of the RPF or TEF methodology.

Within the general field of combination toxicology, dose-additivity is in practice often considered a worst case assumption for many chemical groups at low doses (Feron et al., 1998; 2002; Feron and Groten, 2002) although synergistic effects are the worst case assumption from a theoretical point of view. It has been proposed by various official bodies that dose-additivity would be an appropriate concept for chemicals sharing a common mechanism of action (Dutch Health Council, 2002; COT, 2002). The arguments for assuming dose-additivity for cumulative exposure to ChE-inhibiting compounds mostly relate to indirect evidence. For example, the US-EPA reported that the dose response curves of a couple ChE-inhibiting compounds were largely parallel which indeed is an outcome of the dose-additivity concept. In their discussion, the US-EPA states that there is insufficient evidence to dispose the assumption of dose-additivity (EPA, 2001). However, there is also some evidence that a straight forward dose-addition might not be a correct assumption for cumulation of (all) ChE-inhibiting compounds (see section 4.3 for a detailed discussion).

Based on the type of information included in the publications of (Singh, 1986; Pope and Padilla, 1990; Richardson et al., 2001; Mileson et al. 1998) it is not clear at all that combined / concurrent exposure to ChE-inhibiting compounds will actually follow a dose-additivity concept that allows an RPF like approach. In the

situation that cumulative exposure to ChE-inhibiting compounds will become an actual part of the authorisation procedures for pesticides and – therefore – also an integral part of how the Inspection Service deals with their task, more direct data are needed on this subject to conclude how to proceed with cumulative exposure to ChE-inhibiting compounds.

From a pragmatic point of view the risk of cumulative exposure to OPs can be handled using the RPF approach for the time being. Additional research on the concept of dose additivity is however, strongly recommended.

One way to go forward is to investigate the interaction of OPs first in-vitro. In such an approach (e.g. using a factorial design), one could try to substantiate or to dispose the hypothesis of dose-additivity for OPs. Such testing could be directed to the enzyme acetylcholine esterase but also to effects of OPs on muscarinergic and cholinergic receptors which have been linked to other types of neurotoxicity by ChE-inhibiting compounds (e.g. Smulders, 2004).

8.3.3. *Probabilistic exposure calculations*

The present available probabilistic exposure calculations are not yet able to provide the necessary output for risk assessment of cumulative exposure to multiple compounds in multiple food products. See for review Pieters et al. (2005) and Slob and Bakker (2004). In particular for acute risk assessment, one would like to know what fraction of the population has how much probability to exceed the limit value (ARfD). Such answers cannot be obtained from the present Monte Carlo based calculations. For a single food products a statistical model has recently become available (Slob and Bakker, 2004).

Further developments of dietary intake models that include multiple food products are needed to provide giving the necessary information on the fraction of the population exceeding a limit value and frequency of such events .

8.4. Cumulative risk assessment and setting of MRLs

Maximum Residue Levels (MRLs) for pesticides are currently set on the principles of Good Agricultural Practice (GAP). Residue data from field trail studies for single active ingredients are used to establish the level of the MRL. The total intake is compared to the ADI and ARfD using a number of formulas (deterministic). This calculation is performed in both the authorisation procedures and for inspection purposes. In addition, probabilistic calculations of the intake can be made.
The pros and cons of probabilistic methods for setting an MRL are being discussed now on the international level (e.g. Codex Alimentarius). An WHO expert

workshop on this issue is planned in 2005. At present, there is no consensus how to implement probabilistic methods in the setting of the MRL.

In addition to this, using cumulative exposure to pesticides – or to OPs in particular – in the setting of an MRL is even more complex. One could think of a procedure to take into account a certain level of background exposure to OPs in general. How this should be quantified is dependent on a large number of factors. A discussion on this aspect should be held separately and should use the input of the expert workshop to be held in 2005. This subject needs further and refined evaluation.

8.5. Consequences for policy decisions

Cumulative exposure to pesticides with a common mechanism of action is a fact of life. Various movements (of scientific, political and non-governmental origin) are working towards inclusion of a risk assessment of cumulative exposure to pesticides with a common mechanism of toxicity. Of the various approaches available the RPF-approach appears to be the most adequate method. Although some uncertainty exists on the fundamental concept of dose additivity for OPs (and maybe other groups of pesticides), one can move forward with this issue by assuming that (for the time being) the dose additivity concept holds true for OPs. An approach that could be followed is the use of a 'group-ADI' or a 'group-ARfD' for OPs such as is sometimes done for other types of substances also (see also Appendix). Such a group-based limit value can be derived when a number of choices have been made. These choices are: the dose metric for RPF calculations (mg or mmoles), the selection of appropriate endpoints for both acute and chronic toxicity, and the selection of an index compound.

In addition, also choices have to be made on the type of data to use, how to handle non-detect samples, and the type of probabilistic methods to be used.

Although the issue can be dealt with in a pragmatic way, still several problems could arise in the policy area when introducing cumulative risk assessment for pesticides with a common mechanism of toxicity. This is illustrated by two fictive cases.

Case 1

Toxicological evaluation for the authorisation of a new OP pesticide reveals that there is no health risk due to the use of this single substance (intakes are below the ADI and ARfD according to current procedures). However, additional cumulative dietary risk assessment shows a potential risk of the total cumulative exposure. What should the policy decision be to obtain an acceptable cumulative exposure? Refusal of the new OP or a restriction in the use of already accepted OPs ? If one decides to restrict other OPs, which ones are to be restricted and which ones not ? Will the restriction be based on the level of active substances or at the level of

pesticidal products ? Should a comparative risk assessment be performed to decide which scenario of restriction is optimal (in terms of cumulative exposure reduction, public health risks, operator safety, or environmental risks) indicating which pesticide use is restricted and if so, how should this be done? These questions indicate that many policy decisions (risk management) have to be made. This requires also international agreement.

Case 2

The Dutch Inspection Service samples a box of oranges. The analysis shows that for two OP pesticides the residue level exceeds the MRL. However, individual risk assessment for each pesticide separately as well as the cumulative exposure for the two compounds together reveal no acute health risk (both OPs result in an intake below the ARfD). Nevertheless, when total cumulative dietary exposure is included (background exposure to other OPs through the diet) the exposure shows a potential risk (total cumulative intake above the ARfD). What should be done ? The Inspection Service could remove the sampled oranges from the market although the products by themselves have no acute health risks. On the other hand, besides the fact the product has an unacceptable residue level of pesticides, one could also interpret this finding as a reason for having more strict controls in order to reduce the background exposure in general.

These two cases illustrate the problems that could be associated with introducing cumulative risk assessment in the authorisation and inspection procedures. Any way, when for example MRLs are going to be set taking into account cumulative exposure an internationally agreed procedure is needed (see also above). If such international agreement is not reached, problems will arise in setting MRLs and mutual recognition, both at the EU level as well as at the worldwide Codex Alimentarius level.

Besides the fact the choices and agreements have to be made in the risk assessment process, the illustrations also show the need for developing choices and agreements in the risk management area.

8.6. Recommendations

The issue of cumulative exposure to pesticides with a common mechanism of toxicity is a fact of life. Various movements argue to incorporate cumulative exposure into the risk assessment (in particular the authorisation) of pesticides. Selection criteria should be formulated to select groups of pesticides for which a cumulative exposure assessment should be performed.

As a start, the group of OP pesticides can be the first group for which a cumulative exposure assessment could be performed. For the time being, a pragmatic approach can be followed assuming that the dose additivity concept holds true for OP pesticides. In this respect the RPF-approach appears to be the most adequate method to be used.

Guidance documents should be prepared in which several choices are made how to perform an cumulative assessment (dose metric, endpoints, acute and chronic, setting of RPFs, use of residue data, handling non-detect samples, type of approach to use for the dietary intake calculations).

Because the authorisation process of plant protection products is largely performed on the EU level, the approach to be used and the guidance documents for such an approach should ultimately reach consensus on the EU level. It is recommended, however, to prepare proposals for such an approach within The Netherlands by RIVM, CTB and RIKILT (and others).

In addition to procedures for risk assessment, it is recommend to also work on proposals for risk management since the inclusion of risk assessment of cumulative exposure will urge for new type of decisions to be made by policy makers.

In the meantime, additional research on cumulative exposure (and possibly aggregated exposure) of OP pesticides is strongly recommended. This will provide the information to either support or to reject the concept of dose-additivity for OPs. New research may diminish the level of uncertainty attached to the current approaches.

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Appendix :

Reaction RIVM/SIR on proposals from the EU-parliament (december 2004).

**COMPROMISE AMENDMENTS for draft recommendation on
second reading for MRLs in pesticides - rapporteur Mr Sturdy**

Within the European parliament concern exists with respect to cumulative exposure to certain plant protection products. In this phase, the issue is tried to be handled by introducing cumulative exposure into the definitions of health based limit values for single substances.

1) Scientifically this is invalid
2) The proposed approach will confront the risk assessor with definitions that cannot be handled, which will lead to useless discussions within the EU authorisation proces, and misinterpretation.
3) Because the prosposed definitions are not in line with international consensus, the European Union will confront itself with unnecessary international problems e.g. with respect to trade and health safety issues.

Risk assessment is based on two parts: I) toxicological effects of a substance and II) exposure to the substance. It is problematic to incorporate cumulative <u>exposure</u> into <u>toxicological</u> limit values such as the ARfD and ADI, because these limit values are compound specific.

The risk of cumulative exposure to plant protection products should be dealt with in the area of 'exposure to the substance' (e.g. when setting an MRL).

If different plant protection products have cumulative effects based on a common mechanism of action, a group-acute reference dose and a group-ADI may be proposed taking into account the relative potency of the different plant products. These 'group-limits' could then be expressed in toxicological equivalents, an approach also used for dioxin-like compounds.

<u>The formal definitions might then be transformed into:</u>

(i)"*acute reference dose*: means the estimate of the amount of substance in food, expressed on a body weight basis, that can be ingested over a short period of time,

usually during one day, without appreciable risk to the consumer on the basis of*, the data produced by appropriate studies and taking into account sensitive groups within the population (e.g. children and the unborn);*

(j)"*acceptable daily intake*": means the estimate of the amount of substance in food expressed on a body weight basis, that can be ingested daily over a lifetime, without appreciable risk to any consumer on the basis of all known facts at the time of evaluation, *taking into account sensitive groups within the population (e.g. children and the unborn).*

In addition to this definitions, a group limit value can be proposed for substances with a common mechanism of action:

"*Group ARfD*": means the estimate of the total amount of substances with a common mechanisms of action in food, expressed as toxic equivalents of an index compound on a body weight basis, that can be ingested during one day without appreciable risk to the consumer on the basis of*, the data produced by appropriate studies and taking into account sensitive groups within the population (e.g. children and the unborn);*

(j)"*Group ADI*": means the estimate of the amount of substances with a common mechanisms of action in food, expressed as toxic equivalents of an index compound on a body weight basis, that can be ingested daily over a lifetime, without appreciable risk to any consumer on the basis of all known facts at the time of evaluation, *taking into account sensitive groups within the population (e.g. children and the unborn).*