Combined actions of pesticides in food

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Making of regulations, co-ordination, research and development, take place in the Administrations center in Moerkhoej. The 11 Regional Authorities handle the practical inspection of food and veterinary matters, including import/export etc.

The central administration of The Danish Veterinary and Food Administration employ a staff of approx. 550 full-time employees, whilst the 11 regional authorities employ a further approx.1.600 full-time employees.

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List of abbreviations

2,4-D:	2,4-dichlorophenoxyacetic acid
ACh/Ch:	Acetylcholine/choline ratio
AChE:	Acetylcholinesterase activity
ADI:	Acceptable daily intake
AL_n:	Maximum acceptable level of compound n
ALT:	Alanine aminotransferase
AST:	Aspartate aminotransferase
ATPase:	Adenosine triphosphatase
DDT:	Dichlorodiphenyltrichloroethane
cDNA:	Complementary DNA (deoxyribonucleic acid)
DEHP:	Di(2-ethylhexyl)phthalate
d_n:	Dose (or concentration) levels chemical n
D_n:	Dose (or concentration) levels of compound n that produces the same level of response as produced by the mixture
ED_{10}:	10 % effect dose
E_n:	Exposure of compound n
EPN:	O-ethyl O-p-nitrophenyl phenylphosphonothioate
$f(d_1,d_2)$:	Fraction of total possible effect produced by the dose d_1 and d_2
$f_b(d_1,d_2)$:	Fraction of total possible effect produced by the dose d_1 and d_2 taken the background effect, f(0,0), into consideration
$f_{expected}$:	Fraction of total possible effect for a mixture expected from the model of Bliss independence
FQPA:	U.S. Food Quality Protection Act
GST-P:	Glutathion S-transferase placental form
HCH:	Hexachlorocyclohexane
HEPT:	Heptachlor
hER:	Human estrogen receptor
HI:	Hazard index
I_c:	Combination index
i.p.:	Inter peritoneal
JMPR:	Joint Meeting on Pesticide Residues
MCL:	Maximum contamination level
MOE_n:	Margin of exposure of compound n
MOE_T:	Combined margin of exposure
MOS:	Margin of safety
NOAEL:	No observed adverse effect level
OP:	Organophosphorus pesticide
PAH:	Polycyclic aromatic hydrocarbon
PBO:	Piperonyl butoxide
PCB:	Polychlorinated Biphenyl
PDBu:	Phorbol-12,13-dibutyrate
PMA:	Phorbol-12-myristate-13-acetate

POD:	Point of departure
RfD:	Reference dose
RNA:	Ribonucleic acid
RPF:	Relative potency factor
SCE:	Sister chromatid exchanges
SGPT:	Serum glutamic pyruvic transaminase
TCE:	Trichloroethylene
TDI:	Tolerable daily intake
TEF:	Toxicity equivalency factor
TEQ:	Toxicity equivalent
U.S. EPA:	United States Environmental Protection Agency
WOE:	Weight of evidence

Summary

The objective of this report is to examine whether there is a scientific basis for using a general standard formula in the risk assessment of pesticide mixtures. This is done in order to ascertain and/or improve the current toxicological risk assessment of pesticide mixtures which humans are exposed to via food.

There is no internationally accepted procedure for the toxicological evaluation of exposure to multiple residues of pesticides in crops except for the few groups of pesticides sharing a group ADI e.g. those listed under dithiocarbamates. The risk assessment of pesticide residues in food is based on toxicological evaluation of the single compounds although humans are exposed to more than one pesticide at the same time that potentially possess a similar or different toxic effect. These compounds may interact causing a higher or lower or toxic effect than would be expected from the single compounds. Consequently potential combined actions of pesticides need to be addressed in the risk assessment process.

The evaluation of the toxicological properties of a pesticide mixture requires detailed information on the composition of the mixture and the mechanism of action of each of the individual compounds. In order to perform a risk assessment, proper exposure data are also needed. Most often such detailed information is not available. The mixture of pesticide residues that a person would be exposed to via the food may change over time in composition and quantity. It is not possible to examine the effect of all mixtures because there are too many combinations and furthermore a sufficient number of dose levels are not feasible. In addition, high dose levels of a pesticide mixture may have different types of effects than low dose levels and high to low dose extrapolation may be meaningless.

Two different kinds of approaches for health risk assessment of chemical mixtures have been recommended, namely whole mixture approaches and component-based methods. The assessment on whole mixtures can be done on the mixture of concern, on a sufficiently similar mixture, or on a group of similar mixtures. These assessments would be ideal for risk assessment of pesticide residues in food, however they are not applicable here since they are very data intensive.

This leaves the single compound approaches as the more realistic ones. For mixtures in which the compounds are toxicologically similar (e.g. same mechanism of action), four methods based on Loewe additivity have been suggested: the hazard index, the relative potency factor method, and the special type of the relative potency factor method named the toxicity equivalency factor, and the margin of exposure. These methods differ by the required data on toxicological processes but in all cases the exposure levels are added after having been multiplied by a scaling factor that accounts for differences in the toxicological potency. For compounds acting independently (different mechanism of action) the response addition approach have been suggested, and for compounds that interact, use of interaction hazard index have been suggested.

Several reports have suggested that since the pesticides are found in food at levels well below their respective no observed adverse effect levels (NOAEL), the approaches based on toxicological similar mechanism (same mechanism of action) and toxicological independence (different mechanism of action) should be used for risk assessment of pesticide residues. In fact, it has been suggested that methods for toxicologically similar compounds could be used in most cases, even when the compounds are not toxicologically similar.

The present knowledge about combined toxic effects of mixtures of pesticides (the active substances of pesticide formulations) that have been published in the scientific literature are summarised and evaluated in this report in order to test the hypothesis behind the risk assessment models presented. The *in vivo* studies on pesticide mixtures were performed at high doses (ten times NOAEL or higher) compared to the expected pesticide residues in food. Studies employing high doses have shown both additivity and interactions such as synergism or coalism as well as antagonism. None of the studies reported in the literature used low pesticide doses, in range of normally found residues. Further, the number of theoretical possible pesticide mixtures is enormous compared to the number of mixtures studied and published in the scientific literature and the overall quality of the data in the studies is not good enough to reach a clear conclusion. Therefore it is concluded that there is no scientific background for establishing a general standard formula for risk assessment of pesticide mixtures in food. However, studies on other chemical mixtures at low doses have been found not to demonstrate a risk different from that of the single compounds in the mixture. The authors had concluded that combined exposure to arbitrarily chosen chemicals did not demonstrate more than an additive action when all chemicals in the mixture were administrated at their own individual NOAELs.

At the moment the best suggestion for a feasible risk assessment of pesticide mixtures in crops is to make a case-by-case evaluation in which the available chemical and toxicological data on the pesticides are evaluated in a weight of evidence process. Then the hazard index with the acceptable daily intake (ADI) or NOAEL as the acceptable level in the denominator should be used. However, when the weight of evidence process points out that the compounds in the mixture share a common mechanism (e.g. for the organophosphorus pesticides), the toxicity equivalency factor (TEF) approach should be used, if possible.

Ten examples on risk assessment of pesticide residues found in specific crops to be consumed in Denmark are shown in the report. The assessment did only consider the risk from eating the actual crops and it did not take the overall exposure of pesticides from food in general and from other sources into account. The risk assessments are based on data on chemical structures, toxicology (including toxic effects, target organs, and mode of actions), metabolism, and metabolites of single compounds. Such data are presented for about 80 pesticides that have been found in food available in retail shops in Denmark, the food being domestically produced or ingested. The ten examples have shown that the suggested risk assessment method is feasible for use in practice and that the actual levels of pesticide residues in the examples are not expected to constitute a risk to humans.

The area risk assessment of pesticide mixtures is relatively new and further research is required. Indeed there is a glaring need for further experimental studies on combined actions of pesticides to clarify the exact role of the models and statements presented in this report.

Sammendrag (Summary in Danish)

Formålet med denne rapport har været at undersøge, om der er videnskabelig basis for at bruge en generel standardformel i risikovurderingen af blandinger af pesticider. Dette er gjort i et forsøg på at forbedre den nuværende toksikologiske risikovurdering af de blandinger af pesticider, som mennesker bliver eksponeret for via levnedsmidler.

Der er i dag ingen internationalt accepteret metode til at foretage en toksikologisk evaluering af eksponeringen for blandinger af pesticider fundet som rester i afgrøder. En undtagelse er dog enkelte grupper af pesticider, der har en gruppe-ADI (acceptabel daglig indtagelse) f.eks. dithiocarbamater. Risikovurderingen af pesticidrester i levnedsmidler er i dag baseret på en toksikologisk vurdering af de enkelte stoffer, selvom mennesker eksponeres for mere end et stof af gangen, der kan have samme eller forskellige effekter. Disse stoffer kan interagere og derved medføre en højere eller lavere toksisk effekt, end man ville forvente udfra de enkelte stoffer alene. Derfor er det nødvendigt i risikovurderingsprocessen at overveje om der kan forekomme kombinationseffekter.

Evalueringen af de toksikologiske egenskaber af en pesticidblanding kræver detaljeret information om sammensætningen af blandingen og virkemåden af de enkelte stoffer. For at udføre en risikovurdering er det nødvendigt at have gode data vedrørende eksponeringen. Ofte er så detaljeret information ikke tilgængeligt. Den blanding af pesticidrester som en person vil blive eksponeret for via levnedsmidler, vil variere i mængde og sammensætning over tiden. Det er ikke muligt at undersøge effekten af alle blandinger, fordi der er så mange kombinationsmuligheder og det er ikke muligt at opnå et tilstrækkeligt antal dosisniveauer. En pesticidblanding ved høje doser kan have andre effekter end den vil have ved lave doser, og derfor vil en ekstrapolering mellem dosisniveauer være meningsløs.

Der er i rapporten blevet anbefalet to forskellige typer af metoder til risikovurderingen af kemiske blandinger, nemlig metoder baseret på data for hele blandingen og metoder baseret på data for enkeltstoffer. De først nævnte metoder kan enten baseres på data for den aktuelle blanding, på en blanding der ligner tilstrækkeligt meget, eller på en gruppe af lignende stoffer. Disse metoder kunne give det mest realistiske billede ved risikovurderingen af pesticider i levnedsmidler, men det er desværre ikke muligt at anvende dem pga. manglende data.

Det er således mere realistisk at anvende de metoder, der er baseret på data for de enkelte stoffer i blandingen. For de blandinger, hvor stofferne toksikologisk ligner hinanden, er der foreslået fire metoder, der alle er baseret på Loewe additivitet. Det er: ”hazard index”, ”relative potency factor”, ”toxicity equivalency factor” (der er en speciel type af ”relative potency factor”) samt ”margin of exposure”. I disse fire metoder adderes eksponerings niveauerne efter, at de er blevet multipliceret med en skalerings faktor, og metoderne adskiller sig kun fra hinanden i de data vedr. toksikologiske processer, der skal sættes ind i ligningerne. I de tilfælde, hvor stofferne agerer uafhængigt af hinanden, er det blevet foreslået at benytte metoden ”response addition”, og for de tilfælde, hvor stofferne interagerer, er ”interaction hazard index” blevet foreslået.

Pesticider forekommer i levnedsmidler i niveauer, hvor der i forsøg med de enkelte stoffer ikke er fundet effekter. Flere rapporter har derfor foreslået, at de metoder, der er baseret på toksikologisk lignende mekanismer og toksikologisk forskellige mekanismer kan benyttes i risikovurderingen af pesticidrester. Forfatterne til disse rapporter mener, at det er forsvarligt at undlade at se på hele blandingen, og at man kan se bort fra interaktioner. Det er sågar blevet foreslået, at metoderne for stoffer, der toksikologisk ligner hinanden kan bruges i langt de fleste tilfælde – også i tilfælde hvor stofferne i blandingen ikke er toksikologisk ens.

Den nuværende viden om toksikologiske kombinationseffekter af pesticidblandinger (de aktive stoffer i pesticid formuleringer), der har været publiceret i den videnskabelige litteratur, er resumeret og evalueret i denne rapport. Dette er gjort for at teste hypoteserne bag de risikovurderingsmodeller, der er præsenteret i rapporten. De publicerede *in vivo* studier af pesticid blandingerne var alle udført ved høje doser (ti gange NOAEL eller højere) sammenlignet med de fund, der er gjort af pesticidrester i levnedsmidler. Studierne viste både additivitet og interaktioner (både synergisme, coalisme og antagonisme). Det teoretisk mulige antal pesticidblandinger er enormt sammenlignet med det antal blandinger, der er undersøgt, og den generelle kvalitet af dataene i de foreliggende studier er ikke god nok til at lave en klar konklusion. Derfor må det konkluderes, at der ikke er videnskabelig baggrund for at opstille en generel standard formel til risikovurdering af pesticidblandinger i levnedsmidler. Studier af andre kemiske stoffer ved lave doser har vist, at risikoen ved blandingerne ikke var forskellig fra den for de enkelte stoffer i blandingen. Forfatterne bag disse studier konkluderede, at eksponering for en blanding af vilkårligt valgte stoffer højst gav en additiv effekt, når alle stofferne blev givet ved deres individuelle NOAEL.

Indtil videre er den mest anvendelige metode til risikovurdering af blandinger af pesticider i afgrøder, at foretage en evaluering af de kemiske og toksikologiske data fra sag til sag i en ”weight of evidence” proces. Derefter kan ”hazard index” benyttes med enten det acceptable daglige indtag (ADI) eller NOAEL indsat i nævneren som det acceptable niveau. Hvis ”weight of evidence” processen viser, at stofferne i blandingen agerer efter den samme mekanisme (f.eks. som for organophosphat pesticiderne), så bør ”toxicity equivalency factor” (TEF) metoden bruges i stedet, hvis der foreligger anvendelige data til dette.

I denne rapport er der gennemgået ti eksempler på risikovurdering af pesticidrester fundet i udvalgte afgrøder til forbrug i Danmark. Vurderingen tager kun højde for risikoen ved at spise den aktuelle afgrøde og tager således ikke højde for den totale eksponering for pesticider fra f.eks. et helt måltid eller fra andre kilder. Risikovurderingerne er baseret på data vedr. kemisk struktur, toksikologi (inkl. toksiske effekter, målorganer og virkemåde), metabolisme og metabolitter af de enkelte stoffer. Sådanne data er præsenteret for omkring 80 stoffer, der er fundet som rester i levnedsmidler til salg i detailhandelen i Danmark. De ti eksempler på blandinger viser, at den foreslåede risikovurderingsmetode er anvendelig til brug i praksis, og at de aktuelle niveauer af pesticider i eksemplerne vurderet efter denne metode ikke forventes at udgøre en sundhedsmæssig risiko for mennesker.

Risikovurdering af pesticidblandinger er et relativt nyt område, og det er nødvendigt med yderligere forskning på området. Der er i høj grad brug for flere eksperimentelle studier af

kombinationseffekter af pesticider for at klarlægge om de modeller og udsagn, der er præsenteret i denne rapport kan underbygges af data.

1 Introduction

1.1 The objective of the project

The current practice in risk assessment of pesticide residues in food is generally based upon data from studies on single compounds. However, humans may concurrently be exposed to a number of pesticides that possesses a similar or different toxic effect. These compounds may interact causing a lower or higher toxic effect than would be expected from the single compounds. The present report summarises and evaluates the present knowledge about combined toxic effects of mixtures of pesticides (the active substances of pesticide formulations) that are published in the scientific literature. The objective is to examine whether there is a scientific basis for using a general standard formula in the risk assessment of pesticide mixtures. The selected approach is then applied to findings in Danish food. This is done in order to ascertain and/or improve the current risk assessment of pesticide mixtures humans are exposed to via food.

1.2 The limit of this project

This project primarily focuses on pesticides that are found in food available in retail shops in Denmark. The highest residue concentrations of pesticides are found in fruit and vegetables. A lot of these fruits and vegetables originate from foreign countries and these products sometimes contain residues of pesticides that are not allowed for use in Denmark.

Mixtures of pesticides are often found in groundwater and drinking water in Denmark but in concentrations normally much lower than in fruits and vegetables. However these pesticide mixtures are beyond the objective of this project.

Other chemical compounds and circumstances may have an influence on the toxic effect of pesticides, but this project is only dealing with combined action of the active substances of the selected pesticides. The influence of e.g. food additives, type of product, drugs, industrial chemicals, exposures in the working environment, and vehicles in pesticide formulations on the toxic effects of the pesticides is not taken into account in this project.

1.3 Combined actions of pesticide mixtures

Interactions between drugs administered to humans have been known for many years in the field of pharmacology. However these experiences are not directly useful for predicting toxic effects of mixtures of pesticides because the pesticide exposure levels of the general human population are relatively low compared to the doses used in therapy, and interactions occur-

ring at high doses may not be representative for low-dose exposures (Könemann and Pieters, 1996).

Humans are exposed to more than one pesticide at the same time and these may interact to give a higher or a lower toxic effect than that of the single compounds. Nevertheless a major part of the resources in toxicology research are devoted to studies on single compounds. The low level of activity in examinations of combined actions is not due to ignorance of the importance of the issue. It is rather a reflection of the difficulty, complexity, and controversy surrounding this area of research (el-Masri et al., 1997, Simmons, 1995).

Interactions between chemicals may be of a physicochemical and/or biological nature. When chemicals interact chemically a more or a less toxic product may appear. It is supposed that physicochemical interactions will normally only occur at high doses and therefore have less importance at low dose scenarios (Könemann and Pieters, 1996). Physicochemical interactions will therefore not be considered in this report.

The assessment of the toxicological properties of a pesticide mixture requires detailed information on the composition of the mixture and the mechanism of action of each of the individual compounds. In order to perform a risk assessment, proper exposure data are also needed. Most often such detailed information is not available. The mixture of pesticide residues that a person would be exposed to via the food may change over time in composition and quantity. It is not possible to test for all mixtures because there are too many combinations and furthermore a sufficient number of dose levels are not feasible. In addition, high dose levels of a pesticide mixture may have different types of effects than low dose levels and high to low dose extrapolation may be meaningless.

There is no internationally accepted procedure for the toxicological assessment of intake of multiple residues of pesticides as residues in crops, except for the few groups of pesticides sharing a group ADI e.g. those listed under dithiocarbamates. The considerations concerning the health assessment of mixtures of pesticides found as residues in crops have previously led to the following two practices used by the Danish Veterinary and Food Administration:

- Summation of the concentrations of all residues and applying the ADI for the most toxic pesticide found – this is the most restrictive assessment.
- Using the sum of the percentage of the ADI of the individual pesticides found.

However, the report from the Bichel-commitee recommended that knowledge of combined action should be taken more into account in the risk assessment of pesticides (Bichel-udvalget, 1999).

A better understanding of the combined effects between different chemicals based on this report is anticipated to improve the safety evaluations of pesticide mixtures performed by the Danish Veterinary and Food Administration and provide a more rational basis for making risk assessments on combined exposure to pesticides.

1.4 Structure of this report

The terminology for describing combined action is far from consistent in the literature. In many situations the authors of articles have stated that the mixture of concern is expected to produce an additive effect. But since there is more than one definition of additivity such a statement is ambiguous. Different conclusions may be drawn from the same data depending on the reference model used in the evaluation. Therefore the terminology that will be used in the evaluation of the toxicological studies in this report is introduced in chapter 2.

Chapter 3 is a short introduction to some models for risk assessment of various chemical mixtures that have been suggested in the literature. This is followed by a discussion of the applicability of the models in risk assessment of pesticide residues in food. In chapter 4, models are selected for use in risk assessment of residues of pesticides in food.

A relatively small number of studies on combined actions of pesticides have been published in the scientific literature. These studies are summarised and evaluated in chapter 5 based on the models and terminology introduced in chapter 2. This evaluation is done in order to test the hypothesis behind the models presented and discussed in chapter 3 and 4. In chapter 6 some examples of risk assessment of relevant pesticide mixtures found in food in Denmark are shown. The risk assessments are based on data on single compounds and these data are presented.

2 Definitions of combined action

There is some confusion regarding the concepts and terminology used to describe combined action. In the following two different models describing additivity will be presented. None of these models are perfect but if one remembers the assumptions on which each model is based, they can be used to describe combined actions.

In 1992 a group of scientists met at the Fifth International Conference on the Combined Effects of Environmental Factors. Their presentations made it clear that a consensus on concepts and terminology for combined action assessment was needed. They discussed this subject and reached an agreement, which they afterwards published (Greco et al., 1992).

The description of additivity in this chapter is primarily based on this agreement in which the authors proposed that both Loewe additivity and Bliss independence is taken into consideration as empirical reference standards. The consensus terminology is presented in table 2.1. The word additivity will in this report be used as a common term for the terms Loewe additivity, Bliss independence and inertism.

A combined effect of a mixture of two chemicals is called synergistic (e.g. Loewe synergism and Bliss synergism) when the effect is greater than predicted from the reference model. When the combined effect is less than predicted the effect is called antagonistic (e.g. Loewe antagonism and Bliss antagonism).

	Both agents effective individually – similar mechanism	Both agents effective individually – dissimilar mechanism	Only one agent effective individually	Neither agent effective individually
Synergism: combination effect greater than predicted	Loewe synergism	Bliss synergism	Synergism	Coalism
Additivity: combination effect equal to prediction from reference model	Loewe additivity	Bliss independence	Inertism	Inertism
Antagonism: combination effect less than predicted	Loewe antagonism	Bliss antagonism	Antagonism	

Table 2.1: Consensus terminology for combined actions of mixtures of two compounds. (Table modified from Greco et al., 1992).

The second and third column in table 2.1 show the two reference models described by Loewe and Bliss that can be used in cases where individual compounds in the mixture is effective (Loewe and Muischnek, 1926; Bliss, 1939). The assumptions for the two models are described later. The two columns to the right describe the situations where only one or none of the compounds in the mixture is effective alone.

For each model the term for additivity is shown in the middle, the term for a synergistic effect is shown above that, and the term for an antagonistic effect is shown in the lowest row.

Greco and co-workers states that in cases with more than two compounds in a mixture it is not fruitful to use these names (Greco et al., 1992). Therefore in this chapter simple mixtures consisting of only two compounds are taken into consideration. It is assumed that there is only one response of interest and that the dose-response relationships for both compounds are monotonic. This means that an increase in dose of one of the compounds cannot lead to a decrease in response. Finally it is assumed that the samples are big enough to ignore random variation. (NAP, 1989)

The two models have so far not been used by many scientists to interpret combined actions of pesticides within the area of toxicology.

2.1 Model for Loewe additivity and departure from additivity

The model for Loewe additivity assumes that the compounds in the mixture behave as if they are dilutions of each other (Krishnan et al., 1997, Svendsgaard and Hertzberg, 1994). This means that the compounds act on the same biological site by the same mechanism of action and differ only in their potencies.

The dose-response curves for the single compounds in a mixture are allowed to be nonparallel (on a linear-log graph). Sometimes the term “dose additivity” is used as another word for Loewe additivity but this is most often in cases in which the dose-response curves for the compounds are parallel (on a linear-log graph) (Svendsgaard and Greco, 1995).

Another name for Loewe additivity is simple similar action.

The combination index, I_c, for two compounds in a mixture is defined as:

$$I_c = \frac{d_1}{D_1} + \frac{d_2}{D_2}, \quad \text{for Loewe additivity and}$$

where d_1 and d_2 are the dose (or concentration) levels of each chemical in the mixture and D_1 and D_2 are the dose (or concentration) levels of the single compounds that produce the same level of response as produced by the mixture (Berenbaum, 1989, Svendsgaard and Hertzberg, 1994).

To evaluate a set of data from an experiment of combined effects it is necessary to have the dose-response curves for each compound in the mixture to identify D_1 and D_2. The method is as follows:

1. Find the response or effect of the mixture

2. Find the dose (or concentration) of each compound that corresponds to this effect on the dose-response curves for the single compounds – this gives D_1 and D_2.

3. Determine d_1 and d_2, which are the doses (or concentrations) used in the experiment.

4. Calculate the combination index, I_c, using the data found above.

5. Evaluate the calculated I_c for the mixture:

   When $I_c = 1$ the combined action is called Loewe additivity.
   When $I_c > 1$ the combined action is called Loewe antagonism.
   When $I_c < 1$ the combined action is called Loewe synergism.

The equation for Loewe synergism and antagonism of two compounds can be illustrated in an isobologram (iso = equal, bol = effect) as a concave-up isobole and a concave-down isobole, respectively (see figure 2.1). Each isobole is made up by points of different combinations of the two compounds where each combination gives rise to the same biological effect (Gessner, 1995, Altenburger et al., 1990).

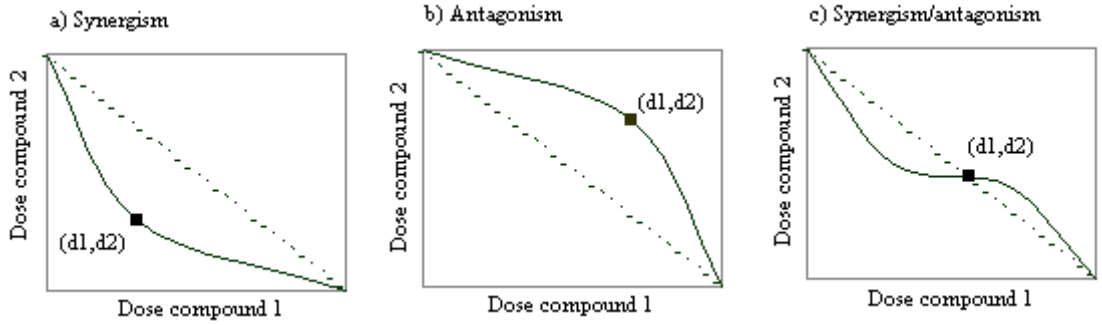


Figure 2.1: Isoboles for two compounds (1 and 2). Each isobole is made up by points of different combinations of the two compounds (d_1,d_2) where each combination gives rise to the same biological effect. The stippled line illustrates Loewe additivity. a) Shows synergism that is the doses of compound 1 and 2 cause a response smaller than expected. b) Shows antagonism that is the doses d_1 and d_2 should be higher to cause a certain response. c) Shows synergism and antagonism - and additivity in the point where the curve crosses the stippled line. (Modified from NAP, 1989)

2.1.1 Example of the use of the Loewe additivity model

In an *in vitro* study the inhibition of acetylcholinesterase by the active metabolites of chlorpyrifos and azinphos-methyl (chlorpyrifos-oxon and azinphos-methyl-oxon) were examined (Richardson et al., 2001). The article includes a huge amount of data on inhibition of acetylcholinesterase activity in serum and whole homogenised rat brains by different concentrations of the two compounds in mixture and by each compound alone.

The two compounds act by the same mechanism and they are therefore expected to match the Loewe additivity model. An example on the Loewe additivity model is the mixture of chlorpyrifos-oxon and azinphos-methyl-oxon in the mixture with I10 + I70 which means at the concentration of the two compounds that gives an inhibition of the acethylcholinesterase activity of 10 and 70 % respectively. This mixture was reported to inhibit the acetylcholinesterase activity with 74.13 %.

The concentration-effect curves made from the data in the article are shown in figure 2.2. D_1 and D_2, the concentration of each compound that corresponds to the effect of the mixture (74.13 %) are found from the concentration-effect curves. D_1 and D_2 are reported to be 5.38 nM and 81.08 nM for chlorpyrifos-oxon and azinphos-methyl-oxon respectively. The concentrations of the single compounds to give the actual inhibition (I10 + I70) are reported to be d_1 = 0.49 nM and d_2 = 73.35 nM. Now the combination index, I_c can be calculated:

$$I_c = \frac{d_1}{D_1} + \frac{d_2}{D_2} = \frac{0.49}{5.38} + \frac{73.35}{81.08} \cong 0.996$$

This is very close to 1, which means Loewe additivity.

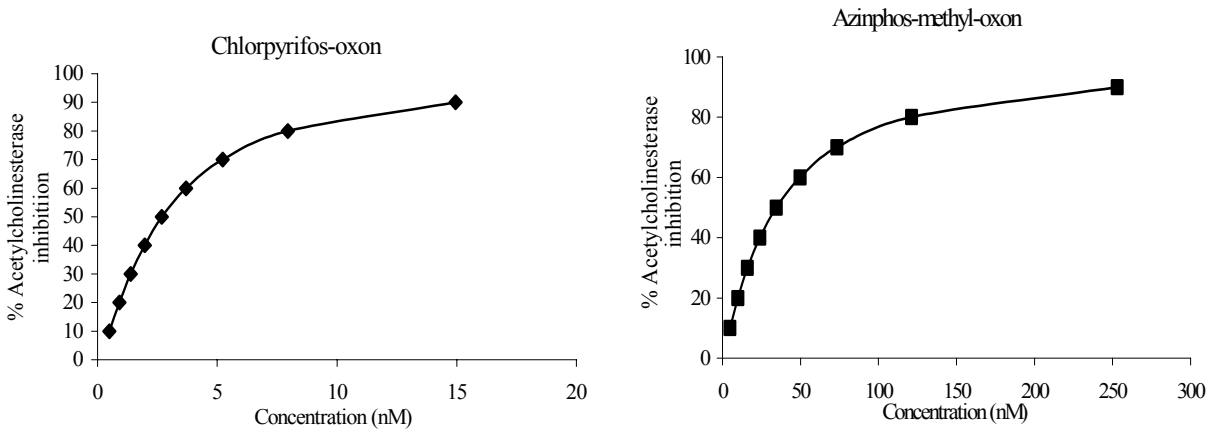


Figure 2.2: Concentration-effect curves for inhibition of acetylcholinesterase activity in brain in vitro by chlorpyrifos-oxon and azinphos-methyl-oxon. (Data from Richardson et al., 2001).

In the article they calculate the expected % inhibition of acetylcholinesterase activity by converting the concentration of one compound to the toxicologically equivalent concentration of

the other compound. In this specific case their model gives the same conclusion as the Loewe additivity model.

2.2 Bliss independence and departure from independence

The theoretical basis for the Bliss independence model is probabilistic independence. This means that the two compounds in the mixture do not interfere with each other but they both contribute to a common result. The model assumes that the compounds in the mixture do not act by the same mechanism. The nature and site of action may also differ among the compounds but this is not always the case. Greco et al. point out that Bliss independence cannot be defended for compounds that do not demonstrate a maximum effect (Greco et al., 1992).

Other names for Bliss independence are simple dissimilar action and response additivity. The latter term is often used in cancer risk assessment of chemical mixtures (Seed et al., 1995, Teuschler and Hertzberg, 1995) and in cases in which the responses are small (Svendsgaard and Greco, 1995).

To use the Bliss independence model for evaluating a set of data from an experiment it is necessary to know the effect of each compound alone and of the mixture and to know the background response. A further condition is that the compounds must demonstrate a maximum effect because this maximum level is used to establish the fractions of total possible effect produced by a particular dose of the single compounds and of the mixture.

In the case of no background effect the fraction of total possible effect for the mixture expected from the model of Bliss independence, $f_{expected}$, is (Bliss, 1939, NAP, 1989, Könemann, 1996):

$$\begin{aligned} f_{expected} &= f(d_1,0) + f(0,d_2) \times [1 - f(d_1,0)] \\ &= f(d_1,0) + f(0,d_2) - f(d_1,0) \times f(0,d_2), \end{aligned} \qquad (1)$$

where $f(d_1,0)$ and $f(0,d_2)$ is the fractions of total possible effect of single compound exposure to the two compounds 1 and 2 at doses d_1 and d_2 respectively. Bliss independence occurs if the measured effect of the mixture (stated as a fraction of total possible effect) equals $f_{expected}$.

If compound 1 has a fraction of total possible effect of $f(d_1,0)$ then compound 2 can act only on the remaining fraction $1- f(d_1,0)$ assuming that the maximum fraction of total possible effect is 1 (Svendsgaard and Hertzberg, 1994). Then the fraction of total possible effect of compound 2 will be $f(0,d_2) \times [1- f(d_1,0)]$ and the expected fraction of total possible effect for the mixture will be as shown in equation (1).

With a background effect, f(0,0), the above equation assuming Bliss independence can be rewritten as follows (NAP, 1989):

$$f_{expected,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2), \qquad (2)$$

where $f_b(d_1,0)$ and $f_b(0,d_2)$ are defined:

$$f_b(d_1,0) = \frac{f(d_1,0) - f(0,0)}{1 - f(0,0)} \qquad (3)$$

$$f_b(0,d_2) = \frac{f(0,d_2) - f(0,0)}{1 - f(0,0)} \qquad (4)$$

If the background is zero, the factor f(0,0) drops out, and $f_{expected,\,b}$ becomes equal to $f_{expected}$ for all d_1 and d_2.

To evaluate the combined effect of a mixture of two compounds the measured effect in the study (expressed as fraction of total possible effect) have to be adjusted for the background effect in the same way as the effect of the single compounds (NAP, 1989):

$$f_b(d_1,d_2) = \frac{f(d_1,d_2) - f(0,0)}{1 - f(0,0)} \qquad (5)$$

The way to use the Bliss independence model is as follows:

1. Calculate the effect of the mixture (expressed as a fraction of total possible effect) allowing for background using equation (5). This is the actual measured effect in the study.

2. Calculate $f_b(d_1,0)$ and $f_b(0,d_2)$ using equation (3) and (4).

3. Use equation (2) to calculate the expected fraction $f_{expected,b}$ assuming Bliss independence.

4. Compare $f_b(d_1,d_2)$ and $f_{expected,b}$:

   When $f_b(d_1,d_2) = f_{expected,b}$ then the combined action is called Bliss independence
   When $f_b(d_1,d_2) < f_{expected,b}$ then the combined action is called Bliss antagonism
   When $f_b(d_1,d_2) > f_{expected,b}$ then the combined action is called Bliss synergism

2.2.1 Example of the use of the Bliss independence model

Wu et al. (1996) examined the influence of cimetidine on the inhibition of acetylcholinesterase activity by diazinon in rat brain 3 hours after dosing. The two compounds act by different mechanisms and the Bliss independence model is therefore used to evaluate the data. Brain acetylcholinesterase activity in the control group is reported to be 7.09 µmole/min/g tissue, corresponding to 100% - or an inhibition of 0 %. The activity in rats treated with cimetidine (80 mg/kg, i.p.) or diazinon (50 mg/kg, i.p.) was reported to be 6.83 and 6.09 µmole/min/g tissue, corresponding to 4 % and 14 % inhibition respectively. The mixture of

cimetidine and diazinon (80 + 50 mg/kg, i.p.) was reported to inhibit the acetylcholinesterase activity to 5.23 µmole/min/g tissue, corresponding to 26 % inhibition compared with the control group.

In this example the background value is zero. The expected fraction of total possible effect for the mixture from the model of Bliss independence is calculated from equation 1:

$$f_{expected} = f(d_1,0) + f(0,d_2) - f(d_1,0) \times f(0,d_2)$$
$$= 0.04 + 0.14 - 0.04 \times 0.14 = 0.17 \qquad \text{(that is 17 \%)}$$

This is less than the measured inhibition in the study (26 %). Therefore, a weak Bliss synergism is indicated.

2.3 Mixtures containing no or only one active compound

The last two columns in table 2.1 show the terms used in situations where only one or neither of the compounds is effective at the actual dose (or concentration) when given or added alone. The leading adjectives are omitted in these cases since the term inertism can be understood as a special case of both Loewe additivity and Bliss independence, synergism as a special case of both Loewe and Bliss synergism, and antagonism as a special case of both Loewe and Bliss antagonism.

In cases where only one compound is observed to have an effect on the examined endpoint, the combined action can be evaluated by looking at the data. If the effect of the mixture is equal to that of the active compound, the combined action is called inertism. If the effect of the mixture is greater or lower than that of the active compound, it is called synergism or antagonism respectively.

If neither of the compounds is effective when given alone, the terms inertism and coalism are used. Inertism is used when none of the compounds is effective alone and neither the mixture. If the single compounds do not have an effect on the examined endpoint at the actual dose (or concentration) but the mixture has an effect, then the combined action is called coalism.

3 Methods for risk assessment of mixtures

Various approaches have been suggested in the scientific literature for use in the evaluation of the health risks from exposure to mixtures of chemicals but there is no internationally accepted procedure. The most important approaches are summarised in this chapter. This is followed by a discussion of the advantages and disadvantages of methods for use in risk assessment of pesticide residues in food.

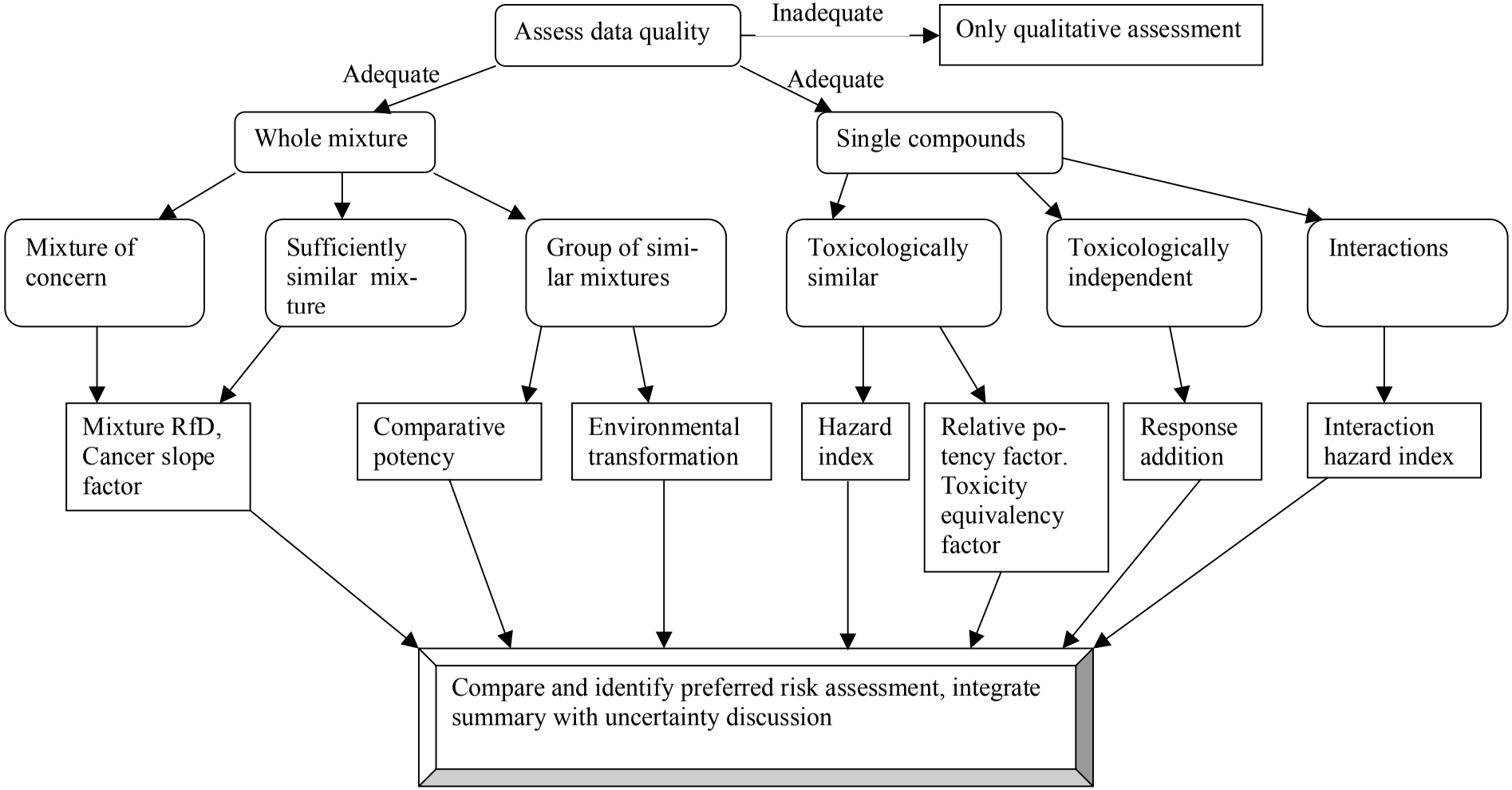
In 1986 The Environmental Protection Agency in USA (U.S. EPA) recommended three approaches for health risk assessment of chemical mixtures (U.S. EPA, 1986, Mumtaz, 1995): 1) the mixture of concern approach, 2) the similar mixture approach and 3) the component-based method.

When making a risk assessment of exposure to a mixture, the most appropriate method should be used. Which of the methods that is considered appropriate depends on the toxic effect, the available data on toxicity of the mixture or similar mixtures, the predicted interactions among the compounds in the mixture and on the quality of the exposure data. However the U.S. EPA points out that it is ideal to conduct all three assessments when possible in order to make the best risk assessment and to use all the available data – in particular the incorporation of interaction data when available. The uncertainties for the risk assessment should be clearly discussed and the overall quality of the risk assessment should be characterised (U. S. EPA, 1986).

The guidance was supplemented in 2000 (U.S. EPA, 2000b). The flow chart for the different types of mixture assessments is shown in figure 3.1 and is described below. In this new guidance there are three methods for whole mixture assessment and four compound-based methods.

First step in the procedure is to assess the quality of the data. When the data are adequate for an assessment, it should be decided whether there are data available for an assessment on the whole mixture or only on the components. The whole mixture based methods are only described briefly in the present report since data for these methods are rarely available.

Figure 3.1: Flow chart of the risk assessment approach used by U.S. EPA (figure modified from U.S. EPA, 2000b).



3.1 Data available on whole mixtures

U.S. EPA describes three kinds of assessments on whole mixtures (U.S. EPA, 2000b). The assessment can be done on 1) the mixture of concern, 2) a sufficiently similar mixture or 3) on a group of similar mixtures:

1) The mixture of concern approach requires that the toxicity data are available on the specific mixture of concern. These data have to be adequate for deriving the dose-response toxicity in order to estimate a reference dose (RfD) or a cancer slope factor (in studies on cancer risk). A cancer slope factor is defined as a "plausible upper-bound estimate of the probability of a response per unit intake of a chemical over a lifetime. Usually the cancer slope factor is the upper 95th % confidence limit of the dose-response curve." (RAGS, 2001).

   The mixture of concern approach is the preferred approach as it is the most direct and the simplest method and it entails the fewest uncertainties.

2) A mixture is sufficiently similar when the compounds in the mixture are almost the same as in the mixture of concern and when the compounds are present in almost the same proportions. The method assumes that the composition of the sufficiently similar mixture is functionally the same as that of the mixture of concern. In addition the data should be adequate to account for the actual endpoints. The strategy is then to estimate the dose-response toxicity value in order to estimate either a cancer slope factor or a reference dose (RfD).

3) When data are available on a group of similar mixtures either the comparative potency factor or the geographic site-specific approach can be used. A group of similar mixtures could be mixtures of the same compounds but in slightly different ratios. It could also be a mixture consisting of the same compounds as the mixture one wants to evaluate but lacking one or more compounds or having one or more additional compounds. Examples of a group of similar mixtures are mixtures that are generated by the same commercial process or emission source but differ slightly in composition or ratios.

   The comparative potency method is data intensive and it involves some statistical modelling and toxicological judgement. Short-term data on several similar mixtures including the mixture of concern are used in this method. The strategy of the method is to estimate a dose-response value using the data on similar mixtures and similar assays to extrapolate to the mixture of concern. (U.S. EPA, 2000b) The method has been used for mixtures formed by combustion of for instance diesel (Albert et al., 1983, Lewtas, 1985, Lewtas et al., 1983, Schoeny et al., 1989).

   The other method to be used when data on a group of similar mixtures are available is the geographic site-specific assessment. The method requires intensive data material on both toxicity and exposure of the compounds in the mixture. A range of toxicity values are estimated based on toxicity data on the mixtures. These are then adjusted

for alterations in the mixture's composition caused by environmental factors to produce a risk estimate for the total mixture. This method has been used for cancer assessment of PCB's but it is complicated to use (U.S. EPA, 2000b).

3.2 Data available on single compounds in the mixture

As seen in figure 3.1 U.S. EPA has set up four different methods based on data on single compounds. These methods approximate the toxicity of mixtures that have not been experimentally tested. The component-based methods are used more often than the whole mixture methods, since one rarely have the needed data on the mixture of concern or on a similar mixture. In another document U.S. EPA has suggested a fifth method (the margin of exposure, MOE) that also will be described in this section (U.S. EPA, 2000a).

When the compounds in the mixture are toxicologically similar (i.e. cause the same critical effect, act on the same molecular target issue, or act by the same mechanism), one could use the hazard index, the relative potency factor method (or the special type of the relative potency factor method named the toxicity equivalency factor) or the margin of exposure – methods that are based on Loewe additivity. These methods differ by the required data on toxicological processes but in all cases the exposure levels are added after having been multiplied by a scaling factor that accounts for differences in the toxicological potency. For compounds that act similarly the U.S. EPA has performed guidance for risk assessment of mixtures consisting of pesticides that have a common mechanism (U.S. EPA, 2000a). The whole risk assessment will be described in section 3.2.1 followed by a more detailed description of the methods for dose response assessment.

When the compounds act independently, one can use response addition, and when the compounds interact, one can use the interaction hazard index. These methods will be described in section 3.2.2 and 3.2.3.

3.2.1 Toxicologically similar compounds in mixtures / common mechanisms of toxicity

EPA has proposed "guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity" (U.S. EPA, 2000a). Chemicals act via a common mechanism of toxicity if they cause the same critical effect, act on the same molecular target issue or act by the same biochemical mechanism of action or share a common toxic intermediate (Botham et al., 1999) i.e. the term common mechanism is another word for "toxicologically similar". The risk assessment method for mixtures that consist of compounds that are toxicologically similar suggested by U.S. EPA will be described in the beginning of this section.

Whether the combined toxicity of a mixture is greater than, equal to or less than the toxicity caused by one of the compounds alone depends on several factors. Pharmacokinetics/-dynamics of each compound causing the common toxic effect and the interactions that may take place between the compounds of course has an impact on the extent of the toxicity. The

patterns of exposure and the duration of the common toxic effect are other factors, one should consider.

There are four steps in the general process of risk assessment for mixtures: 1) the hazard assessment and characterisation, 2) dose response assessment and characterisation, 3) exposure assessment and characterisation and 4) risk characterisation. These steps will be described in the following (U.S. EPA, 2000a).

1) The hazard assessment and characterisation step identifies the potential health effects that the pesticide can cause. First one has to identify the chemicals that have a common mechanism of toxicity. U.S. EPA has described a procedure for that in "Guidance for identifying pesticide chemicals and other substances that have a common mechanism of toxicity" (U.S. EPA, 1999). Second the conditions for expression of the risk via route, use pattern, and duration of exposure should be described. A specific toxicity endpoint for a certain exposure duration (e.g., acute, chronic) shared by each chemical in the mixture should be specified. U.S. EPA uses the weight of the evidence approach to evaluate and characterise the toxicity endpoints of concern and for evaluation of risk to the human population. If one has observed clear species (strain or sex) differences, data from the most sensitive test animal should be used.

2) The dose response assessment and characterisation step determines the health effects that occur at different levels of exposure. A uniform point of departure must be selected, normalised, and adjusted. Then a method for combining common toxicity must be selected. U.S. EPA considers Loewe additivity to be an appropriate approach of risk assessment of mixtures because it assumes that the chemicals act on similar biological systems and draw out a common response. Both the margin of exposure (MOE) method and the relative potency factor (RPF) method can be used to evaluate the toxicity of a mixture assuming that the compounds act additively. The margin of exposure is calculated by dividing the point of departure (POD) by the measured or estimated exposure from a given route. The point of departure on each compound's dose-response curve can be determined as the toxic potency of the compound relative to the other compounds. In the relative potency factor method the potency of each compound is expressed in relation to the potency of an index chemical. These methods will be described in more details below.

3) The exposure assessment and characterisation step express how much of the pesticide humans are exposed to via different exposure routes. First the routes of exposure (food, drinking water, and various non-agricultural uses) are identified and then the frequencies, durations, and magnitude of exposures are determined. The co-occurrences should be found and highly exposed sub-populations must be identified. Based on these data realistic exposure scenarios must be developed.

4) In the risk characterisation step the risk of health effects that could result from exposure to the pesticides are identified. The exposure should be matched with the relevant toxicological values in terms of route and duration. Then the cumulative risk of each individual compound on a daily basis should be calculated by maintaining appropriate spatial, temporal and demographic characteristics of data. U.S. EPA uses Monte Carlo analysis to

make an iterative process of multiplication of residue concentrations in foods by one-day consumption of these foods. The results are characterised and interpreted. The major chemical contributors to risk, the exposure scenarios of concern and the sensitive sub-populations are identified and it is discussed how well the data support the conclusions. Finally the uncertainties and the uses of assumptions are identified.

The groups of pesticides that have been examined for common mechanisms by various scientists, are organic phosphorus, chlorinated hydrocarbon and carbamate pesticides:

Mileson and co-workers tested the hypothesis that organic phosphorus pesticides act by a common mechanism of toxicity, that is, by inhibiting acetylcholinesterase activity by phosphorylation and thereby accumulating acetylcholine in the nervous system (Mileson et al., 1998). They proposed six alternative hypotheses and evaluated these based on available data in the literature. They rejected all six alternative hypotheses and accepted the original hypothesis and concluded: "OP pesticides act by a common mechanism if they inhibit acetylcholinesterase activity by phosphorylation and elicit any spectrum of cholinergic effects".

The Office of Pesticide Programs in the U.S. EPA has evaluated data on the chloroacetanilide pesticides acetochlor, alachlor, butachlor, metolachlor and propachlor in order to evaluate whether these compounds share a common mechanism (U.S. EPA, 2001a). They used the weight of evidence approach for this evaluation and the conclusion was that acetochlor, alachlor and butachlor can be grouped based on a common mechanism of toxicity for nasal turbinate tumours.

The weight of evidence were found to support the grouping of the dithiocarbamates mancozeb, maneb, metiram, ziram and thiram based on a common mechanism of toxicity for distal peripheral neuropathy (U.S. EPA, 2001b). The other three dithiocarbamates (ferbam, Na-dimethyldithiocarbamate and metam sodium) in the candidate group could not be included in this group.

Seven thiocarbamates was found to produce a common toxic effect namely neuropathy but the specific mechanism has not been established (U.S. EPA, 2001c). However the U.S. EPA concluded that the thiocarbamates could be grouped based on the common toxicity supported by similarity in structure and metabolism.

3.2.1.1 Hazard index

The hazard index (HI) method is based on the same assumptions as the Loewe model i.e. that the compounds in the mixture behave as if they are dilutions of each other (as described in chapter 2). The compounds in the mixture act on the same biological site by the same mechanism of action and differ only in their potencies. In the hazard index the Loewe model is expanded to account for more than two compounds.

In the hazard index approach the doses are standardised by using health-based values such as the acceptable daily intake (ADI). The hazard index can be calculated by the following equation:

$$HI = \frac{E_1}{AL_1} + \frac{E_2}{AL_2} + ... + \frac{E_n}{AL_n} = \sum_{i=1}^{n} \frac{E_i}{AL_i},$$

where E_1, E_2, E_n, and E_i are the levels of exposure of each compound in the mixture and AL_1, AL_2, AL_n, and AL_i is the maximum acceptable level for each compound. The "acceptable level" is often a regulatory goal for exposure to the i^{th} compound e.g. acceptable daily intake (ADI), reference dose (RfD) or margin of safety (MOS). Since this method is based on an assumption of additivity it can lead to errors if a synergistic or antagonistic action occurs.

If the hazard index exceeds 1, the mixture has exceeded the maximum acceptable level (e.g. ADI or RfD) and there might thus be a risk. However Seed and co-workers point out that an uncertainty factor between 1 and 100 should be taken into account in deciding whether a hazard index represents a risk (Seed et al., 1995). The uncertainty factor should reflect the confidence in the information that is available on the interactions between the single compounds. It should also reflect the concentration of or the number of the compounds in the mixture since a synergistic response is more likely to occur at high doses or when the number of compounds in the mixture is high (NRC, 1989).

The hazard index makes most sense when it is specific to a single toxic effect. Therefore the U.S. EPA recommends a separate hazard index for each toxic effect of interest (U.S. EPA, 2000b, Hertzberg et al., 1999).

The hazard index is specifically recommended for mixtures of compounds that are toxicologically similar and all have dose-response data. However the U.S. EPA notices that in practice the method is used on mixtures of compounds that has the same target organ because of the lack of information of mechanisms and pharmacokinetics (U.S. EPA, 2000b).

3.2.1.2 Relative Potency Factor and Toxicity Equivalency Factor approach

In the relative potency factor (RPF) method it is assumed that the relative potencies of chemical mixtures are the same in various bioassays used for toxicity testing of certain endpoints (Mumtaz, 1995). Short-term assays of complex mixtures are used instead of long-term *in vivo* assays when the latter are not available. The strategy is to scale the dose of all compounds in the mixture relative to the potency of an index compound and then add the scaled doses. The method is based on Loewe additivity, nevertheless the relative potency factors are supposed to account for data on mixtures with different modes of action. The relative potency factors should also account for conflicting or missing data (U.S. EPA, 2000b).

The toxicity equivalency factor (TEF) is a special case of relative potency factor. It is also used to estimate the toxicity of complex mixtures of toxicologically or structurally related compounds when insufficient *in vivo* toxicity data are available. A compound in the mixture is

chosen as an index compound. The doses of the compounds in the mixture are then normalised according to potency to the same scale and afterwards summed. To evaluate a set of data of combined effects it is necessary to have the dose-response curve for the index compound and to know the effect of the other compounds in the mixture (Seed et al., 1995, U.S. EPA, 2000b).

In the toxicity equivalency factor method it is assumed that the compounds in the mixture act by the same biologic or toxic pathway (Safe, 1998) i.e. the same assumption as for Loewe additivity. This is the most important assumption that has to be fulfilled to make sure that it would not make a difference whether another index compound were chosen. Other assumptions are that the effects of each compound in the mixture are essentially additive at submaximal levels of exposure and that the dose-response curves are parallel (Safe, 1998).

The toxicity equivalency factor approach requires a larger set of data than necessary for most relative potency factors. Therefore the term toxicity equivalency should not be used for the general case (U.S. EPA, 2000b).

The toxicity equivalent (TEQ) is calculated by multiplying the concentration of each compound (C_i) in a mixture by the relative potency of the individual compounds in the mixture (TEF_i):

$$TEQ = \sum C_i \times TEF_i$$

The resulting TEQ is assumed to be an equivalent dose of the index compound and it can therefore be compared to the RfD of the index compound (Botham et al., 1999). If the TEQ is greater than the RfD, the mixture may constitute a risk.

This approach was initially developed to estimate the potential toxicity of mixtures of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. The National Research Council Committee on Pesticides also used it to estimate the risk for infants and children due to exposure of pesticides in the diet (Botham et al., 1999). Safe (1998) has suggested using it for estimating total intakes of potential dietary and environmental estrogens.

The UK Pesticide Safety directorate has decided to use the toxicity equivalency approach for assessment of combined risk from exposure to mixtures of acetylcholinesterase inhibitors (organophosphorus compounds and carbamates) (PSD, 1999). Despite clear differences in the action of carbamates and organophosphorus compounds, the index compounds selected for all acetylcholinesterase inhibitors were either aldicarb (carbamate) or chlorpyrifos (OP).

The method is complicated to use and requires some statistical modelling and judgement of the toxicity equivalency factor or relative potency factor.

3.2.1.3 Margin of exposure

The margin of exposure (MOE) for a single compound is calculated as the ratio of the point of departure (POD) to the measured or estimated exposure from a given route.

$$MOE = \frac{POD}{Exposure}$$

The POD is defined as "a point of estimate of the dose or exposure level that is used to depart from the observed range of empirical response (or incidence) data for purpose of extrapolation risk to the human population" (U.S. EPA, 2000a). The POD is preferably the dose corresponding to a given effect level e.g. ED_{10}. The margin of exposure approach is often used to determine the acceptability of acute risks for single chemicals. MOEs of >10 or >100 are usually considered acceptable when derived from toxicological data from human and animal studies. These levels are chosen since they are numerically the same as the typical uncertainty factors that are used in calculating e.g. a reference dose from NOAEL (Wilkinson et al., 2000).

The combined margin of exposure (MOE_T) is the reciprocal of the sum of the reciprocal of MOEs of each compound in the mixture (Wilkinson et al., 2000):

$$MOE_T = \frac{1}{1/MOE_I + 1/MOE_{II} + 1/MOE_n}$$

A MOE_T higher than 100 is usually considered acceptable when derived from toxicological data from animal.

Using the no observed adverse effect level (NOAEL) for the reference compound, the toxicity equivalent (TEQ) can also be used to derive the MOE:

$$MOE = NOAEL - TEQ$$

(Botham et al., 1999).

3.2.2 Toxicologically independent compounds in mixtures

3.2.2.1 Response addition

The method called response addition by U.S. EPA is the same as the Bliss independence model described in chapter 2. The assumptions for the method are also described in that chapter. The method is easy to use if the right data are available.

For mixtures with more than two compounds equation (1) and (2) in chapter 2 can be extended (U.S. EPA, 2000b). The fraction of total possible effect for a mixture expected from

Bliss independence model without a background ($f_{expected}$) and with a background f(0,…,0) ($f_{expected, b}$) is defined:

$$f_{expected} = 1 - (1 - f(d_1, 0, ..., 0)... \times ...(1 - f(0, ...0, d_n)$$

and

$$f_{expected,b} = 1 - (1 - f_b(d_1, 0, ..., 0)... \times ...(1 - f_b(0, ...0, d_n)$$

3.2.3 Interactions

The interaction-based hazard index uses the weight of evidence approach as a quantitative modifier to the hazard index in risk assessments involving interactions of multiple compounds (U.S. EPA, 2000b, Mumtaz et al., 1992, Mumtaz et al., 1998). It assumes that binary interactions are the most important and information on binary interactions is used to modify risk assessments for chemical mixtures. It is also assumed that compounds in a mixture act by similar mechanisms (U.S. EPA, 2000b).

In the first steps of the interaction-based hazard index approach the mechanistic understanding and the toxicological significance is connected. This forms the basis of the risk assessment. Thereafter the binary mixtures are grouped in three modifying categories used to alter the rating of the risk assessment. The three modifying categories are duration/sequence of exposure, *in vivo*/*in vitro* and route of exposure.

This classification is used to set up a quantitative interaction matrix by the aid of a set of default weighting factors and a lot of calculations. The calculations include the hazard index and interaction factors for each binary mixture. The normalised site-specific weight of evidence is calculated and used to adjust the hazard index for the uncertainty of interactions. And finally the adjusted hazard index can be evaluated.

As indicated above this method is very complicated and it requires a great deal of calculations and assumptions on the interactions of the compounds.

3.2.3.1 Physiologically based toxicokinetic / toxicodynamic

Exposure to multiple chemicals may cause alterations in the toxicokinetics and/or toxicodynamics of the individual chemicals resulting in a change in the toxicity predicted based on the summation of the effects of the compounds (Krishnan et al., 1994). Toxicokinetic interactions occur as a result of one compound altering the metabolism, disposition and elimination of other compounds. They may affect the relationship between administered dose and the dose delivered to the target site. The processes are absorption, distribution, metabolism, and excretion of the compounds. Toxicodynamic interactions include interactions that affect a tissues response or susceptibility to toxic injury but they do not directly affect the metabolism or disposition of the toxic compound (U.S. EPA, 2000b). A large number of processes that determine the mechanisms of action of a compound may be involved. These processes are for in-

stance inhibition of cellular enzymes and damage through binding to proteins or DNA (Larsen et al., in preparation).

In a toxicokinetic model the processes of absorption, distribution, metabolism and excretion of chemicals are described mathematically. In this kind of modelling the whole organism is considered as a single homogeneous compartment or as a multi-compartmental system with elimination occurring in specific compartments of the model. In a physiologically based toxicokinetic model the rat or man is described as a set of tissue compartments. If a direct relationship exists between the concentration of the active metabolite (or parent compound) and the toxic effect the model can be extended to include the toxicodynamic phase (Larsen et al., in preparation).

3.3 Discussion of the methods

3.3.1 Whole mixture approaches

The mixture of concern approach is the most preferred approach but even this method does not guarantee an accurate risk assessment. One of the major problems is (as for the health assessment of individual) high to low dose extrapolation. Another important matter is that in the risk assessment it is necessary to extrapolate between different bioassays i.e. different sensitivities in detecting various toxicity end points (Hertzberg et al., 1999).

The uncertainty in the similar mixture approach lays (in addition to the circumstances mentioned above) in the judgement of the similarity between mixtures. The judgement has to be done on a case-by-case basis and the considerations should imply the uncertainties associated with using data on a dissimilar mixture as well as the uncertainties of using other approaches, e.g. one of the single compound approaches (Hertzberg et al., 1999).

The comparative potency method is developed to estimate long-term cancer risk using information from short-term cancer bioassays and *in vitro* mutagenicity assays as a surrogate for chronic *in vivo* assays. This means that it involves extrapolation across mixtures and across assays. Further it assumes that the ratio of toxic potencies between any two mixtures is the same. These factors in addition to the circumstances mentioned for the mixture of concern approach makes the uncertainties (Hertzberg et al., 1999).

The whole mixture approaches would make the best risk assessment of pesticide residues in food compared to single compound approaches. However they are not very applicable since they require a lot of data, which is rarely available.

3.3.2 Single compound approaches

3.3.2.1 Toxicologically similar compounds

In this review, three approaches have been presented to evaluate the consequences on human health of simultaneous exposure to two or more chemicals that act by a similar/common mechanism of action but have different potencies and exposure characteristics: *the hazard index method, the relative potency factor/the toxicity equivalency factor and the margin of exposure method*. They are all based on Loewe addition and in all methods the exposure levels are added after being multiplied by a scaling factor that accounts for differences in toxicological potency. But the methods differ in the required data on toxicological processes. These approaches have recently been critically evaluated by Wilkinson and co-workers due to the renewed interest activated by the U.S. Food Quality Protection Act (FQPA) that demands the U.S. EPA to consider the combined effects of pesticides and other chemicals that act by a similar mechanism (Wilkinson et al., 2000). The required data, applicability, assumptions, advantages and disadvantages of the methods are summarised in table 3.1 at the end of this chapter.

The advantages of the *hazard index method* are that it is transparent and understandable. It often relates directly to what is called a "long-used and well-understood measure of acceptable risk" namely the reference dose, RfD. This circumstance is according to Wilkinson and co-workers on the other hand also a disadvantage. They state that RfD is not appropriate to use as a point of departure because it is obtained by using an uncertainty factor (which is subjective) and it is therefore not a true measure of relative toxicological potency. Using the NOAEL instead of RfD in the denominator eliminates this disadvantage because it makes the calculation more transparent since then no uncertainty factor is included (Wilkinson et al., 2000).

The uncertainty factors included in the RfD as well as in the ADI for pesticides have been established by a group of scientists within FAO/WHO after evaluation of the present data on each compound including uncertainties and lack of data in the available data package. Since an uncertainty factor has to be included in the calculation of the hazard index anyhow, the use of the ADI's (where individual safety factors are included) as the maximum acceptable level, AL_n, instead of NOAEL's (where the safety factors are not included) form a better well-digested evaluation.

U.S. EPA notice that although the hazard index is based on an assumption of similar mechanism, in practice the method is used on mixtures of compounds that has the same target organ because of the lack of information of mechanisms and toxicokinetics (U.S. EPA, 2000b).

The *relative potency factor method (RPF)* and *the toxicity equivalence factor method (TEF)* rely on the availability of dose-response data for at least one compound in the mixture (the index compound). They also rely on the scientific judgement as to the toxicity of the other individual compounds in the mixture and of the whole mixture. The toxicity equivalence factor method requires a strong degree of toxicologically similarity and it is applied to all endpoints. This implies that the method is only applicable for a few mixtures. The relative po-

tency factor requires toxicologically similarity for specific conditions i.e. endpoint, route of exposure and duration. The two methods are very uncertain since they may not account for the departures from the assumptions. Both methods are complicated to use and require some statistical modelling and judgement of the toxicity equivalence factors or relative potency factors.

The relative potency factor method and the toxicity equivalence factor method have the advantages that they are transparent and understandable. They are based on summation of terms that relate directly to real exposure and toxicity data. One of the disadvantages is that they rely too much on the quality of the toxicology database of the index pesticide since the doses of the compounds are normalised according to potency of the index compound. There are no specific guidance criteria available for the selection of the index compound. U.S. EPA has suggested that the index compound should be the compound in the mixture that is the best studied and has the largest body of scientific data of acceptable quality (U.S. EPA, 2000b). The methods can be criticised for using data on well-studied compounds to improve the acceptability of compounds that have poor toxicological databases.

The judgement of the scientific data for use in the relative potency factor method should be assembled and evaluated by a cross-disciplinary group of scientists. These people should have an expertise of the given chemical group or an understanding of the relevance of the various toxicological assays to human health risks (U.S. EPA, 2000b).

The advantage of *the margin of exposure for mixtures (MOE_T)* is that it is derived from data that are directly related to the actual exposure and toxicity data of each member of the mixture. Further it requires a single consideration of uncertainty at the end of the process. However, this last consideration has to be done with care. There are no criteria for defining the magnitude of an acceptable MOE_T. Usually the MOE_T is assumed to be acceptable when it is above 100 since this is numerically the same as the typical uncertainty factor that is used in calculating e.g. a reference dose from NOAEL. However, if the toxicological data for the compounds differ in quantity and quality, such an assumption will not be appropriate. Wilkinson et al. suggest, that it could be necessary to make a stepwise reduction of the acceptable MOE_T as the size of the group increases (Wilkinson et al., 2000).

In *the hazard index* and *the toxicity equivalency factor/relative potency factor* the doses of the compounds in the mixture are normalised to a common scale and then summed (Seed et al., 1995). Botham and co-workers claim that if the database is sufficiently rich the toxicity equivalency factor approach improves on the hazard index approach since it provides a better basis for standardizing toxic dose metrics for the compounds in the mixture (Botham et al., 1999). On the other hand they say that if the uncertainties are too broad the toxicity equivalency factor approach will provide only a little increased advantage over that of the hazard index.

As mentioned earlier the U.S. EPA recommends using the hazard index method when the compounds in the mixture act on the same target organ (but not necessarily by the same mechanism). As a consequence of this, the hazard index is more applicable than the relative potency factor method, which assumes similarity of mechanism (U.S. EPA, 2000b).

The *relative potency factor* method is more dependent on the assumption of parallel dose-response curves than the margin of exposure method (EPA, 2000a).

As mentioned earlier, Wilkinson and co-workers have evaluated the approaches to determine the cumulative effects of chemicals with a common mechanism of toxicity. They conclude that the "serious difficulties associated with defining "common mechanism of toxicity" and "concurrent exposure" combined with the current paucity of data and methodology required to conduct cumulative risk assessment suggest that the procedure is not yet ready for use in pesticide regulation" (Wilkinson et al., 2000).

3.3.2.2 Toxicologically independent mixtures

Only one method for risk assessment of toxicologically independent mixtures is presented namely *the response addition method*. The required data, applicability, assumptions, advantages and disadvantages of the method are summarised in table 3.1 at the end of this chapter. To use this method it is necessary to know the fraction of total possible effect of each of the compounds in the mixture and of the mixture itself. Mathematically the method is easy to use. Unfortunately the applicability is low since such data are seldom available.

3.3.2.3 Interaction hazard index

U.S. EPA has suggested the interaction hazard index approach for mixtures consisting of interacting compounds. The required data, applicability, assumptions, advantages and disadvantages of the method are summarised in table 3.1 at the end of this chapter. There are four important features in the interaction hazard index approach (Seed et al., 1995). Firstly the interaction mechanism should be well understood. Secondly the data from other related compounds should be consistent with the proposed mechanism. Thirdly the toxicological significance of this interaction should be able to be demonstrated, and fourthly the *in vivo* data of the interaction should be available from long-term studies and relevant route of exposure.

U.S. EPA has pointed out some very important weaknesses of the interaction hazard index approach (U.S. EPA, 2000b): There is no guidance for selection of the uncertainty factors for interactions used in the method and the steps in determining the binary weight of evidence are complex. The weighting factors used in the method lack support from empirical assessments of key experimental variables. Further the interaction hazard index approach is supposed to account for (pair wise) interactions, but the method may be too simple in that the interaction information is only represented by the uncertainty factor, which is multiplied with the entire additive hazard index. The magnitude of the interaction is not included in the method.

The fact that a qualitative/subjective evaluation of data is used as the basis for quantitative modelling makes this model less applicable.

Advantages of physiologically based models are that they allow extrapolation of target tissue dosimetry across routes of exposure, among species, and from high to low doses (Conolly,

2001, Medinsky and Klaassen, 1996). The disadvantages of these models are that they are very data intensive, they require support by extensive mechanistic research and the model includes a lot of calculations. The most important disadvantage is that the parameters to be used in the model are difficult to determine (Medinsky and Klaassen, 1996, Botham et al., 1999).

Obviously these models have some huge advantages but since the parameters for many processes are not known they can only be used in few cases.

Procedure	Required data	Applicability	Assumptions	Advantages	Disadvantages
Hazard Index (HI)	Maximum acceptable level for each compound (e.g. RfD or ADI)	Good dose-response data, exposure data at low levels. HI is also used for compounds with similar target organ	Loewe addition – toxicological similarity	Transparent, understandable, relates directly to long-used and well-understood measure of acceptable risk e.g. RfD or ADI	RfD (or ADI) is not an appropriate point of departure – it involves an uncertainty factor (subjective)
Relative Potency Factor (RPF)	Toxicity data for each compound, dose-response data for the index compound	Some data available – restricted by similarity and to specific conditions	Loewe addition – toxicological similarity, but for specific conditions (end point, route, duration). It is supposed to account for mixtures with different mode of action	Transparent, understandable, relates directly to real exposure and toxicity data	Complicated to use. Relies on the availability of dose-response data for the index compound.
Toxicity Equivalency Factor (TEF)	Toxicity data for each compound, dose-response data for the index compound	Data seldom available – restricted by strong similarity – few chemical classes will qualify. Applied to all end points	Loewe addition – strong degree of toxicological similarity	Transparent, understandable, relates directly to real exposure and toxicity data	In some cases complicated to use. Relies on the availability of dose-response data for the index compound
Margin Of Exposure for mixtures (MOE_T)	Point of departure		Loewe addition – toxicological similarity	Relates directly to real exposure and toxicity data	No criteria for defining the magnitude for an acceptable MOE_T
Response Addition	Fractions of total possible effect	Data seldom available	Toxicological independence – Bliss independence	Mathematically easy	Data applicability is low
Interaction Hazard Index	Maximum acceptable level for each compound, a number of weighting factors	Limited data on interactions	Binary interactions are most important	Supposed to account for interactions (binary)	Complex to determine the binary weight of evidence. Weighting factors are not supported by experimental data. No guidance for selecting uncertainty factors for interactions and interactions are only represented by these

Table 3.1: Six methods for risk assessment of mixtures based on data on single compounds. The required data, applicability, assumptions, advantages, and disadvantages of each method that have been mentioned in the literature are summarised, see section 3.3.2.

4 Selection of methods for risk assessment of pesticide residues in food

In the previous chapter the most important existing methods for risk assessment of mixtures of various chemicals were presented. The whole mixture approaches presented would be the ideal risk assessment of pesticide residues in food, however they are not applicable here since they are demanding a large number of data, which is rarely available. This leaves "the single compound approaches" as the more realistic ones.

In chapter 3 the single compound approaches were divided into three categories depending on whether the compounds in the mixture acted by a similar mechanism, independently or whether the compounds interacted. In this chapter it will be discussed whether the approach for interactions or one of the other approaches will be the most preferred for risk assessment of pesticide mixtures.

4.1 Interactions versus additivity

One of the main points to consider is whether there is no interaction or interaction in the form of either synergism or antagonism between the pesticides in a mixture. When mixtures consist of more than two compounds and when the toxicity targets is complex, both additivity, synergism, and antagonism can occur at the same time and it can be problematic to make an overall interpretation.

It is very difficult to predict the toxic interactions. If the actual exposure is very low it is often unclear whether knowledge about the combined action at higher concentrations is relevant for the low exposure level. Knowledge about combined actions has often been gained for considerably higher concentrations than the levels actually found in food – often at levels far beyond the threshold doses for the effect of the single substances such that the combined action (if any) is seen more clearly.

Hertzberg and co-workers has pointed out another problem. They noticed that many studies failed to identify what the "no-interaction" hypothesis was (for example which reference model to use). This means that conclusions regarding interactions from such an article are difficult (if not impossible) to interpret. Overall this means that conclusions regarding toxicological interactions are only weakly supported by empirical studies (Hertzberg et al., 1999).

Neither the approaches for toxicologically similar compounds nor the approach for toxicologically independent compounds presented in chapter 3 will accurately predict risks for compounds that exhibit toxicological interactions. At the moment there exist no applicable risk assessment methods that account for interactions. The interaction-based hazard index approach presented by Mumtaz and co-workers seems to be the only method at present that take toxicological interactions into account but it uses a number of assumptions and unknown pa-

rameters that have not been determined from experimental or epidemiological data. Conolly has stated that one of the greatest dangers in trying to describe mechanisms quantitatively is the use of speculative assumptions about the mechanisms rather than the lack of knowledge as such (Conolly, 2001). This is exactly the problem with the interaction-based hazard index approach and this makes the method less useful.

4.2 Toxicologically similar compounds versus toxicologically independent compounds

As the knowledge about the properties of various combinations of chemicals is very limited the relevant mode of action for a mixture is rarely known. In practise mixtures often consist of combinations of compounds with different modes of action.

Many groups of scientists have suggested that "dose additivity" (Loewe additivity) makes the best model for the interpretation of the combined action of mixtures of various chemicals (not only pesticides) at low doses and therefore they suggest using one of the methods based on toxicological similarity.

Ikeda evaluated publications on combined action of chemicals in the leading toxicological journals in the period 1981-1987. He concluded that in most cases additive effects or less than additive effects were seen but in a few cases more than additive effects were seen. However he concluded, "the most practical approach in evaluating the combined effect of chemicals seems to be the assumption of additive effects" (Ikeda, 1988).

U.S. EPA suggest that "additivity" (corresponding to Loewe additivity with the definitions in the present report) is likely when the compounds in a mixture are present at low doses and when the compounds have the same toxic effect and act via the same mechanism (U.S. EPA 1986). However the definition of "low dose" is vague (Seed et al., 1995).

From the results of experimental short-term toxicity studies Feron and co-workers concluded that combined exposure to arbitrarily chosen chemicals did not demonstrate more than an additive action when all chemicals in the mixture were administrated at their own individual no adverse effect levels (NOAELs) whereas no clear evidence of toxicity was found at slightly lower dose levels. The examined compounds had either different target organs and/or differ in the mode of action. Exposure levels at or below the individual NOAELs of the compounds in a mixture are therefore not expected to be associated with a greater hazard than exposure to the individual chemicals. However, both synergistic and antagonistic effects may be seen at exposure levels higher than the NOAELs. This makes it clear that it is important to define the meaning of the term "low" levels in reference to the threshold of the individual compounds in the mixture (Larsen et al., in preparation; Feron et al. 1995a, Groten et al. 1997, Jonker et al. 1990, Jonker et al. 1993, Jonker et al. 1996).

Feron and co-workers are of the opinion that the use of the "dose addition" approach to the risk assessment of chemical mixtures is only scientifically justifiable when all the chemicals

in the mixture act in the same way, by the same mechanism, and thus differ only in their potencies. Application of the "dose addition" model to mixtures of chemicals that act by mechanisms for which the additivity assumptions are invalid could greatly overestimate the risk (Larsen et al., in preparation; Feron et al., 1995b, Cassee et al., 1998).

When the mixture consists of toxicologically independent compounds the response addition method should be used. However, in the risk assessment of pesticide residues the necessary data for the response addition method are not available and therefore the method is not applicable.

4.3 Conclusion

According to the above mentioned groups of scientists it would thus be reasonable to use the approaches based on toxicologically similarity and toxicologically independency for risk assessment of pesticide residues in food since these compounds are found at levels well below the NOAELs for the compounds. However, the approach suggested for toxicologically independent compounds (the response addition method) is not applicable because of lack of data. If the conclusions made by the above mentioned groups are valid and their assumptions hold true the methods for toxicologically similar compounds could be used in most cases, that is, the hazard index, the relative potency factor/the toxicity equivalency factor, or the margin of exposure could be used for risk assessment of pesticide mixtures in food.

From the discussion in chapter 3 it is seen that for toxicologically similar acting compounds the hazard index is the most appropriate method in most cases. The advantages of the hazard index method are that it is transparent, understandable and it relates directly to a long-used well-understood measure of acceptable risk (e.g. ADI). The method involves (as well as other methods) considerations on uncertainty factors but since an uncertainty factor is included in ADI the method is simple to use. The method has been used for mixtures of compounds that had the same target organ but differed in mode of action. In figure 4.1 the hazard index method is suggested for use also for mixtures of pesticides (found in food) that are toxicologically independent, since these compounds are found at levels well below their respective NOAELs and they are therefore not expected to cause more than an additive effect.

It is not possible to set up a general standard formula for risk assessment of pesticide mixtures in food and, therefore, The Danish Veterinary and Food Administration suggest to use the flow chart shown in figure 4.1. The risk assessment has to be done on a case-by-case evaluation in which the available chemical and toxicological data on the pesticides are evaluated in a weight of evidence process. Then the hazard index with the acceptable daily intake, ADI, as the acceptable level in the denominator should be used. However, in cases where the weight of evidence points out that the compounds in the mixture share a common mechanism the toxicity equivalency factor should be used instead of the hazard index. This concerns for instance the organophosphorus pesticides, the chloroacetanilides, the dithiocarbamates, and the thiocarbamates.

This method is the best suggestion for risk assessment of pesticide mixtures at the moment. The subject risk assessment of pesticide mixtures is relatively new and more research still has to be done. Fenner-Crisp has pointed out some of the areas where work should be focused. These includes “further elucidation of mechanisms of toxicity, development of tools for the refinement of the estimation of cumulative effect, development of more and better exposure data that are not chemical-specific (e.g., human activity patterns and residue monitoring techniques), and evolution and validation of models – both biological and exposure” (Fenner-Crisp, 2000).

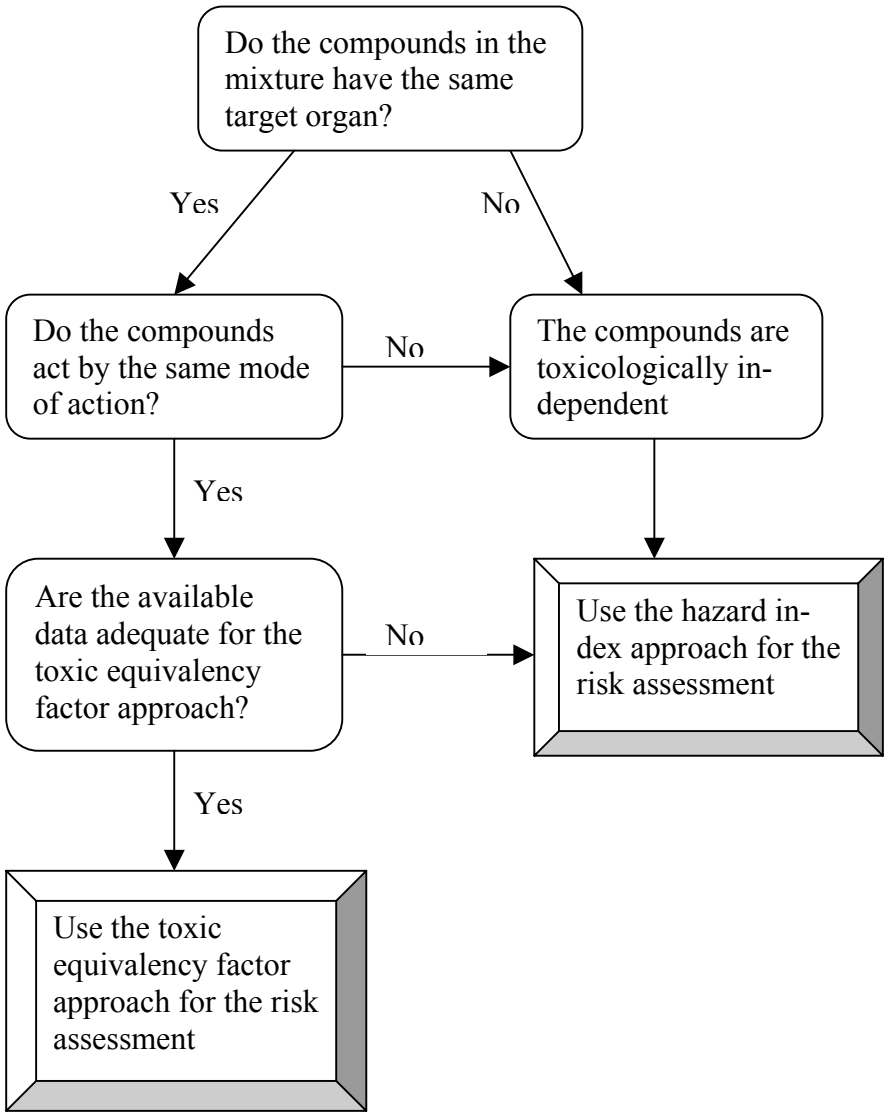


Figure 4.1: Flow chart of the risk assessment approach for pesticide mixtures found in food.

5 Studies on combined actions described in the literature

The literature study that will be presented in this chapter was performed in order to test the hypotheses presented in chapter 4. This is done in order to clarify whether the models presented and discussed in chapter 3 could be supported by experimental studies on combined actions of pesticides reported in the scientific literature. Another purpose was to find out whether there were any articles reporting clear-cut synergism at low doses.

5.1 Literature search

The literature search concentrated on studies of effects of mixtures of pesticides on animals exposed orally. As the number of *in vivo* studies was low, *in vitro* studies were also included. Articles concerning mixtures of pesticides in combination with non-pesticides are included to a limited extent.

A computer-supported online literature search was performed and it covered the period from 1980 to the middle of 2001. The databases Medline, Silverplatter Medline, Toxline and Toxline Plus were searched. The following keywords were used and combined:

- Pesticide
- Toxicology
- Mixture
- Interaction/Chemical interaction
- Cumulative risk/effect
- Combined effect
- Additive/additivity
- Synergistic/synergism
- Antagonistic/antagonism
- Potentiation
- Loewe additivity/antagonism/synergism
- Bliss independence/antagonism/synergism
- Increase
- Enhancement
- Reduction

Further references cited in these publications were sought, and searches on selected authors were performed. Although this literature search sought completeness, undoubtedly some studies were missed, particularly those not published in English.

5.2 Evaluation of the studies described in the literature

The data from the studies described in the literature were analysed either quantitatively or qualitatively depending on the quality of the data. For such an analysis to be made, the effects of both the single compounds and the mixture must be known. In this report an analysis is called quantitative either when it was possible to apply one of the models presented in chapter 2 (Loewe or Bliss) to predict the combined effect or when none or only one of the compounds was observed to have an effect by itself on the examined endpoint. The word qualitative is used in cases where the data is not adequate for an analysis by the right model, but the data is nevertheless strong enough so that a comparison between the effects of single compounds and the mixture can be made. An interpretation based on a qualitative analysis is therefore more uncertain than a quantitative analysis.

The most important factor that made data from the studies inapplicable for evaluation was that the effects of single compounds were not examined. In some studies on mixtures of compounds with similarity in mechanism the Loewe model should have been used to interpret the combined action but because of lack of or inadequate dose-response curves it was not possible to interpret the combined action. Another factor that made data inapplicable for evaluation was that compounds did not demonstrate a maximum effect, which is a condition that should be fulfilled for using the Bliss independence model. In some studies the mixtures consisted of too many compounds or the study reported too few data, which also made the evaluation impossible.

The literature search ended up with 47 relevant articles concerning studies on combined action of pesticides and 18 articles about mixtures of pesticides and non-pesticides. In appendix 1 (mixtures of pesticides) and appendix 2 (mixtures of pesticides and non pesticides) tables with information on these studies are shown. Compounds, doses, exposure and species (or *in vitro* tests) used in the studies are listed as well as the effects reported. In the column "Why these compounds?" the arguments of the authors for choosing the actual mixture is listed - for example whether the compounds are widely used or commonly found, they belong to the same chemical group, have a specific effect or act by the same mechanism and so on. Finally the combined action identified by the authors as well as the ones presented here are stated. This project's interpretation of the data from the articles is based on the models and terminology described in chapter 2.

5.2.1 Combined action of mixtures of pesticides

There were 47 articles concerning combined effects of pesticide mixtures. Some authors described more than one study per article so the total number of studies was 50. 22 of these studies were done *in vitro* and 28 *in vivo*. Most of the *in vivo* studies were done on rats (21) with only a few on mice (5) and dogs (2).

It was possible to make a quantitative analysis of the data from 21 of the 50 studies concerning pesticide mixtures. Of these 21, 15 studies were concerned with mixtures where only one

or neither of the compounds was effective individually. Furthermore it was possible to make a qualitative analysis of some of the data in 2 of the 21 studies. The combined action in the rest of the 50 studies could not be interpreted due to inadequacy of the data.

In the following, the studies in which it was possible to make a quantitative or qualitative analysis are summarised. The considerations and calculations leading to the interpretations are shortly described. It should be pointed out that the interpretations of the data are not necessarily in agreement with the interpretations of the authors of the articles. In appendix 1 all studies on combined action of pesticides are listed. In the column "Interpretation" both the interpretation from this project and that of the authors are shown.

The 21 studies in which some of the data were adequate for a quantitative or qualitative analysis are listed in table 5.1 and 5.2. The first table lists the 10 *in vivo* studies and table 5.2 lists the 11 *in vitro* studies.

5.2.1.1 *In vivo* studies

In the 10 *in vivo* studies, there were adequate data for a quantitative analysis of 28 mixtures. 12 of these mixtures were found to have an additive action (Bliss independence or inertism). Nine mixtures had a combined action less than additive (Bliss/Loewe antagonism, antagonism), 3 different mixtures were found to act synergistic (Bliss synergism, synergism) and 4 mixtures showed coalism *in vivo*. The *in vivo* studies will be summarised in the following starting with the mixtures acting additively followed by the mixtures showing antagonism and ending with the mixtures showing synergism. Table 5.3 gives a survey of the number of each type of combined action found in the studies.

In only one of the 12 mixtures indicating additivity more than one compound were active. This was in one study by Sedrowicz and co-workers where the combined action could be described by the Bliss independence model (Sedrowicz et al., 1996). In the other 11 mixtures the combined action were found to be inertism. Chlorfenvinphos and cypermethrin were given alone and in mixture to rats at doses similar to 3.5 and 15 times NOAEL respectively (5 % LD_{50} of each compound) and the effect on the intestinal transport of leucine was studied. The percentages of leucine absorbed during perfusion and retaining in the liver were as follows:

Control: 0.77 % ~ 0.9923
Chlorfenvinphos: 0.48 % ~ 0.9952
Cypermethrin: 0.29 % ~ 0.9971
Mixture: 0.17 % ~ 0.9983

The fractions of total possible effect for the compounds and the mixture can now be calculated:

$$f_b(d_1,0) = \frac{f(d_1,0) - f(0,0)}{1 - f(0,0)} = \frac{0.9952 - 0.9923}{1 - 0.9923} = 0.3766$$

$$f_b(0, d_2) = \frac{f(0, d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.9971 - 0.9923}{1 - 0.9923} = 0.6234$$

$$f_b(d_1, d_2) = \frac{f(d_1, d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.9983 - 0.9923}{1 - 0.9923} = 0.7792$$

And the expected fraction of total possible effect for the mixture is:

$$\begin{aligned} f_{expected,\,b} &= f_b(d_1,0) + f_b(0, d_2) - f_b(d_1,0) \times f_b(0, d_2) \\ &= 0.3766 + 0.6234 - 0.3766 \times 0.6234 = 0.7652 \end{aligned}$$

This is almost the same as what was measured in the study (0.7792) indicating Bliss independence.

Mixtures of toxaphene (50 mg/kg/day) and parathion (5 mg/kg/day) or 2,4-D (50 mg/kg/day) or all three compounds were given to mice and the effect on hepatic cytochrome P-450 content was examined (Chaturvedi et al., 1991). They also examined the effect of single compound treatment. They found that only toxaphene had an effect individually (1.5 nmol/mg microsomal protein compared with 0.96 nmol/mg microsomal protein for the control). The effect of the mixtures with toxaphene was reported to have an effect between 1.5 and 1.7 nmol/mg microsomal protein, that is, the same as the effect of toxaphene indicating inertism for the combined action of all three mixtures. The doses were high, at least 50 × NOAEL.

The same authors also examined the effect of parathion, 2,4-D, and a mixture of the two compounds on hepatic cytochrome P-450 content in mice (Chaturvedi et al., 1991). Neither the compounds nor the mixture had an effect which shows inertism.

The level of aspartate aminotransferase (AST) was found to increase to 227.6 U/L (compared with 156.2 U/L for the control) in serum from rats treated with dimethoate. Mixtures of dimethoate (20×NOAEL) and carbaryl (0.7×NOAEL) or endosulfan (10×NOAEL) were reported to increase the level of aspartate aminotransferase to a level between 207.8 and 217.8 U/L (Selmanoglu et al., 2001). Neither carbaryl nor endosulfan had a statistically significant effect on this parameter. The effect level of the mixture is the same as that of dimethoate alone leading to the interpretation of inertism.

Parathion (12.5×NOAEL) inhibited the activity of acetylcholinesterase in brain in mice by up to 83 %. Toxaphene (143×NOAEL) did not show an effect. A mixture of the two compounds was reported to inhibit the acetylcholinesterase activity by 71 % which shows inertism (Kuntz et al., 1990).

Akay and co-workers examined the effect on immune and haematological parameters of individual and combined exposure of endosulfan (10×NOAEL), dimethoate (20×NOAEL) and carbaryl (0.7×NOAEL) to rats. The level of IgG was reduced due to an exposure to endosulfan (from 15650 mg/L for the control to 11571 mg/L). Mixtures of endosulfan and dimethoate

and of endosulfan, dimethoate and carbaryl were reported to inhibit the level of IgG to 11155 mg/L and 10941 mg/L respectively indicating inertism (Akay et al., 1999).

A mixture of endosulfan (10×NOAEL) and dimethoate (20×NOAEL) or carbaryl (0.7×NOAEL) was found to increase the number of monocytes in the blood of treated rats. The number of monocytes was increased from 0.15×10^9/L in the control group to 0.48×10^9/L and 0.41×10^9/L. Dimethoate and carbaryl did not have an effect on the number of monocytes but endosulfan increased the number by the same level as the mixtures (0.52×10^9/L) indicating inertism. At lower doses (1/10 and lower) of the pesticides no effect was found (Akay et al., 1999).

9 mixtures had a combined effect less than additive. The Bliss model could be used to describe two mixtures and the Loewe model could be used in one case. In the remaining 6 mixtures only one of the compounds had an effect.

The oxidative liver DNA damage was examined in rats treated with a mixture of thiabendazole, fenarimol, diphenylamine and chlorothalonil at doses corresponding to 1-100 times NOAEL (Lodovici et al., 1997). Single compound exposures showed that only diphenylamine and chlorothalonil increased the levels of 8-OH-2-deoxyguanosine relative to 2-deoxyguanosine in liver DNA. The Loewe model (see chapter 2) can be used to interpret the data reported in the article. Step one is to find the effect of the mixture. In this study the level of 8-OH-2-deoxyguanosine relative to 2-deoxyguanosine of the mixture is 3.4×10^5. The authors presented dose response curves for the two compounds and from these curves the dose of each compound that correspond to the effect of the mixture (3.4×10^5) could be found to 0.14 mg/kg/day (= D_1) and 0.5 mg/kg/day (= D_2) for diphenylamine and chlorothalonil respectively. The doses of the two compounds in the mixture were 0.14 mg/kg/day (d_1) and 0.13 mg/kg/day (d_2) respectively. The combination index can now be calculated:

$$I_c = \frac{d_1}{D_1} + \frac{d_2}{D_2} = \frac{0.14}{0.14} + \frac{0.13}{0.5} = 1.26$$

As the combination index is higher than 1, Loewe antagonism is indicated.

The white blood cell count was enhanced in rats given a mixture of hexachlorocyclohexane (HCH) and isoproturon compared with single compound exposure at doses corresponding to 25×NOAEL and 7×NOAEL, respectively (Raizada et al., 2001). The white blood cell counts were 3755 mm^3, 2975 mm^3, 6187 mm^3 and 7125 mm^3 for hexachlorocyclohexane, isoproturon, the mixture of the two compounds and the control, respectively, When the counts for the control group is fixed to 100 % (~ 0 % inhibition), the inhibition of white blood cell count by the compounds can be calculated:

Hexachlorocyclohexane:

$$\frac{3755}{7125} = 0.53 \sim 47\ \%\ \text{inhibition}$$

Isoproturon:

$$\frac{2975}{7125} = 0.42 \sim 58\ \%\ \text{inhibition}$$

Mixture:

$$\frac{6187}{7125} = 0.87 \sim 13\ \%\ \text{inhibition}$$

The Bliss independence model is then used to calculate the combined action of the two compounds:

$$f_{expected} = f(d_1,0) + f(0,d_2) - f(d_1,0) \times f(0,d_2)$$
$$f_{expected} = 0.47 + 0.58 - 0.47 \times 0.58 = 0.72 \sim 72\%$$

This is a much higher inhibition than the measured 13 %, that is an indication of Bliss antagonism.

Parathion (12.5×NOAEL) and 2,4-D (50×NOAEL) given to mice were reported to hydrolyse 1.8 and 6.2 µmol acetylcholinesterase per min per g brain respectively on day 15 (maximum effect were seen here) (Kuntz et al., 1990). The acetylcholinesterase activity in the brains in the control group was 11 µmol/min/g brain corresponding to 100 % or an inhibition of 0 %. The acetylcholinesterase activities of parathion and 2,4-D are then converted to 83 % (= $f(d_1,0)$) and 44 % (= $f(0,d_2)$) inhibition respectively. A mixture of the two compounds was reported to give an activity on 3.2 µmol/min/g brain corresponding to 71 % inhibition of acetylcholinesterase. The two compounds act by different mechanisms so the Bliss independence model should be used as reference model. The fraction of total possible effect for the mixture expected from the Bliss model can be calculated (from equation 1 in chapter 2):

$$f_{expected} = f(d_1,0) + f(0,d_2) - f(d_1,0) \times f(0,d_2)$$
$$f_{expected} = 0.83 + 0.44 - 0.83 \times 0.44 = 0.90 \qquad (90\ \%)$$

This means that the expected fraction of total possible effect is 90 %. This is higher than the 71 % inhibition measured in the study so Bliss antagonism is indicated.

Krechniak et al. (1994) examined the induction of hepatic microsomal enzymes in rats by lindane and carbaryl. Lindane was the only compound that had an effect when given alone but the mixture inhibited this effect by about 15 % at high doses giving rise to an antagonistic effect. The two compounds have the same target organs: liver and kidney. At the lowest dose level the two compounds did not interact (inertism). The dose levels were 3 to 12 times NOAEL for carbaryl and between 22 and 88 times NOAEL for lindane.

In another study dimethoate (20×NOAEL) and endosulfan (10×NOAEL) were given to rats alone and in a mixture (Selmanoglu et al., 2001). When given as single compounds only dimethoate had an effect on the serum values of both glucose (122.6 mg/dL compared to 209.0 mg/dL for the control group) and urea nitrogen (36.0 mg/dL compared with 26.3 mg/dL for

the control group). The mixture of endosulfan and dimethoate was reported to perform less effect on these parameters (194.6 mg glucose/dL and 26.0 mg urea nitrogen/dL). This indicates an antagonistic effect.

Endosulfan (10×NOAEL) were reported to inhibit the level of IgM in rats from 806 mg/L (control group) to 527 mg/L (Akay et al., 1999). Mixtures of endosulfan (10×NOAEL), dimethoate (20×NOAEL) and/or carbaryl (0.7×NOAEL) had a less inhibiting effect on the IgM level (670, 627, 787 mg/L respectively). This indicates antagonism. At lower doses (1/10 and lower) no effect was found.

The level of IgG in rats treated with endosulfan (10×NOAEL) alone was 11571 mg/L compared to 15650 mg/L for the control group (Akay et al., 1999). A mixture of endosulfan (10×NOAEL) and carbaryl (0.7×NOAEL) had a less inhibiting effect on the level of IgG than endosulfan alone (IgG level: 13045 mg/L). This indicates antagonism. At lower doses (1/10 and lower) no effect was found.

Metribuzin (10000 ppb) were reported to inhibit the acetylcholine/choline ratio in hippocampus of rats by about 50 % whereas a mixture of aldicarb (10 ppb) and metribuzin (10000 ppb) did not inhibit the ratio (Boyd et al., 1990). This indicates antagonism.

In the 7 mixtures that had an effect higher than additive only one or neither of the compounds were effective individually.

Kuntz et al. (1990) examined the effect of a mixture at high doses of toxaphene (143×NOAEL) and 2,4-D (50×NOAEL) on serum glutamic pyruvic transaminase activity (SGPT) in mice. Only toxaphene had an effect given alone (reported to be 67 % higher than the control) but the mixture had an SGPT activity that was almost twice as high as that for toxaphene alone indicating synergism. The two compounds have different mode of action but they both have an effect on the liver.

A mixture of EPN (O-ethyl O-p-nitrophenyl phenylphosphonothioate) and malathion at “safe levels” (reported to be the highest no effect level) inhibited acetylcholinesterase in erythrocytes in dogs (Williams et al., 1958). Both compounds are organophosphorus insecticides that are known as inhibitors of acetylcholinesterase. EPN had no effect at the actual dose level. Although the doses were chosen at “the highest no effect level”, malathion inhibited acetylcholinesterase by 10 % when given alone. This indicates that the dose level used in the study was not a no effect level for this parameter. The mixture had an inhibiting effect of 67 % indicating a synergistic effect.

In the same study on dogs two organophosphorus pesticides (EPN and Systox) were found to inhibit acetylcholinesterase in plasma by 24.4 % (= $f(d_1,0)$) and 14 % (= $f(0,d_2)$) at levels reported to be the highest no effect levels by the authors and the mixture was reported to inhibit the acetylcholinesterase by 58 % (Williams et al., 1958). The two compounds are expected to act by the same mechanism since they are both organophosphorus pesticides, therefore the Loewe model should be used to analyse the data. However the data presented in the article are

not adequate for such an analysis. A qualitative evaluation of the data suggests a synergistic effect.

Mixtures of dimethoate (20×NOAEL) and endosulfan (10×NOAEL) and/or carbaryl (0.7×NOAEL) increased alanine aminotransferase (ALT) in serum from rats (from about 60 U/L to 80 U/L) even though none of the compounds had an effect when given alone (Selmanoglu et al., 2001) indicating coalism. The three compounds had different modes of action and target organs.

A mixture of dimethoate (20×NOAEL) and carbaryl (0.7×NOAEL) was found to change the number of monocytes and granulocytes in the blood from treated rats. The number of monocytes was increased from 0.15×10^9/L in the control group to 0.38×10^9/L and the number of granulocytes decreased from 0.56×10^9/L to 0.29×10^9/L (Akay et al., 1999). Neither of the compounds had an effect of the monocyte nor granulocyte counts, which indicates coalism. At lower doses (1/10 and lower) of the pesticides no effect was found.

The interpretations of the data from the published *in vivo* studies on combined actions of pesticides are divided into the types of combined actions that were described in chapter 2. In each case the number of mixtures is shown in table 5.3.

	Both agents effective individually – similar mechanism	Both agents effective individually – dissimilar mechanism	Only one agent effective individually	Neither agent effective individually
Synergism: combination effect greater than predicted	Loewe synergism: 0	Bliss synergism: 0	Synergism: 2 +1? (Kuntz et al., 1990, Williams et al., 1958)	Coalism: 4 (Selmanoglu et al., 2001, Akay et al., 1999)
Additivity: combination effect equal to prediction from reference model	Loewe additivity: 0	Bliss independence: 1 (Sedrowicz et al., 1996)	Inertism: 10 (Chaturvedi et al., 1991, Selmanoglu et al., 2001, Kuntz et al., 1990, Akay et al., 1999, Krechniak et al., 1994)	Inertism: 1 (Chaturvedi et al., 1991)
Antagonism: combination effect less than predicted	Loewe antagonism: 1 (Lodovici et al., 1997)	Bliss antagonism: 2 (Raizada et al., 2001, Kuntz et al., 1990)	Antagonism: 6 (Krechniak et al., 1994, Selmanoglu et al., 2001, Akay et al., 1999, Boyd et al., 1990)	

Table 5.3: The interpretations of the data from the published in vivo studies on combined actions of pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures found is shown in this table. "+1?" means that the interpretation is based on a qualitative analysis.

5.2.1.2 *In vitro* studies

The 11 *in vitro* studies described 26 mixtures on which a quantitative analysis could be done and 1 mixture that could be analysed qualitatively. Synergism (Loewe synergism, synergism) were seen more often than in the *in vivo* studies, in fact it was seen in 12 of the mixtures. In addition two of the studies showed coalism. Nine mixtures acted additively (Loewe additivity, Bliss independence, inertism) and 4 antagonistic (Bliss antagonism, antagonism). In most of the studies only one or neither of the compounds were effective individually. The *in vitro* studies will be summarised in the following starting with the mixtures acting additively followed by the mixtures showing antagonism and ending with the mixtures showing synergism. Table 5.4 gives a survey of the number of each type found from the data in the studies.

Chlorpyrifos-oxon and azinphos-methyl-oxon are the active metabolites of the two organophosphorus pesticides chlorpyrifos and azinphos-methyl. Inhibition of acetylcholinesterase activity in whole homogenised rat brains by mixtures of these two compounds at various con-

centrations was examined (Richardson et al., 2001). This study was used in chapter 2 as an example of how to use the Loewe model. The calculations indicated Loewe additivity for the inhibition of brain acetylcholinesterase.

Acetylcholinesterase in plasma from rats was inhibited by the three carbamates aldicarb, carbofuran and oxamyl when given individually and in mixtures (Iyaniwura, 1989). To be able to evaluate the combined action of mixtures of the three carbamates the Loewe model is extended, although Greco and co-workers have warned against it (Greco et al., 1992). However, an extended version of the Loewe model corresponds to the hazard index method presented in chapter 3, which is recommended by the U.S. EPA. The carbamates were mixed in equal proportions. At a total carbamate concentration of 10^{-7} M the acetylcholinesterase activity was inhibited by 58 %. The concentration of each compound that corresponds to this effect is found on the dose-response curves for the single compounds: $D_1 = 3.3\times10^{-7}$ M (aldicarb), $D_2 = 2.3\times10^{-7}$ M (carbofuran) and $D_3 = 5.0\times10^{-8}$ M (oxamyl). Then the combination index can be calculated:

$$I_c = \frac{d_1}{D_1} + \frac{d_2}{D_2} + \frac{d_3}{D_3}$$

$$I_c = \frac{3.3\times10^{-8}\,\mathrm{M}}{3.3\times10^{-7}\,\mathrm{M}} + \frac{3.3\times10^{-8}\,\mathrm{M}}{2.3\times10^{-7}\,\mathrm{M}} + \frac{3.3\times10^{-8}\,\mathrm{M}}{5\times10^{-8}\,\mathrm{M}} = 0.91$$

As I_c is approximately 1, this suggests Loewe additivity.

At lower carbamate doses (10^{-8} M in total) the inhibition of acetylcholinesterase of the mixture was 42 %. The concentration of each compound that corresponds to this effect is found on the dose-response curves for the single compounds: $D_1 = 1\times10^{-7}$ M (aldicarb), $D_2 = 1.6\times10^{-8}$ M (carbofuran) and $D_3 = 5.6\times10^{-9}$ M (oxamyl). Then the combination index can be calculated:

$$I_c = \frac{3.3\times10^{-9}\,\mathrm{M}}{1\times10^{-7}\,\mathrm{M}} + \frac{3.3\times10^{-9}\,\mathrm{M}}{1.6\times10^{-8}\,\mathrm{M}} + \frac{3.3\times10^{-9}\,\mathrm{M}}{5.6\times10^{-9}\,\mathrm{M}} = 0.83$$

That is, weak Loewe synergism is indicated at lower doses.

The inhibitory influence of DDT (1 µg/ml) and dieldrin (varying: 4-7 µg/ml) and mixtures of the two compounds on gap junction-mediated intercellular communication (i.e. metabolic cooperation) between Chinese hamster V79 cells was examined (Aylsworth et al., 1989). The recovery of 6-thioguanine-resistant V79 cells was used to indicate the inhibition of metabolic cooperation. The recoveries of these cells at 4 µg dieldrin/ml were reported to be 32.7 %, 46 % and 58 % for DDT, dieldrin, and a mixture of the two respectively. The background level was 18 %. The fractions of total possible effect for the compounds and the mixture can now be calculated:

$$f_b(d_1,0) = \frac{f(d_1,0) - f(0,0)}{1 - f(0,0)} = \frac{0.327 - 0.18}{1 - 0.18} = 0.18$$

$$f_b(0,d_2) = \frac{f(0,d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.46 - 0.18}{1 - 0.18} = 0.34$$

$$f_b(d_1,d_2) = \frac{f(d_1,d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.58 - 0.18}{1 - 0.18} = 0.49$$

The expected fraction of total possible effect for the mixture is:

$$f_{expected,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2)$$
$$= 0.18 + 0.34 - 0.18 \times 0.34 = 0.46$$

This is almost the same as measured in the study ($f_b(d_1,d_2) = 0.49$) indicating Bliss independence. At higher concentrations of dieldrin (5, 6, 7 µg/ml) Bliss independence was also indicated.

At a lower dose of dieldrin (3 µg/ml) the inhibitory influence of the mixture was smaller. The recoveries of 6-thioguanine-resistant V79 cells were reported to be 32.7 %, 44 %, and 30 % for DDT, dieldrin, and a mixture of these two. The background level was 18 % (Aylsworth et al., 1989). The fractions of total possible effect for the compounds and the mixture can then be calculated:

$$f_b(d_1,0) = \frac{f(d_1,0) - f(0,0)}{1 - f(0,0)} = \frac{0.327 - 0.18}{1 - 0.18} = 0.18$$

$$f_b(0,d_2) = \frac{f(0,d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.44 - 0.18}{1 - 0.18} = 0.32$$

$$f_b(d_1,d_2) = \frac{f(d_1,d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.30 - 0.18}{1 - 0.18} = 0.15$$

And the expected fraction of total possible effect for the mixture is:

$$f_{expected,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2)$$
$$= 0.18 + 0.32 - 0.18 \times 0.32 = 0.44$$

This is higher than measured in the study ($f_b(d_1,d_2) = 0.15$) indicating Bliss antagonism.

A mixture of malathion and carbofuran inhibited acetylcholinesterase activity in aggregate cultures of neural cells from foetal rat brain by 56.7 %, which is almost at the same level as the effect of malathion alone (42.3 %) (Segal and Fedoroff, 1989). This indicates inertism. They also examined the effect of two mixtures of triallate and fenitrothion or carbofuran. Triallate did not itself inhibit acetylcholinesterase (reported to be 3.1 % inhibition compared with

2.6 % for the vehicle) and the inhibitions of the two mixtures (49.0 % and 56.9 %) were at the same level as carbofuran (53.4 %) and fenitrothion (46.8 %) respectively, which indicates inertism.

Induction of the formation of foci in MCF-7 focus assay by endosulfan (0.001-10 µM) and dieldrin (0.001-10 µM) were examined (Arcaro et al., 1998). Only endosulfan was found to have an effect and this was only at the highest concentration. A mixture of the two compounds caused the same effect as endosulfan alone indicating inertism.

The inhibition of 17β-estradiol from recombinant human estrogen receptors (hER) extracted from Sf9 insect cells infected with a baculovirus containing cDNA of the hER was examined by Arnold and co-workers (Arnold et al., 1997). They found that dieldrin inhibited the binding of 17β-estradiol by 10 % and a mixture of toxaphene and dieldrin also inhibited by 10 % indicating inertism.

The effect of benomyl (30 µg/ml) and pirimiphos-methyl (30 µg/ml) on ^{3}H-leucine incorporation into the human leukaemia cell line HL-60 cell protein was examined after 4 hours (Marinovich et al., 1994). They found that benomyl inhibited the incorporation, and that pirimiphos-methyl had no effect when given alone. However a mixture of the two compounds (30:30 µg/ml) did not have an effect after 4 hours, which suggest antagonism. At lower doses of pirimiphos-methyl (15 and 7.5 µg/ml) and after 24 hours the incorporation was inhibited by the same extent as that of benomyl itself indicating inertism.

The sister chromatic exchanges (SCE) and chromosome aberrations induced in Chinese hamster ovary cells were found to be increased by captafol whereas polyoxin had no effect (Wang et al., 1987). The sister chromatic exchanges and chromosome aberrations caused by captafol at the highest examined concentration was reported to be 57 SCEs/cell and 50 % aberrant cells respectively. A mixture of the compounds at the same concentration level caused a smaller effect than that of captafol alone (34 SCEs/cell and 28 % aberrant cells) indicating antagonism.

Fenitrothion and carbofuran individually inhibited the acetylcholinesterase activity in aggregate cultures of neural cells from foetal rat brain by 24.8 and 32.1 % respectively. The mixture was reported to inhibit acetylcholinesterase by 36.8 % (Segal and Fedoroff, 1989). The Loewe model should be used for evaluating the combined effect of a mixture of these two compounds. The data in the study however, are not adequate for such an analysis. A qualitative analysis suggests a weak antagonism.

A mixture of pirimiphos-methyl and benomyl was reported to inhibit protein synthesis *in vitro* (human neuroblastoma cell line, SH-SY5Y) by 76 % though benomyl inhibited by 53 % and pirimiphos-methyl alone did not have an effect (Marinovich et al., 1996). This indicates synergism. A mixture of benomyl, dimethoate, diazinon, azinphos-methyl and pirimiphos-methyl was reported to give an inhibition of 56 % compared to 31 % inhibition by benomyl at the same concentration. The other compounds in the mixture did not inhibit the protein synthesis (Marinovich et al., 1996). This indicates synergism.

A mixture of azinphos-methyl, dimethoate and diazinon were found to inhibit protein synthesis though only one compound (azinphos-methyl) had an effect when added alone to human neuroblastoma cell line, SH-SY5Y. The inhibition of the mixture was reported to 75 % and azinphos-methyl inhibited by 50 % (Marinovich et al., 1996). This indicates synergism.

Although both malathion and fenitrothion are known as inhibitors of acetylcholinesterase activity only malathion had an effect when added alone (42.3 % inhibition) to neural cells from foetal rat brain. A mixture of the two compounds inhibited acetylcholinesterase activity by 56.7 % (Segal and Fedoroff, 1989), which indicates synergism.

Gilot-Delhalle et al. (1983) examined the mutation frequency of four organophosphorus compounds (trichlorfon, parathion-methyl, malathion and azinphos-methyl). Mixtures of trichlorfon and one of the other three compounds were examined. Only trichlorfon had an effect individually but the mixtures had an effect of about 200-250 % of that of the single compound indicating synergism.

Azinphos-methyl (60 µg/ml) was found to inhibit the ^{3}H-leucine incorporation into the human leukaemia cell line HL-60 cell protein by 60 % whereas dimethoate (100 µg/ml) and diazinon (40 µg/ml) had no effect (Marinovich et al., 1994). A mixture of these three compounds at the same concentrations inhibited the incorporation by 89 % indicating synergism. At lower concentrations neither the individually compounds nor the mixture had an effect on the incorporation.

Arnold and co-workers examined the inhibition of 17β-estradiol from recombinant human estrogen receptors (hER) extracted from Sf9 insect cells infected with a baculovirus containing cDNA of the hER (Arnold et al., 1997). They examined the inhibition of toxaphene, dieldrin, chlordane and alachlor and mixtures of these compounds. Dieldrin inhibited the binding of 17β-estradiol by 10 %. Dieldrin was the only compound that had an effect individually but the following mixtures inhibited the binding by 25-30 %: dieldrin + chlordane, dieldrin + toxaphene + chlordane and dieldrin + aldrin. This indicates synergism. A mixture of toxaphene and chlordane inhibited 17β-estradiol by 20 % indicating coalism.

A mixture of dimethoate, omethoate, deltamethrin and benomyl induced an increase of sister-chromatid exchanges (up to 3.3 SCE/cell-background) in human lymphocytes (Dolara et al., 1992). Neither of the compounds was effective individually so the combined action was coalistic.

The interpretations of the data from the published *in vitro* studies on combined actions of pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures is shown in table 5.4.

	Both agents effective individually – similar mechanism	Both agents effective individually – dissimilar mechanism	Only one agent effective individually	Neither agent effective individually
Synergism: combination effect greater than predicted	Loewe synergism: 1 (Iyaniwura, 1989)	Bliss synergism: 0	Synergism: 11 (Marinovich et al., 1996, Segal and Fedoroff, 1989, Gilot-Delhalle et al., 1983, Marinovich et al., 1994, Arnold et al., 1997)	Coalism: 2 (Arnold et al., 1997, Dolara et al., 1992)
Additivity: combination effect equal to prediction from reference model	Loewe additivity: 2 (Richardson et al., 2001, Iyaniwura, 1989)	Bliss independence: 1 (Aylsworth et al., 1989)	Inertism: 6 (Segal and Fedoroff, 1989, Arcaro et al., 1998, Arnold et al., 1997, Marinovich et al., 1994)	Inertism: 0
Antagonism: combination effect less than predicted	Loewe antagonism: 0	Bliss antagonism: 1 (Aylsworth et al., 1989)	Antagonism: 2 +1? (Marinovich et al., 1994, Wang et al., 1987, Segal and Fedoroff, 1989)	

Table 5.4: The interpretations of the data from the published in vitro studies on combined actions of pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures found is shown in this table. "+1?" means that the interpretation is based on a qualitative analysis.

5.2.2 Combined action of mixtures of pesticides and non-pesticides

In this review, 18 articles on combined actions of mixtures of pesticides plus non-pesticides were found including 13 *in vivo* studies (8 on mice, 5 on rats), 6 *in vitro* studies, and one case study on one human. Data from 5 (2 *in vivo* and 3 *in vitro* studies) of the 19 studies on combined actions of mixtures of pesticides plus non-pesticides could be interpreted quantitatively, see table 5.5. These studies will be summarised in the following starting with the *in vivo* studies followed by the *in vitro* studies.

5.2.2.1 *In vivo* studies

In the 2 *in vivo* studies, there were adequate data for a quantitative analysis of in total 5 mixtures. 1 of these mixtures was found to have an additive action (Bliss independence). 1 mix-

ture could be described by Bliss antagonism and 3 mixtures were found to act synergistic (Bliss synergism, synergism) *in vivo*. The *in vivo* studies will be summarised in the following starting with the mixtures acting additively followed by the mixtures showing antagonism and ending with the mixtures showing synergism. Table 5.6 gives a survey of the number of each type found from the data in the studies.

The effect on testicular tissues in mice of exposure to lead oxide (50 mg/kg bw/day), chlordane (75 or 275 mg/kg bw/day), and a mixture of these compounds were examined by Al-Omar and co-workers (Al-Omar et al., 2000). They examined the effect on testis weight and the diameter of the seminiferous tubules after 2, 3, 4, and 5 weeks exposure. The effect changed over time but overall the mixture acted Bliss independent on these parameters.

The activity of acetylcholinesterase in red blood cells in rats was reported to be 1.180, 0.395, and 0.538 µmole/min/ml for cimetidine (2×80 mg/kg), diazinon (50 mg/kg), and a mixture of the two respectively. The background level was 1.283 µmole/min/ml (Wu et al., 1996). The inhibition of acetylcholinesterase can then be calculated to be 8 %, 69 %, and 58 % for cimetidine, diazinon, and the mixture, respectively. The expected fraction of total possible effect for the mixture is then:

$$f_{expected,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2)$$
$$= 0.08 + 0.69 - 0.08 \times 0.69 = 0.72 \qquad \sim 72\%$$

This is higher than measured (58 %) indicating Bliss antagonism.

They also examined the carboxylesterase activity in plasma and liver (Wu et al., 1996). They found the following activities of carboxylesterase in the plasma: 174.5, 34.3, and 18.2 µmole/min/ml for cimetidine, diazinon and a mixture of the two compounds. The background level was 178.3 µmole/min/mg. These activities correspond to 2 %, 81 %, and 10 % inhibition of carboxylesterase by cimetidine, diazinon and the mixture, respectively. The expected fraction of total possible effect for the mixture is then:

$$f_{expected,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2)$$
$$= 0.02 + 0.81 - 0.02 \times 0.81 = 0.81 \quad \sim 81\ \%$$

This is higher than measured (10 %) indicating Bliss antagonism.

The activity of carboxylesterase in liver was reported from the same study to be 776, 115, 182, and 778 µmole/min/mg for cimetidine, diazinon, the mixture, and the control group, respectively. Inhibition of carboxylesterase activity can then be calculated to be 0.3, 85, and 77 % for cimetidine, diazinon, and the mixture, respectively. The expected fraction of total possible effect for the mixture is then:

$$f_{expected,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2)$$
$$= 0.003 + 0.85 - 0.003 \times 0.85 = 0.85 \quad \sim 85\%$$

This is a bit larger than measured (77 %) indicating weak Bliss antagonism.

Wu and co-workers also examined the effect of cimetidine (2×80 mg/kg) and diazinon (50 mg/kg) on the inhibition of acetylcholinesterase activity in brain as well as the inhibition of carboxylesterase activity in brain in the study on rats (Wu et al., 1996). The activity of acetylcholinesterase in brain was reported to 6.83, 6.09, and 5.23 μmole/min/mg for cimetidine, diazinon, and a mixture of the two compounds. The background level was 7.09 μmole/min/mg. These activities correspond to 4 %, 14 %, and 26 % inhibition of acetylcholinesterase activity by cimetidine, diazinon, and the mixture respectively. The expected fraction of total possible effect for the mixture is then:

$$f_{expected,\,b} = f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0) \times f_b(0,d_2)$$
$$= 0.04 + 0.14 - 0.04 \times 0.14 = 0.17 \qquad \sim 17\ \%$$

This is lower than measured (26 %) indicating weak Bliss synergism.

The activity of carboxylesterase of diazinon in brain was reported to be 67.3 μmole/min/mg compared to 78.9 μmole/min/mg for the control group. Cimetidine had no effect. The activity of the mixture was reported to 46.8 μmole/min/mg, which indicates synergism.

Al-Omar and co-workers investigated the effect on testicular tissues in mice of exposure to lead (50 mg/kg bw/day), chlordane (75 or 275 mg/kg bw/day), and a mixture of these compounds (Al-Omar et al., 2000). The number of sertoli cells, spermatogenia, spermatids, and primary and secondary spermatocytes were examined after 2, 3, 4, and 5 weeks exposure. The effect changed over time but the overall interpretation is that the compounds acted by Bliss synergism on these parameters.

The interpretations of the data from the published *in vivo* studies on combined actions of mixtures of pesticides plus non-pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures found is shown in table 5.6.

	Both agents effective individually – similar mechanism	Both agents effective individually – dissimilar mechanism	Only one agent effective individually	Neither agent effective individually
Synergism: combination effect greater than predicted	Loewe synergism: 0	Bliss synergism: 2 (Wu et al., 1996, Al-Omar et al., 2000)	Synergism: 1 (Wu et al., 1996)	Coalism: 0
Additivity: combination effect equal to prediction from reference model	Loewe additivity: 0	Bliss independence: 1 (Al-Omar et al., 2000)	Inertism: 0	Inertism: 0
Antagonism: combination effect less than predicted	Loewe antagonism: 0	Bliss antagonism: 1 (Wu et al., 1996)	Antagonism: 0	

Table 5.6: The interpretations of the data from the published in vivo studies on combined actions of mixtures of pesticides plus non-pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures found is shown in this table.

5.2.2.2 *In vitro* studies

In the 3 *in vitro* studies, there were adequate data for a quantitative analysis of in total 9 mixtures. For 6 mixtures the combined action were addition (Loewe additivity, Bliss independence) and 3 mixtures acted synergistic (Bliss synergism, synergism). In the following these articles will be summarised starting with the mixtures acting additively followed by the mixtures showing antagonism and ending with the mixtures showing synergism. Table 5.7 gives an overview over the number of each type found from the data in the studies.

Jett and co-workers examined the effect of chlorpyrifos-oxon (1-180 nM) and four polycyclic aromatic hydrocarbons (PAH) (2-50 μM) on acetylcholinesterase activity *in vitro* (Jett et al., 1999). They examined both the effect of single compounds and of mixtures of chlorpyrifos-oxon and one of the PAHs (benzo[a]pyrene, pyrene, anthracene, fluoranthene). All the compounds were found to inhibit the acetylcholinesterase activity and therefore the Loewe model is used to make a quantitative analysis of the data. An analysis indicates Loewe additivity for all 4 mixtures. One of the highest combination indexes was achieved in the following example. The mixture of 180 nM (= d_1) chlorpyrifos-oxon and 2.8 μM (= d_2) anthracene was reported to give 50 % acetylcholine activity. The concentrations of each compound giving this effect are found from the dose-response curves for the single compounds: $D_1 = 1.86 \times 10^{-7}$ M

(chlorpyrifos-oxon), $D_2 = 1.74\times10^{-5}$ M (anthracene). Then the combination index can be calculated:

$$I_c = \frac{d_1}{D_1} + \frac{d_2}{D_2} = \frac{1.8\times10^{-7}\,\mathrm{M}}{1.86\times10^{-7}\,\mathrm{M}} + \frac{2.8\times10^{-6}\,\mathrm{M}}{1.74\times10^{-5}\,\mathrm{M}} \cong 1.16$$

As I_c is approximately 1, this suggests Loewe additivity.

Mixtures of DDT (0.5 µg/ml) and oleic acid (6 µg/ml) and of the single compounds were found to affect the metabolic cooperation (that is the gap junction-mediated intercellular communication) between Chinese hamster V79 cells measured as the recovery of 6-thioguanine-resistant V79 cells (Aylsworth et al., 1989). The recoveries was reported to be 34 %, 26 %, 28 %, and 21 % for the mixture, oleic acid, DDT, and for the control respectively. The fractions of total possible effect for the compounds and the mixture are calculated:

$$f_b(d_1,0) = \frac{f(d_1,0) - f(0,0)}{1 - f(0,0)} = \frac{0.26 - 0.21}{1 - 0.21} = 0.063$$

$$f_b(0,d_2) = \frac{f(0,d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.28 - 0.21}{1 - 0.21} = 0.089$$

$$f_b(d_1,d_2) = \frac{f(d_1,d_2) - f(0,0)}{1 - f(0,0)} = \frac{0.34 - 0.21}{1 - 0.21} = 0.16$$

The expected fraction of total possible effect for the mixture is:

$$\begin{aligned} f_{expected,\,b} &= f_b(d_1,0) + f_b(0,d_2) - f_b(d_1,0)\times f_b(0,d_2) \\ &= 0.063 + 0.089 - 0.063\times0.089 = 0.15 \end{aligned}$$

This is almost the same as measured in the study (0.16) indicating Bliss independence. At higher concentrations of DDT Bliss synergism was indicated as described below.

Mixtures of 12-O-tetradecanoylphorbol-13-acetate (TPA: 0.025 ng/ml) and DDT (500-2000 ng/ml) were found to affect the metabolic cooperation between Chinese hamster V79 cells measured as the recovery of 6-thioguanine-resistant V79 cells (Aylsworth et al., 1989). A quantitative analysis showed Bliss independence at these DDT concentration levels.

Mixtures of varying concentrations of oleic acid (1.5-12 µg/ml) and 2.0 µg/ml DDT were found to affect the metabolic cooperation between Chinese hamster V79 cells measured as the recovery of 6-thioguanine-resistant V79 cells (Aylsworth et al., 1989). The effect of individually addition of the two compounds was also examined. The effect of the mixtures was higher than that of single compounds and a quantitative analysis of the data showed Bliss synergism.

The same group also examined the effect of mixtures of 6 µg/ml oleic acid and varying concentrations of DDT (1.0-3.0 µg/ml) and of the single compounds (Aylsworth et al., 1989). A quantitative analysis of the data indicated Bliss synergism. At 0.5 µg/ml DDT the combined effect could be interpreted as Bliss independence as described above.

The metabolic cooperation between Chinese hamster V79 cells measured as the recovery of 6-thioguanine-resistant V79 cells was found to be affected by mixtures of 12-O-tetradecanoylphorbol-13-acetate (TPA: 0.025 ng/ml) and DDT (3000-4000 ng/ml) (Aylsworth et al., 1989). A quantitative analysis indicated that the combined action was Bliss synergistic at these concentrations.

In an *in vitro* study a mixture of pyrethrin (100 µM) and piperonyl butoxide (400 µM) was reported to inhibit the synaptosome ATPase of rat brain by 49.3 %. Piperonyl butoxide inhibited the synaptosome by 14.5 % whereas pyrethrin did not have an effect (Kakko et al., 2000). This indicates synergism.

The interpretations of the data from the published *in vitro* studies on combined actions of mixtures of pesticides plus non-pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures found is shown in table 5.7.

	Both agents effective individually – similar mechanism	Both agents effective individually – dissimilar mechanism	Only one agent effective individually	Neither agent effective individually
Synergism: combination effect greater than predicted	Loewe synergism: 0	Bliss synergism: 2 (Aylsworth et al., 1989)	Synergism: 1 (Kakko et al., 2000)	Coalism: 0
Additivity: combination effect equal to prediction from reference model	Loewe additivity: 4 (Jett et al., 1999)	Bliss independence: 2 (Aylsworth et al., 1989)	Inertism: 0	Inertism: 0
Antagonism: combination effect less than predicted	Loewe antagonism: 0	Bliss antagonism: 0	Antagonism: 0	

Table 5.7: The interpretations of the data from the published in vitro studies on combined actions of mixtures of pesticides plus non-pesticides are divided into the types of combined action that were described in chapter 2. In each case the number of mixtures found is shown in this table.

5.3 Conclusion and suggestions for further studies on combined action

To summarise this evaluation of data from studies on combined effects of pesticides and of mixtures of pesticides and non-pesticides, it was noted that the literature search ended up with 47 relevant articles with studies of combined effects of pesticides and 18 articles on combined actions of mixtures of pesticides and non-pesticides. The data from these studies were analysed in a way depending on the quality of the data. Only a small number of the articles presented data adequate for such an analysis.

For mixtures of pesticides, the data from 10 *in vivo* studies and 11 *in vitro* were found to be adequate for an interpretation. The articles on *in vivo* studies described the effect of 28 mixtures. Of these mixtures 43 % acted additively (Bliss independence or inertism), 32 % had an effect less than additivity (Bliss/Loewe antagonism, antagonism) and 25 % had an effect greater than additivity (synergism, coalism). The 11 *in vitro* studies described 27 pesticide mixtures of which 33 % of the mixtures acted additively (Loewe additivity, Bliss independence, inertism), 15 % antagonistic (Bliss antagonism, antagonism) and 52 % synergistic (Loewe synergism, synergism) or coalistic.

For mixtures of pesticides and non-pesticides there were adequate data for a quantitative analyse in 2 *in vivo* and 3 *in vitro* studies. The *in vivo* studies described the effect of 5 mixtures and the *in vitro* studies described 9 mixtures. 20 % of the 5 mixtures were found to have an additive action (Bliss independence), 20 % of the mixtures could be described by Bliss antagonism and 60 % of the mixtures were found to act synergistic (Bliss synergism, synergism). In the *in vitro* studies 67 % of the mixtures had an additive action (Loewe additivity, Bliss independence) and 33 % of the mixtures acted synergistic (Bliss synergism, synergism).

The *in vivo* studies on pesticide mixtures were performed at high doses (10×NOAEL or higher). The main reason why the studies were not done at low doses is of course that at low doses no effect of single compounds can be seen and therefore it is expected that the mixture also do not display an effect. A combined action will with high probability not be observed at this level.

In some of the studies an evident synergistic or coalistic action was found. In the *in vivo* studies on combined action of pesticides 14 % were found to have a coalistic action. In these cases neither of the compounds were effective individually. Nevertheless an effect was seen when given together. No relation between dose level and the observed combined action was found and it can therefore not be excluded that pesticides in a mixture act by synergism or coalism even at low doses.

The number of theoretical possible pesticide mixtures is enormous compared to the number of mixtures studied. This together with the overall quality of the data in the studies presented makes it impossible to reach a clear conclusion. Therefore there is a glaring need for further experimental studies on combined actions of pesticides. This is also essential to clarify the role of the models and statements presented in chapter 3 and 4.

IN VIVO								
Compounds in mixture	**Why these compounds?**	**Species**	**Doses and NOAELs (mg/kg bw/day)**	**Exposure**	**Effects studied/found**	**Interpretation**	**Comments**	**Reference**
Chlorfenvinphos (CF), cypermethrin (CM)	Widely used (alone or in mixture)	Rats	CF: 0.75 (NOAEL: 0.05). CM: 18.0 (NOAEL: 5)	By gavage, in soybean oil, 2 weeks	Leucine absorbed during perfusion and retained in the liver	Bliss independence	High dose of CF	Sedrowicz et al., 1996
Toxaphene and parathion or/and 2,4-D	Widely used pesticides	Mice	T: 50 (NOAEL=0.4). P: 5 (NOAEL=0.4). 2,4-D: 50 (NOAEL=1)	Oral, daily for 7 days	Effect on hepatic cytochrome P-450 content	Inertism (3 mixtures)	Only toxaphene had an effect. High doses	Chaturvedi et al., 1991
Parathion, 2,4-D	Widely used pesticides	Mice	P: 5 (NOAEL=0.4). 2,4-D: 50 (NOAEL=1)	Oral, daily for 7 days	Effect on hepatic cytochrome P-450 content	Inertism	None of the compounds had an effect alone. High doses	Chaturvedi et al., 1991
Dimethoate and endosulfan or carbaryl	Widely used in Turkey	Rats	D: 20.4 (NOAEL: 1.2). E: 6.12 (NOAEL: 0.6). C: 10.1 (NOAEL: 14.7)	3.5 months, by gavage, dissolved in sunflower oil	Changes in aspartate aminotransferase (AST) in serum	Inertism (2 mixtures)	Only dimethoate had an effect. High doses of D and E	Selmanoglu et al., 2001
Parathion, toxaphene	Widely used, different chemical classes	Mice	P: 5 (NOAEL: 0.4) T: 50 (NOAEL: 0.35)	Oral intubation, corn oil, daily up to 14 days	Inhibit AChE (brain)	Inertism	Only parathion had an effect. High doses	Kuntz et al., 1990
Endosulfan + dimethoate, carbaryl	Wide use	Rats	E: 6.12 (NOAEL: 0.6) D: 20.4 (NOAEL: 1) C: 10.1 (NOAEL: 14.7)	Oral, 3.5 month	Immune parameter, IgG	Inertism (2 mixtures)	Only endosulfan had an effect. High doses of E and D	Akay et al., 1999
Endosulfan and dimethoate or carbaryl	Wide use	Rats	E: 6.12 (NOAEL: 0.6) D: 20.4 (NOAEL: 1) C: 10.1 (NOAEL: 14.7)	Oral, 3.5 month	Monocytes	Inertism (2 mixtures)	Only endosulfan had an effect. High doses of E and D	Akay et al., 1999
Thiabendazole, fenarimol, diphenylamine, chlorothalonil	Commonly found in foods of central Italy	Rats	0.02-0.149 (NOAELs: T: 10, F: 1.2, D: 7.5, C: 3)	10 d, corn oil	Oxidative liver DNA damage – levels of 8-OH-2-deoxyguanosine relative to 2-deoxyguanosine in DNA	Loewe antagonism	Only D and C had an effect. 1/100 the calculated human exposure through food in Italy. High doses	Lodovici et al., 1997

Hexachlorocyclohexane (HCH), isoproturon	Widely used pesticides. HCH found in food + body tissues of workers	Rats	HCH: 12.5 (NOAEL: 0.5). Isoproturon: 22.5 (NOAEL: 3)	90 days, orally, by gavage, in peanut oil,	Enhanced white blood cell counts	Bliss antagonism	High doses	Raizada et al., 2001
Parathion, 2,4-D	Widely used, different chemical classes	Mice	P: 5 (NOAEL: 0.4) 2,4-D: 50 (NOAEL: 1)	Oral intubation, corn oil, daily up to 14 days	Inhibit AChE (brain)	Bliss antagonism	High doses	Kuntz et al., 1990
Lindane, carbaryl	Frequently found in food and feeds	Rats	L:C: 11:44, 22:150, 44:176 (NOAELs: L: 0.5, C: 14.7)	Oral, in soybean oil for 3 days.	Induction of microsomal enzymes	Antagonism at highest doses. Inertism at lower doses	Only lindane had an effect. High doses	Krechniak et al., 1994
Dimethoate, endosulfan	Widely used in Turkey	Rats	D: 20.4 (NOAEL: 1.2). E: 6.12 (NOAEL: 0.6).	3.5 months, by gavage, dissolved in sunflower oil	Changes in glucose, urea nitrogen in serum	Antagonism	Only dimethoate had an effect. High doses	Selmanoglu et al., 2001
Endosulfan + dimethoate and/or carbaryl	Wide use	Rats	E: 6.12 (NOAEL: 0.6) D: 20.4 (NOAEL: 1) C: 10.1 (NOAEL: 14.7)	Oral, 3.5 month	Immune parameters: IgG, IgM	Antagonism (3 mixtures)	Only endosulfan had an effect. High doses of E and D	Akay et al., 1999
Aldicarb, metribuzin		Rats	Aldicarb: 10 ppb Metribuzin: 10000 ppb	90 days, drinking water, ad libitum	Inhibit the ACh/Ch ratio in hippocampus	Antagonism	ACh/Ch ratio: only metribuzin had an effect	Boyd et al., 1990
Toxaphene, 2,4-D	Widely used, different chemical classes	Mice	T: 50 (NOAEL: 0.35) 2,4-D: 50 (NOAEL: 1)	Oral intubation, corn oil, daily up to 14 days	Effects on SGPT	Synergism day 15 (highest effect).	Only 2,4-D had an effect. High doses	Kuntz et al., 1990
EPN, malathion	Same chemical group (OPs)	Dogs	EPN: 20 ppm (~0.5 mg/kg/day). Malathion: 100 ppm (~2.5 mg/kg/day)	6 weeks, in corn oil. Food and water ad libitum	Inhibit AChE (red blood cells)	Synergism	Only malathion had an effect. Doses: ~ highest NOAEL according to the authors	Williams et al., 1958
EPN, Systox	Same chemical group (OPs)	Dogs	EPN: 20 ppm (~0.5 mg/kg/day). Systox: 2 ppm (~0.05 mg/kg/day)	6 weeks, in corn oil. Food and water ad	Inhibit AChE (plasma)	Synergism?	Qualitative analysis. Doses: ~ highest NOAEL according to the	Williams et al., 1958

				libitum			authors	
Dimethoate and endosulfan or/and carbaryl	Widely used in Turkey	Rats	D: 20.4 (NOAEL: 1.2). E: 6.12 (NOAEL: 0.6). C: 10.1 (NOAEL: 14.7)	3.5 months, by gavage, dissolved in sunflower oil	Increased in alanine aminotransferase (ALT) in serum	Coalism (3 mixtures)	None of the compounds had an effect alone. High doses of D and E	Selmanoglu et al., 2001
Dimethoate, carbaryl	Wide use	Rats	D: 20.4 (NOAEL: 1) C: 10.1 (NOAEL: 14.7)	Oral, 3.5 month	Monocytes, granulocytes	Coalism	None of the compounds had an effect alone. High dose of D	Akay et al., 1999

Table 5.1: Pesticide mixtures. In vivo studies in which the data were adequate for either a quantitative or qualitative analysis. The NOAELs are the ones upon which the ADIs are based according to JMPR. Further details on the studies are shown in appendix 1.

IN VITRO							
Compounds in mixture	**Why these compounds?**	***In vitro* test**	**Concentrations**	**Effects studied/found**	**Interpretation**	**Comments**	**Reference**
Chlorpyrifos-oxon, azinphos-methyl-oxon	Same mechanism	Blood + brains from rats	Conc. of each individual compound was chosen so the ChE inhibition was 10-90 %	Inhibit AChE – (brain)	Loewe additivity		Richardson et al., 2001
Aldicarb, carbofuran, oxamyl	Same chemical group (carbamates)	Plasma ChE from rats	10^{-10}-10^{-5} M. Mixtures: 1:1:1	Inhibit AChE (plasma)	Loewe additivity / weak Loewe synergism	1 mixture, varying conc.	Iyaniwura, 1989
DDT, dieldrin		Chinese hamster V79 cells	DDT: 1.0 µg/ml. Dieldrin: 4-7 µg/ml	Inhibition of metabolic cooperation	Bliss independence		Aylsworth et al., 1989
1) Malathion, carbofuran. 2) Triallate, fenitrothion. 3) Triallate, carbofuran	Same mechanism	Neural cells from the foetal rat brain	10 µL aliquots of 95 % ethanol solutions of the pesticides to cultures	Inhibit AChE (brain)	Inertism (3 mixtures)	Only carbofuran and fenitrothion had an effect	Segal and Fedoroff, 1989
Endosulfan, dieldrin	Chemicals reported to have an (weak) estrogenic activity	MCF-7 focus assay	0.001-10 µM	Formation of foci	Inertism	Only endosulfan had an effect - and only at highest conc.	Arcaro et al., 1998
Toxaphene, dieldrin		Recombinant human estrogen receptor	Toxaphene: 200 nM Dieldrin: 630 nM	Inhibition of 17beta-estradiol binding	Inertism	Only dieldrin had an effect	Arnold et al., 1997
DDT, dieldrin		Chinese hamster V79 cells	DDT: 1.0 µg/ml. Dieldrin: 3 µg/ml	Inhibition of metabolic cooperation	Bliss antagonism		Aylsworth et al., 1989
Pirimiphos methyl, benomyl	Mix found in groundwater and food. Widely used OP + benomyl. Known effect	Human leukaemia cell line HL-60 cells	7.5-30 µg/ml	^{3}H-Leucine incorporation into HL-60 cell protein	1) 1:1 at 4h: antagonism. At lower doses of P and at 24 h: inertism	Only benomyl had an effect	Marinovich et al., 1994
Captafol, polyoxin	Mixture have been applied for registration to commercialise in China	Chinese hamster ovary cells	C: 0.05-3.10 µg/ml. P: 0.0004-0.25 µg/ml	SCE induction. Chromosome aberrations induction	Antagonism	Only captafol had an effect	Wang et al., 1987

Fenitrothion, carbofuran.	Same mechanism	Neural cells from the foetal rat brain	10 µL aliquots of 95 % ethanol solutions of the pesticides to cultures	Inhibit AChE	Weak antagonism?	Qualitative interpretation	Segal and Fedoroff, 1989
Dimethoate (D), azinphos-methyl (A), diazinon (DZ), pirimiphos methyl (P) and benomyl (B)	Mix found in groundwater and food. Widely used Ops + benomyl	Human neuroblastoma cell line, SH-SY5Y	4-15 µg/ml. Based on conc. found in food in Italy	Inhibit protein synthesis	1) P+B, 2) D+DZ+A, 3) D+DZ+A+P+B: synergism	Only one compound in mixture had an effect (B in 1) and 3), A in 2))	Marinovich et al., 1996
Malathion, fenitrothion	Same mechanism	Neural cells from the foetal rat brain	10 µL aliquots of 95 % ethanol solutions of the pesticides to cultures	Inhibit AChE	Synergism	Only fenitrothion had an effect	Segal and Fedoroff, 1989
Trichlorfon + malathion, parathion-methyl or azinphos-methyl	Same chemical group (OPs)	Mutagenicity (yeast)	16-114 mM depending on compound	Mutation frequency	Synergism (3 mixtures)	Only trichlorfon in the mixtures had an effect	Gilot-Delhalle et al., 1983
Dimethoate, diazinon, azinphos-methyl	Mix found in groundwater and food. Widely used OPs. Known effect	Human leukaemia cell line HL-60 cells	100:40:60 µg/ml	^{3}H-Leucine incorporation into HL-60 cell protein	Synergism	Only azinphos-methyl had an effect	Marinovich et al., 1994
1) Dieldrin, chlordane. 2) Dieldrin + alachlor. 3) Dieldrin, chlordane, toxaphene. 4) Toxaphene, chlordane		Recombinant human estrogen receptor	200-630 nM, 1 microM alachlor	Inhibition of 17beta-estradiol binding	1)-3) Synergism. 4) coalism	Only dieldrin had an effect	Arnold et al., 1997
Dimethoate, omethoate, deltamethrin, benomyl	Commonly used pesticides	Human lymphocytes	Total in mixture: 41.5 µg/l	Sister-chromatid exchanges	Coalism	Neither compound was effective individually	Dolara et al., 1992

Table 5.2: Pesticide mixtures. In vitro studies in which the data were adequate for either a quantitative or qualitative analysis. Further details on the studies are shown in appendix 1.

Compounds	Why these compounds?	Doses/concentrations	Exposure	Species/test	Effects studied/found	Interpretation	Comments	Reference
Chlordane, lead oxide	Soil contaminants in Iraq	Chlordane: 75 or 275 mg/kg bw/day. Lead oxide: 50 mg/kg bw/day	Orally. 35 days, chlordane dissolved in corn oil	Mice	Changed testis weight + diameter of the seminiferous tubules	Bliss independence		Al-Omar et al., 2000
Cimetidine, diazinon	Widely used	Cimetidine: 2×80 mg/kg, i.p.. Diazinon: 50 mg/kg i.p.	Intraperitoneal injection of cimetidine at 1 and 24 h prior to diazinon dosing	Rats	Inhibit AChE (RBC), carboxylesterase (plasma, liver)	Bliss antagonism / weak Bliss antagonism		Wu et al., 1996
Cimetidine, diazinon	Widely used	Cimetidine: 2×80 mg/kg, i.p.. Diazinon: 50 mg/kg i.p.	Intraperitoneal injection of cimetidine at 1 and 24 h prior to diazinon dosing	Rats	Inhibit AChE (brain), carboxylesterase (brain)	Weak Bliss synergism, synergism	Carboxylesterase (brain): only diazinon had an effect	Wu et al., 1996
Chlordane, lead oxide	Soil contaminants in Iraq	Chlordane: 75 or 275 mg/kg bw/day. Lead oxide: 50 mg/kg bw/day	Orally. 35 days, chlordane dissolved in corn oil	Mice	Number of Sertoli cells, spermatogonia, spermatids and primary + secondary spermatocytes	Bliss synergism		Al-Omar et al., 2000
Chlorpyrifos-oxon + 1 of 4 PAHs	Chlorpyrifos often used in private homes in USA. PAHs found in house dust	PAHs: 2-50 µM. Chlorpyrifos-oxon: 1-180 nM		*In vitro* (AChE from electric eel and human)	Inhibit AChE	Loewe additivity (4 mixtures)		Jett et al., 1999
DDT, oleic acid		DDT: 0.5 µg/ml O: 6.0 µg/ml		*In vitro* (Chinese hamster V79 cells)	Inhibition of metabolic cooperation	Bliss independence	Bliss synergism at higher DDT concentrations	Aylsworth et al., 1989
DDT, TPA		DDT: 500-2000 ng/ml TPA: 0.025 ng/ml		*In vitro* (Chinese hamster V79 cells)	Inhibition of metabolic cooperation	Bliss independence	Bliss synergism at higher DDT concentrations	Aylsworth et al., 1989

DDT, oleic acid		1) DDT: 1.0-3.0 µg/ml O: 6.0 µg/ml 2) DDT: 2.0 µg/ml O: 1.5-12.0 µg/ml		*In vitro* (Chinese hamster V79 cells)	Inhibition of metabolic cooperation	Bliss synergism		Aylsworth et al., 1989
DDT, TPA		DDT: 3000-4000 ng/ml TPA: 0.025 ng/ml		*In vitro* (Chinese hamster V79 cells)	Inhibition of metabolic cooperation	Bliss synergism		Aylsworth et al., 1989
Pyrethrin, piperonyl butoxide (PBO)	Pyrethrin: widely used. PBO: well known synergist used to intensify the effects of pyrethrins	Pyrethrin: 100 µM. PBO: 400 µM		*In vitro* (cerebral synaptosomes of rat brain	Decreased ATPase activity. Pyrethrin:PBO in 1:4 most efficient mixture.	Synergism		Kakko et al., 2000

Table 5.5: Mixtures of pesticides plus non-pesticides. In vivo and vitro studies in which the data were adequate for quantitative or qualitative analysis. Further details on the studies are shown in appendix 2.

6 Application of risk assessment of pesticide residues found in food in Denmark

In chapter 4 it was suggested that the risk assessment of pesticide mixtures should be done on a case-by-case evaluation in which the available chemical and toxicological data on the pesticides are evaluated in a weight of evidence process. The hazard index with the ADI as the acceptable level in the denominator should be used to evaluate the data. However, in cases where the weight of evidence points out that the compounds in the mixture share a common mechanism the toxicity equivalency factor should be used instead of the hazard index. In this chapter this method will be used on examples of residues of pesticides found in crops to be consumed in Denmark.

To make a weight of evidence approach, knowledge about chemical structures, toxicology (including toxic effects, target organs, and mode of actions), metabolism and metabolites, has to be gathered. In the following the collection of such data will be described and after that, some examples of use of the risk assessment procedure will be described.

6.1 Identification of the active substances of pesticides

The Danish Veterinary and Food Administration carries out a number of spot checks on residues of pesticides in Danish produced and imported food in Denmark every year. The number of samples is more than 2000 per year. These samples are analysed for up to 150 pesticides and metabolites but with a slight variation in number and compounds from year to year. In 1999 The Danish Veterinary and Food Administration analysed 2494 samples for about 150 pesticides and metabolites (Veterinær- og Fødevaredirektoratet, 2000). The samples include fruits and vegetables (analysed for up to 143 pesticides), corn and cereals (analysed for up to 26 pesticides), meat (analysed for up to 8 phosphorous pesticides and 4 pyrethroides) and honey (analysed for 37 pesticides in 1997).

In the period from 1997 to 1999 in total 78 pesticides and three metabolites were found as residues in food in Denmark. In corn and cereals 12 pesticides and one metabolite were found. No pesticides have been found in meat and honey.

The compounds found in fruits and vegetables have been used as fungicides, insecticides, and acaricides (Miljøstyrelsen, 1992, Miljøstyrelsen, 1997, Hayes and Laws, 1991, Compendium of pesticide common names, website). Two plant growth regulators are found in flour and bran. A list of the active compounds of pesticides found as residues in food, and their uses are shown in appendix 3. The designed mode of action of the individual pesticides on the target organism is also shown in this appendix.

6.2 Chemical structure and grouping of the pesticides

The active substances of pesticides in this project were grouped according to their chemical structure and classified according to Hayes and Laws (1991), see table 6.1. This division is used in appendices 3 to 6. In appendix 4 the chemical structure of the compounds are shown (found in Miljøstyrelsen, 1992, Miljøstyrelsen, 1997, Hayes and Laws, 1991, Chemfinder, website).

Chemical groups
Carbamate insecticides
Anticholinesterase carbamates and procarbamates
Organic phosphorus pesticides – acetylcholinesterase-inhibitors
Dimethoxy compounds of category IV
Diethoxy compounds of category IV
Mixed substitute compounds of category IV
Chlorinated hydrocarbon insecticides
DDT and its analoges
Metabolite of DDT
Benzene hexachloride and lindane
Cyclodiene and related compounds
Pesticides derived from plants and other organisms
Pyrethrum and related compounds
Herbicides
Organic phosphorus herbicides
Metabolite of glyphosate
Carbamate herbicides
Fungicides and related compounds
Chloroalkyl thio fungicides
Other aromatic hydrocarbons
Anilino and nitrobenzenoid fungicides
Benzimidazole
Phenylamide (acylalanine type)
Dicarboximide
Azole fungicides
Fungicides not otherwise classified
Plant growth regulators
Quaternary ammonium compounds
Miscellaneous pesticides
Synthetic acaricides
Phenols = metabolites

Table 6.1: The names of the chemical groups that are used in this report to classify the pesticides by their structure. The list is based on the classification used by Hayes and Laws (1991).

6.3 Toxicology of single compounds

Toxicological data on the active substances of the pesticides are listed in appendices 5 and 6. The data are primary from Joint Meeting of Pesticide Residues (JMPR) but for the compounds not been evaluated by JMPR the toxicological data are found in other publications (in Moriya et al., 1983; The e-Pesticide Manual, 1999; IRIS, website; National Toxicology Program, website; TERA, website; and International Labour Organization, website). Appendix 5 contains information from the most important toxicological studies for each of the pesticides. The effects, mode of actions, and target organs of the pesticides are listed together with NOAEL for the effects and the ADI of the compounds. Appendix 6 is partly a summary of appendix 5 (making it more easy to sort out and evaluate the data in the weight of evidence approach) and partly a list of absorption, distribution, excretion, metabolism and major metabolites of the pesticides.

6.4 Risk assessment of pesticide residues found in food in Denmark

In the following ten examples of risk assessment of pesticide residues found in specific crops in Denmark will be shown. The assessment will only consider the risk from eating the actual crops and it does not take the total exposure of these pesticides from other food items and from other sources into account.

Processing effects such as washing, peeling, and heating (boiling or baking) of the fruits and vegetables will reduce the intake of pesticides but these factors are not taken into consideration due to lack of data.

The pesticide mixtures, which will be examined in the following, are identified from data from crop samples in which more than one compound are found as pesticide residues. The Danish Veterinary and Food Administration have carried out the analyses in 1999 (Veterinær- og Fødevaredirektoratet, 2000). The examples will include data from samples in which the highest number of compounds were found and from samples in which the highest concentration of a single compound was found.

Data on each compound used in the weight of evidence approach are presented in appendices 3, 4, 5, and 6 as described above. Only similarities in effects, in mode of actions, and in target organs will be mentioned briefly in this chapter i.e. not all effects of each compound will be described. The flow chart of the risk assessment approach suggested in chapter 4 will be used to decide whether to use the hazard index or the toxicity equivalency factor approach.

Many of the compounds have an effect on the liver. Since a lot of the compounds are metabolised in the liver an effect here will probably be due to this metabolism and is not caused by a similar mode of action. Compounds will therefore not be considered toxicologically similar only based on an unspecific effect on the liver.

When the weight of evidence approach suggest that the hazard index, HI, should be used in the risk assessment, the equation from chapter 3 is to be used:

$$HI = \frac{E_1}{AL_1} + \frac{E_2}{AL_2} + ... + \frac{E_n}{AL_n} = \sum_{i=1}^{n} \frac{E_i}{AL_i}$$

Where E_i is the exposure of each of the compounds and AL_i is the acceptable level of the compounds. ADI for each of the compounds are used as the acceptable level, AL_i. These ADI values can be found in appendix 6.

The exposure, E_i, of each compound in the sample is calculated from the intake of the crop and the residues of the compounds in the sample:

$$\text{Exposure} = (\text{residue} \times \text{intake}) / (\text{weight of person}),$$

where 60 kg is used as the body weight for adults and 15 kg is used for children (1-3 year). The energy intake for a child of that age is about 50 % of that of an adult (Levnedsmiddelstyrelsen, 1996). Data on estimated intake of the actual crops in the present examples are shown in table 6.2 and 6.3. These data are derived from an examination of food intake of the Danes, carried out by the Danish Veterinary and Food Administration in 1995, and data from Statistics Denmark 1987 on food bought in Denmark. The examination from 1995 included 3098 persons of whom 1261 were children and young people (1-14 year), and 1837 were adults. The intake of fruits and vegetables varies a lot from person to person therefore the data are very uncertain.

When the weight of evidence approach shows that the toxicity equivalency factor approach should be used the following equation, which was introduced in chapter 3, must be used:

$$TEQ = \sum C_i \times TEF_i$$

Where C_i is the concentration of each compound in a mixture, that is, the exposure of the compounds calculated from the equation above.

6.4.1 Samples with a high number of pesticides found as residues

Table 6.2 shows results on five samples of crops in which the highest number of compounds were found as residues. The calculated exposure of each compound when eating the actual crop is shown, and this is used for calculating the hazard index. In the following considerations in the weight of evidence approach will be described very shortly and an example of calculating the hazard index will be shown.

6.4.1.1 Example 1: orange, Spain

The first example is a sample of oranges from Spain in which seven pesticides were found: chlorpyrifos, dicofol, imazalil, malathion, ortho-phenylphenol, prothiofos, and tetradifon. The compounds are not similar in chemical structure and do not belong to the same chemical group (see appendix 3 and 4). The toxicological data in appendices 5 and 6 is used to decide whether the mixture should be evaluated by the toxicity equivalency factor approach or the hazard index approach as shown in the flow chart in figure 4.1. The compounds have the following similarities in their toxic action and target organ:

- Dicofol, imazalil, malathion, ortho-phenylphenol, and tetradifon have an effect on the liver but they do not have a common mode of action.
- Dicofol, malathion, and ortho-phenylphenol cause adenomas in the liver and dicofol can also cause liver carcinomas.
- Chlorpyrifos, malathion, and prothiofos act on the nervous system by inhibiting acetylcholinesterase.
- Dicofol and malathion have an effect on the thyroid gland.

In conclusion, there are no common target organ or mode of action for all the compounds in the mixture even though some of the compounds in the mixture have some similarities. The compounds are toxicologically independent and therefore the hazard index is used for risk assessment of this mixture according to figure 4.1.

The average daily intake of orange (including mandarin and clementine) in Denmark is 15 g/person/day for adults. The energy intake of a child is 50 % of that of an adult, which in this case corresponds to an intake of orange of 7.5 g/person/day (see table 6.2).

The residue of chlorpyrifos in sample 1 was 0.04 mg/kg orange, so the exposure to an adult can be calculated:

$$E_{l,adult} = \frac{0.04\text{mg/kg} \times 0.015\text{kg/person/day}}{60\text{kgbw}} = 10^{-5}\,\text{mg/kgbw/day}$$

And to a child:

$$E_{l,child} = \frac{0.04\text{mg/kg} \times 0.0075\text{kg/person/day}}{15\text{kgbw}} = 2.0 \times 10^{-5}\,\text{mg/kgbw/day}$$

Similar calculations are done for the other compounds in the sample, and the results are shown in table 6.2.

The next step is to calculate the hazard index, HI, from the equation shown above. The hazard index for adults is:

$$HI = \frac{10^{-5}}{0.01} + \frac{3.5\times10^{-5}}{0.002} + \frac{9.5\times10^{-4}}{0.03} + \frac{10^{-5}}{0.3} + \frac{3.8\times10^{-4}}{0.4} + \frac{4.5\times10^{-6}}{0.0001} + \frac{1.5\times10^{-5}}{0.03} \cong 0.097$$

and for children:

$$HI = \frac{2\times10^{-5}}{0.01} + \frac{7\times10^{-5}}{0.002} + \frac{1.9\times10^{-3}}{0.03} + \frac{2\times10^{-5}}{0.3} + \frac{7.5\times10^{-4}}{0.4} + \frac{9\times10^{-4}}{0.0001} + \frac{3\times10^{-5}}{0.03} \cong 0.19$$

Both hazard indexes are well below one and it can be concluded that the residues are not expected do constitute a risk.

6.4.1.2 Example 2: oranges, Spain

Example 2 in table 6.2 is another sample of oranges from Spain. It contained the six compounds: chlorpyrifos, diazinon, imazalil, methidathion, tetradifon, and thiabendazole, which belong to six different chemical groups (see appendix 3). The compounds have the following similarities in their toxic action and target organ:

- Imazalil, methidathion, tetradifon, and thiabendazole act on the liver.
- Tetradifon and thiabendazole can have an effect on the kidney.
- Chlorpyrifos, diazinon, and methidathion can affect the nervous system by inhibiting acetylcholinesterase.

This means that there is no overall mode of action or target organ for the compounds in the mixture. The hazard index must be used in the same way as described in example 1. The results of the calculations are shown in table 6.2, and it is seen that the hazard indexes are well below one indicating that the residues will probably not constitute a risk.

6.4.1.3 Example 3: oranges, Spain

Example 3 in table 6.2 is a sample of oranges in which six compounds were found: diazinon, fenthion, imazalil, ortho-phenylphenol, tetradifon and thiabendazole. They belong to different chemical groups, see appendix 3. The compounds have the following similarities in their toxic action and target organ:

- Diazinon, fenthion, imazalil, and ortho-phenylphenol have an effect on the liver.
- Tetradifon and thiabendazole inhibit acetylcholinesterase in brain and erythrocytes.

That is, the compounds in the mixture are toxicologically independent and there are no common target organ or mode of action for all compounds in the mixture. The hazard index is used for risk assessment of this mixture, as shown in table 6.2. The resulting hazard indexes are well below one and the residues are therefore not expected to constitute a risk.

6.4.1.4 Example 4: grapefruits, Cyprus

A sample of grapefruits from Cyprus (example 4 in table 6.2) contained a mixture of the six compounds bromopropylate, carbendazim, imazalil, malathion, phorate, and thiabendazole. The six compounds belong to five different chemical groups but have the following similarities in mode of action and target organ:

- Bromopropylate, carbendazim, imazalil, malathion, phorate, and thiabendazole have an effect on the liver.
- Bromopropylate, malathion, and thiabendazole can affect the thyroid gland but not by the same mode of action.
- Malathion and thiabendazole can affect the kidney weight.
- Malathion and phorate are known to inhibit acetylcholinesterase.

No overall target organ (except the liver) or mode of action was found for all the compounds in the mixture and the hazard index must be used in the risk assessment, see table 6.2. The calculations show hazard indexes well below one for both adults and children, and it is therefore assumed that the residues do not constitute a risk.

6.4.1.5 Example 5: mandarins, clementines, Spain

The sample of mandarins/clementines from Spain presented in example 5 (see table 6.2) contains seven compounds: chlorpyrifos, dicofol, imazalil, malathion, methidathion, tetradifon, and thiabendazole. Only malathion and methidathion belong to the same chemical group namely the one called dimethoxy compounds of category IV. The compounds have the following similarities in mode of action and target organs:

- Dicofol, imazalil, malathion, methidathion, tetradifon, and thiabendazole can affect the liver.
- Dicofol, malathion, and methidathion can cause tumours in the liver.
- Dicofol, malathion, and thiabendazole can affect the thyroid gland but by different mode of actions.
- Chlorpyrifos, malathion and methidathion can affect the nervous system by inhibiting acetylcholinesterase.

In conclusion the compounds in the mixture are toxicologically independent and the hazard index should therefore be used for risk assessment of this mixture, see table 6.2. The calculated hazard indexes are well below one and the residues are therefore not expected to constitute a risk.

Crop	Compounds	Residue (mg/kg)	Exposure = (Residue × intake) / (weight of person) (mg/kg bw/day)		ADI (mg/kg bw)	Hazard Index – adult	Hazard Index – child
			Adult	Child			
1. Orange, Spain (15 g/person/day incl. mandarin/clementine)	Chlorpyrifos	0,04	10^{-5}	2×10^{-5}	0,01		
	Dicofol	0,14	3.5×10^{-5}	7×10^{-5}	0,002		
	Imazalil	3,8	9.5×10^{-4}	1.9×10^{-3}	0.03		
	Malathion	0,04	10^{-5}	2×10^{-5}	0.3		
	Ortho-phenylphenol	1,5	3.8×10^{-4}	7.5×10^{-4}	0.4		
	Prothiofos	0,018	4.5×10^{-6}	9×10^{-6}	0.0001		
	Tetradifon	0,06	1.5×10^{-5}	3×10^{-5}	0.03	9.7×10^{-2}	0.19
2. Orange, Spain (15 g/person/day incl. mandarin/clementine)	Chlorpyrifos	0,06	1.5×10^{-5}	3×10^{-5}	0.002		
	Diazinon	0,04	10^{-5}	2.0×10^{-5}	0,007		
	Imazalil	2,4	6×10^{-4}	1.2×10^{-3}	0.03		
	Methidathion	0,08	2×10^{-5}	4×10^{-5}	0.4		
	Tetradifon	0,02	5×10^{-6}	10^{-5}	0.03		
	Thiabendazole	2,9	7.3×10^{-4}	1.45×10^{-3}	0.1	3.6×10^{-2}	7.3×10^{-2}
3. Orange, Spain (15 g/person/day incl. mandarin/clementine)	Diazinon	0,15	3.8×10^{-5}	7.5×10^{-5}	0.002		
	Fenthion	0,02	5×10^{-6}	10^{-5}	0,007		
	Imazalil	1,2	3×10^{-4}	6×10^{-4}	0.03		
	Ortho-phenylphenol	1,7	4.3×10^{-4}	8.6×10^{-4}	0.4		
	Tetradifon	0,013	3.3×10^{-6}	6.6×10^{-6}	0.03		
	Thiabendazole	1,5	3.8×10^{-4}	7.5×10^{-4}	0.1	3.4×10^{-2}	6.9×10^{-2}
4. Grapefruit, Cyprus (1 g/person/day)	Bromopropylate	0,34	5.7×10^{-6}	1.1×10^{-5}	0.03		
	Carbendazim	0,05	8.3×10^{-7}	1.7×10^{-6}	0.03		
	Imazalil	0,97	1.6×10^{-5}	3.2×10^{-5}	0.03		
	Malathion	0,028	4.7×10^{-7}	9.3×10^{-7}	0.3		
	Phorate	0,015	2.5×10^{-7}	5×10^{-7}	0.0005		
	Thiabendazole	3,9	6.5×10^{-5}	1.3×10^{-4}	0.1	1.9×10^{-3}	3.8×10^{-3}
5. Mandarin, clementine, Spain (15 g/person/day incl. orange)	Chlorpyrifos	0,02	5×10^{-6}	10^{-5}	0,01		
	Dicofol	0,04	10^{-5}	2×10^{-5}	0,002		
	Imazalil	2,9	7.3×10^{-4}	1.45×10^{-3}	0.03		
	Malathion	0,01	2.5×10^{-6}	5×10^{-6}	0.3		
	Methidathion	0,06	1.5×10^{-5}	3×10^{-5}	0.001		
	Tetradifon	0,02	5×10^{-6}	10^{-5}	0.03		
	Thiabendazole	2,2	2.5×10^{-5}	5×10^{-5}	0.1	4.5×10^{-2}	9.0×10^{-2}

Table 6.2: Five examples of samples with more than one pesticide found as residues in a crop. These examples are the ones in which the highest number of compounds is found. In the first column the average daily intake of each crop per person (adults) are shown. The child is expected to have an energy intake of 50 % of the adult. The calculated exposure of each compound when eating the actual crop is shown, and used for calculating the hazard index. To calculate the exposure, 60 kg is used as the body weight for an adult, and 15 kg for a child (1-3 year).

6.4.2 Samples with a high concentration of pesticides residues

Five examples of samples with more than one pesticide residue are shown in table 6.3. The examples are chosen because they contain the highest concentration of a single compound. The exposure of each compound when eating the actual crop is calculated as above and the results are shown in this table. These data are used to calculate the hazard index or the toxicity equivalency factor. In the following considerations in the weight of evidence approach will be described shortly.

6.4.2.1 Example 6: thyme, Denmark

High concentrations of pirimicarb and vinclozolin were found as residues in a sample of thyme from Denmark, see table 6.3. The two compounds belong to different chemical groups, see appendix 3. Pirimicarb affects the nervous system by inhibiting acetylcholinesterase activity whereas vinclozolin has an effect on liver, spleen, prostate, testis and adrenals (see appendix 5 and appendix 6). They are toxicologically independent and therefore the hazard index has to be used. The hazard index for both adults and children are found to be well below 1 (see table 6.3) indicating that the mixture does not constitute a risk.

6.4.2.2 Example 7: Spinach, Denmark

In a sample of spinach from Denmark, residues of dithiocarbamates (analysed as a group of 6 compounds) and metalaxyl are found as shown in table 6.3. The compounds do not have any similarity in chemical structure or toxicology. Dithiocarbamates have an effect on the thyroid gland and some developmental toxicity, whereas metalaxyl decreases the total leucocyte count, increases the serum alkaline phosphatase levels, and increases adrenal and liver weight. The hazard index has to be used in the risk assessment and the calculated hazard indexes are shown in table 6.3. The lowest ADI for the group of dithiocarbamates has been used (ADI for ferbam and ziram) in the calculation. The calculated hazard indexes are below one for both adults and children and are therefore not expected to constitute a risk.

6.4.2.3 Example 8: Mandarin, clementine, South Africa

In example 8 imazalil and thiabendazole were found in mandarin/clementine from South Africa, see table 6.3. The two compounds belong to two different chemical groups but they both affect the liver weight. Thiabendazole causes haemosiderosis in liver but it is not clear how the liver is affected by imazalil so the hazard index approach is used in the risk assessment. The hazard indexes for both adults and for children are well below one and the residues is therefore not expected to constitute a risk.

6.4.2.4 Example 9: Black currant, Denmark

Dithiocarbamates (analysed as a group of 6 compounds) and tolylfluanid was found in black currant from Denmark, see table 6.3. Dithiocarbamates are developmental toxic, mainly tera-

togenic and tolylfluanid are maternal- and embryo toxic. There is not enough data to clarify whether the compounds are toxicologically similar or not, therefore the hazard index has to be used. The lowest ADI for the group of dithiocarbamates has been used (ADI for ferbam and ziram) in the calculation. The hazard indexes are well below one and the residues are therefore not expected to constitute a risk.

6.4.2.5 Example 10: Kiwi, Italy

Two dicarboximides, iprodione and vinclozolin, was found in kiwi from Italy, see table 6.3. They both have an effect on the liver and the adrenal gland. Iprodione has been reported to cause non-neoplastic lesions in the liver and vinclozolin causes hepatocellular carcinomas. Iprodion are reported to cause histopathological changes in the adrenals, and vinclozolin to cause tumours. The toxicological data for the compounds indicate toxicological similarity and the data are considered to be good enough for using the toxicity equivalency factor approach. The data for the two compounds are considered to be of the same quality and therefore it doesn't matter whether vinclozolin or iprodione is chosen as the index compound.

The toxicity equivalency factor, TEF, is calculated from the ADI values for the individual pesticides shown in table 6.3. ADI for iprodione is 0.06 mg/kg bw and ADI for vinclozolin is 0.01 mg/kg bw. If iprodione is chosen to be the index compound, TEF for vinclozolin is 6 (0.06 mg/kg bw / 0.01 mg/kg bw). The toxicity equivalent for adults can then be calculated as described in the beginning of section 6.4:

$$TEQ = \sum C_i \times TEF_i$$
$$= (7 \times 10^{-6}\,\text{mg/kgbw/day}) \times 1 + (2.9 \times 10^{-4}\,\text{mg/kgbw/day}) \times 6 \cong 1.7 \times 10^{-3}\,\text{mg/kgbw/day}$$

This is a factor 35 below ADI for the index compound (iprodione) and therefore the residues are not considered to constitute a risk.

TEQ for children is:

$$TEQ = (1.4 \times 10^{-5}\,\text{mg/kgbw/day}) \times 1 + (5.7 \times 10^{-4}\,\text{mg/kgbw/day}) \times 6 \cong 3.4 \times 10^{-3}\,\text{mg/kgbw/day}$$

This is a factor 17.5 below ADI for iprodione and therefore the residues are not considered to constitute a risk.

Crop	Compounds	Residue (mg/kg)	Exposure = (Residue × intake) / (weight of person) (mg/kg bw/day)		ADI (mg/kg bw)	Hazard Index – adult	Hazard Index – child
			Adult (60 kg/person)	Child (15 kg/person)			
6. Thyme, Denmark (0.05 g/person/day)	Pirimicarb	5,6	4.7×10^{-6}	9.3×10^{-6}	0,02	2.7×10^{-3}	5.5×10^{-3}
	Vinclozolin	30	2.5×10^{-5}	5×10^{-5}	0.01		
7. Spinach, Denmark (1.3 g/person/day)	Dithiocarbamates	22	4.8×10^{-4}	9.5×10^{-4}	0.003 (for ferbam and ziram)	0.16	0.32
	Metalaxyl	1,8	3.9×10^{-5}	7.8×10^{-5}	0.03		
8. Mandarin, clementine, Spain (15 g/person/day incl. orange)	Imazalil	0,41	1.0×10^{-4}	2.0×10^{-4}	0.03	1.8×10^{-2}	3.7×10^{-2}
	Thiabendazole	6	1.5×10^{-3}	3×10^{-3}	0.1		
9. Black currant, Denmark (0.2 g/person/day)	Dithiocarbamates	4.6	1.5×10^{-5}	3.1×10^{-5}	0.003	5.1×10^{-3}	1.0×10^{-2}
	Tolylfluanid	0.96	3.2×10^{-6}	6.4×10^{-6}	0.1		
10. Kiwi, Italy (3 g/person/day)	Iprodione	0.14	7×10^{-6}	1.4×10^{-5}	0.06	-	-
	Vinclozolin	5.7	2.9×10^{-4}	5.7×10^{-4}	0.01	-	-

Table 6.3: Five examples of samples with more than one pesticide found as residues in a crop. These examples are selected because they contain the highest concentrations of a single compound. In the first column the average daily intake of each crop per person (adults) are shown. The child is expected to have an energy intake of 50 % of the adult. The calculated exposure of each compound when eating the actual crop is shown, and used for calculating the hazard index. To calculate the exposure, 60 kg is used as the body weight for an adult and 15 kg for a child (1-3 year). In example 10 the toxicity equivalency factor approach is used in the risk assessment instead of the hazard index, see text.

6.5 Discussion and conclusion

Examples on risk assessment of pesticide residues found in specific crops consumed in Denmark have been shown in this chapter. The assessment did only consider the risk from eating the actual crops and it did not take the total exposure of these pesticides from other food items and from other sources into account. Such an evaluation would be very interesting but it is beyond the objective of this report.

Reduction factors for processing effects (such as washing, peeling, drying, and heating) were not taken into consideration in the calculations because of lack of information.

The data for intake of each food item and with that the exposure data used in the risk assessment are very uncertain. The data are average daily consumption and they cover up huge differences in intake of fruits and vegetables.

Not all crops contain residues of pesticides in the amount, which is found in the presented examples but it can be expected that the compounds could be found if only the detection limits of the analysis were low enough. The examples are kind of worst-case scenarios and if these levels were used in a prediction of the total pesticide intake it would probably lead to an overestimation of the actual intake of pesticides.

The examples have shown that the suggested risk assessment method is feasible for use in practice and the actual levels of pesticide residues in the examples are not expected to constitute a risk to humans.

7 Conclusions and recommendations

The risk assessment of pesticide residues in food is based on toxicological evaluation of the single compounds. In the current process most toxicological data come from guideline studies on individual compounds. Humans are often exposed to more than one pesticide at the same time and consequently potential combined actions of pesticides need to be addressed in the risk assessment process. A major obstacle in doing so is the lack of data from studies on pesticide mixtures employing standard toxicological methods, such as short-term and long-term animal studies. Thus, the regulatory agencies are faced with the situation that they cannot always reliably predict whether the simultaneous exposure to pesticide residues in food constitute an increased risk. As the possible combinations of pesticides are innumerable and experimental testing of all such mixtures is not feasible from obvious reasons, there is a need for science based advise on how exposure to mixtures of pesticides can be dealt with in the risk assessment.

Several reports have suggested that since the pesticides are found in food at levels well below their respective no observed adverse effect levels (NOAEL), the approaches based on toxicological similar mechanism (same mechanism of action) and toxicological independence (different mechanism of action) should be used for risk assessment of pesticide residues. In fact, it has been suggested that methods for toxicologically similar compounds could be used in most cases, even when the compounds are not toxicologically similar.

This review demonstrates that there is no scientific background for establishing a general standard formula for risk assessment of pesticide mixtures in food. The number of experimental animal studies on combined actions of pesticides published in the scientific literature is too low and the overall quality of the data in the studies is not good enough to reach a clear conclusion. The present *in vivo* studies on pesticide mixtures are performed at high doses (ten times NOAEL or higher) compared to the expected pesticide residues in food. Studies employing high doses have shown both additivity and interactions such as synergism or coalism as well as antagonism. None of the studies reported in the literature have used low pesticide doses, in range of normally found residues. However, studies with mixtures at low doses of other chemicals have not been found to demonstrate a risk different from that of the single compounds in the mixture, and it can be concluded from these data that combined exposure to arbitrarily chosen chemicals does not demonstrate more than an additive action when all chemicals in the mixture are administrated at their own individual NOAELs.

At the moment the best suggestion for a feasible risk assessment of pesticide mixtures in crops is to make a case-by-case evaluation in which the available chemical and toxicological data on the actual pesticides are evaluated in a weight of evidence process. Then the hazard index with the ADI's (where the individual safety factors are included) and not the NOAEL's (where the safety factors are not included) as the acceptable level in the denominator is recommended for use. However, when the weight of evidence process points out that the compounds in the mixture share a common mechanism (e.g. for the organophosphorus pesticides), the toxicity equivalency factor approach should be used, if possible.

This method has been tried out in this report for some pesticide mixtures found as residues in single crops in Denmark. The method was found to be feasible for use in practice.

The area risk assessment of pesticide mixtures is relatively new and further research is required. Indeed there is a glaring need for further experimental studies on combined actions of pesticides to clarify the role of the models and statements presented in this report.

8 References

1. Akay MT, Özmen G, Elcüman EA. Effects of combinations of endosulfan, dimethoate and carbaryl on immune and hematological parameters of rats. Vet Hum Toxicol 1999;41(5):296-299.
2. Albert RE, Lewtas J, Nesnow S, Thorslund TW, Anderson E. Comparative potency method for cancer risk assessment application to diesel particulate emissions. Risk Anal 1983;3(2):101-117.
3. Al-Omar M A, Abbas A K, Al-Obaidy S A. Combined effect of exposure to lead and chlordane on the testicular tissues of Swiss mice. Toxicol Lett 2000;115:1-8.
4. Altenburger R, Bödeker W, Faust M, Grimme H. Evaluation of the isobologram method for the assessment of mixtures of chemicals. Combination effect studies with pesticides in algal biotests. Ecotoxicol Environ Saf 1990;20;(1):98-114.
5. Arcaro KF, Vakharia DD, Yang Y, Gierthy JF. Lack of synergy by mixtures of weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides. Environmen Health Perspect 1998;106(Suppl. 4):1041-1046.
6. Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ Jr, McLachlan JA. Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 1996;272(5267):1489-1492.
7. Arnold SF, Vonier PM, Collins BM, Klotz DM, Guillette LJ Jr, McLachlan JA. *In vitro* synergistic interaction of alligator and human estrogen receptors with combinations of environmental chemicals. Environ Health Perspect 1997;105(Suppl 3):615-618.
8. Ashton F M, Crafts A S. Mode of action of herbicides. A Wiley-Interscience Publication. John Wiley & sons, Inc.; 1973.
9. Aylsworth C F, Trosko J E, Chang C C, Benjamin K, Lockwood E. Synergistic inhibition of metabolic cooperation by oleic acid or 12-O-tetradecanoylphorbol-13-acetate and dichlorodiphenyltrichlorethane (DDT) in Chinese hamster V79 cells: Implication of a role for protein kinase C in the regulation of gap junctional intercellular communication. Cell Biol Toxicol 1989;5:27-37.
10. Berenbaum MC, What is synergy? Pharmacol Rev 1989;41:93-141.
11. Bessi H, Rast C, Rether B, Nguyen BA G, Vasseur P. Synergistic effects of chlordane and TPA in multistage morphological transformation of SHE cells. Carcinogenesis (Oxford) 1995;16(2):237-244.
12. Bichel-udvalget: Rapport fra underudvalget om miljø og sundhed. 12. Marts 1999. In Danish. Website: www.mst.dk/udgiv/publikationer/1999/87-7909-424-4/html/
13. Bliss, CI. The toxicity of poisons applied jointly. Ann Appl Biol 1939;26;585-615.
14. Botham P, Chambers J, Kenyon E, Matthews HBS, Sultatos L, Van Pelt, et al. Report of the toxicology breakout group (BOG). In: A Framework for cumulative risk assessment. Mileson B, Faustman E, Olin S, Ryan PB, Ferenc S, Burke T, editors. An ILSI Risk Science Institute workshop report, International Life Sciences Institute; 1999.
15. Boyd CA, Weiler MH, Porter WP. Behavioral and neurochemical changes associated with chronic exposure to low-level concentration of pesticide mixtures. J Toxicol Environ Health 1990;30(3):209-21.

16. Calciu C, Chan HM, Kubow S. Toxaphene congeners differ from toxaphene mixtures in their dysmorphogenic effects on cultured rat embryos. Toxicology 1997;124(2):153-62.
17. Callender TJ, Morrow L, Subramanian K. Evaluation of chronic neurological sequelae after acute pesticide exposure using SPECT brain scans. J of Toxicol Environ Health 1994;41(3):275-284.
18. Cassee FR, Groten JP, van Bladeren PJ. Toxicological evaluation and risk assessment of chemical mixtures. Crit Rev Toxicol 1998;28(1):73-101.
19. Chaturvedi A K. Toxicological evaluation of mixtures of ten widely used pesticides. J Appl Toxicol 1993;13(3):183-188.
20. Chaturvedi AK, Kuntz DJ, Rao NGS. Metabolic aspects of the toxicology of mixtures of parathion, toxaphene and/or 2,4-D in mice. J Appl Toxicol 1991;11(4):245-251.
21. Chemfinder. Website: www.chemfinder.com
22. Chu I, Secours V, Villeneuve DC, Valli VE, Nakamura A, Colin D, et al. Reproduction study of toxaphene in the rat. J Environ Sci Health [B] 1988;23(2):101-126.
23. Chu I, Villeneuve DC, Sun CW, Secours V, Procter B, Arnold E, et al. Toxicity of toxaphene in the rat and beagle dog. Fundam Appl Toxicol 1986;7(3):406-418.
24. Compendium of pesticide common names. Website: www.hclrss.demon.co.uk/
25. Conolly RB. Toxicological highlight: Biologically motivated quantitative models and the mixture toxicity problem. Toxicol Sci 2001;63:1-2.
26. Corbett JR. The Biochemical Mode of Action of Pesticides. London: Academic Press; 1974.
27. Dolara P, Salvadori M, Capobianco T, Torricelli F. Sister-chromatid exchanges in human lymphocytes induced by dimethoate, omethoate, deltamethrin, benomyl and their mixture. Mutat Res 1992;283(2):113-118.
28. Dolara P, Torricelli F, Antonelli N. Cytogenetic effects on human lymphocytes of a mixture of fifteen pesticides commonly used in Italy. Mutat Res 1994;325(1):47-51.
29. Dolara P, Vezzani A, Caderni G, Coppi C, Torricelli F. Genetic toxicity of a mixture of fifteen pesticides commonly found in the Italian diet. Cell Biol Toxicol 1993;9(4):333-343.
30. Duke SO. Overview of herbicide mechanisms of action. Environ Health Perspect 1990;87:263-271.
31. el-Masri HA, et al., Integrated approaches for the analysis of toxicologic interactions of chemical mixtures. Crit Rev Toxicol 1997;27(2):175-97.
32. Eroschenko VP, Johnson TJ. Estradiol and pesticide methoxychlor do not exhibit additivity or synergism in the reproductive tract of adult ovariectomized mice. J Toxicol Environ Health A 2000;60(6):407-421.
33. Fenner-Crisp PA. FQPA science issues: common mechanism of toxicity and cumulative risk assessment. Regul Toxicol Pharmacol 2000;31:208-310.
34. Feron V J, Groten J P, van Zorge J A, Cassee F R, Jonker D, van Bladeren P J. Toxicity studies in rats of simple mixtures of chemicals with the same or different target organs. Toxicol Lett 1995a;82-83:505-512.
35. Feron V J, Groten JP, Jonker D, Cassee FR, van Bladeren PJ. Toxicology of chemical mixtures: challenges for today and the future. Toxicology 1995b;105(2-3):415-27

36. Gaughan LC, Engel JL, Casida JE. Pesticide interactions: effects of organophosphorus pesticides on the metabolism, toxicity, and persistence of selected pyrethroid insecticides. Pest Biochem Physiol 1980;14:81-85.
37. Germolec DR, Yang RSH, Ackermann MF, Rosenthal GJ, Boorman GA, Blair P, Luster MI. Toxicology studies of a chemical mixture of 25 groundwater contaminants II. Immunosuppression in B6C3F$_1$ mice. Fundam Appl Toxicol 1989;13:377-387.
38. Gessner, P K. Isobolographic analysis of interactions: an update on applications and utility. Toxicology 1995;105(2-3);161-179.
39. Gilot-Delhalle J, Coluzzi A, Moutschen J, Moutschen-Dahmen M. Mutagenicity of some organophosphorous compounds at the ade-6 locus of Schizosaccharomyces pombe. Mutat Res 1983;117:139-148.
40. Gomes J, Dawodo AH, Lloyd O, Revitt DM, Anilal SV. Hepatic injury and disturbed amino acid metabolism in mice followling prolonged exposure to organophosphorus pesticides. Hum Exp Toxicol 1999;18(1):33-37.
41. Greco W R, Unkelbach H D, Pöch G, Sühnel J, Kundi M, Bodeker W. Consensus on concepts and terminology for interaction assessment: the Saaresilkä agreement. Arch Complex Environ Stud 1992;4:65-69.
42. Groten JP, Schoen ED, van Bladeren PJ, Frieke Kuber C, van Zorge JA, Feron VJ. Subacute toxicity of a mixture of nine chemicals in rats: detecting interactive effects with a fractionated two-level factorial design. Fundam Appl Toxicol 1997;36:15-29.
43. Gyorkos J, Denomme MA, Leece B, Homonko K, Valli VE, Safe S. Reconstituted halogenated hydrocarbon pesticide and pollutant mixtures found in human tissues: effects on the immature male Wistar rat after short-term exposure. Can J Physiol Pharmacol 1985;63(1):36-43.
44. Hayes W Jr., Laws, ER Jr., editors. Handbook of pesticide toxicology. Volume 1-3. Academic Press; 1991.
45. Heindel JJ, Chapin RE, Gulati DK, George JD, Price CJ, Marr MC, et al. Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. Fundam Appl Toxicol 1994;22(4):605-621.
46. Hertzberg RC, Rice G, Teuschler LK. Methods for health risk assessment of combustion mixtures. In: Hazardous waste incineration: evaluating the human health and environmental risks. Roberts S, Teaf C, Bean J, editors. Boca Raton, FL. CRC Press;1999. p. 105-148.
47. Hrelia P, Maffei F, Vigagni F, Fimognari C, Flori P, Stanzani R et al. Interactive effects between trichloroethylene and pesticides at metabolic and genetic level in mice. Environ Health Perspect 1994;102(Suppl. 9):31-34.
48. Hurley PM. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ Health Perspect 1998;106(8):437-45.
49. Ikeda, M. Multible exposure to chemicals. Regul Toxicol Pharmacol 1988;8;414-421.
50. International Labour Organization, International Training Centre. International programme on chemical safety. Website: www.itcilo.it/english/actrav/telearn/osh/kemi/alpha2.htm

51. IRIS Integrated Risk Information System, U.S. EPA website: www.epa.gov/iriswebp/iris/index.html
52. Ito N, Hagiwara A, Tamano S, Futacuchi M, Imaida K, Shirai T. Effects of pesticide mixtures at the acceptable daily intake levels on rat carcinogenesis. Food Chem Toxicol 1996/1997);34(11-12):1091-1096.
53. Ito N, Hagiwara A, Tamano S, Hasegawa R, Imaida K, Hirose M, et al. Lack of carcinogenicity of pesticide mixtures administered in the diet at acceptable daily intake (ADI) dose levels in rats. Toxicol Lett (Shannon) 1995a;82-83(Spec. issue):513-520.
54. Ito N, Hasegawa R, Imaida K, Kurata Y, Hagiwara A, Shirai T. Effect of ingestion of 20 pesticides in combination at acceptable daily intake levels on rat liver carcinogenesis. Food Chem Toxicol 1995b;33(2):159-163.
55. Ito N, Imaida, K, Hirose M, Shirai T. Medium-term bioassays for carcinogenicity of chemical mixtures. Environ Health Perspect 1998;106(Supp. 6)1331-1336.
56. Iyaniwura TT. Relative inhibition of rat plasma and erythrocyte cholinesterases by pesticide combinations. Vet Hum Toxicol 1991;33(2):166-168.
57. Iyaniwura, TT. An *in vitro* evaluation of the potential toxicities and interactions of carbamate pesticides. Toxicol In Vitro 1989;3(2):91-93.
58. Jett D A, Navoa R V, Lyons Jr. M A. Additive inhibitory action of chlorpyrifos and polycyclic aromatic hydrocarbons on acetylcholinesterase activity *in vitro*. Toxicol Lett 1999;105:223-229.
59. JMPR: Evaluations of the toxicity of pesticide residues in food. FAO / WHO. 1965.
60. JMPR: Evaluations of the toxicity of pesticide residues in food. Geneva: FAO / WHO. 1966.
61. JMPR. 1968 Evaluations of some pesticide residues in food. Geneva: FAO / WHO; 1969.
62. JMPR. 1969 Evaluations of some pesticide residues in food. Rome: FAO / WHO; 1970.
63. JMPR. 1970 Evaluations of some pesticide residues in food. Rome: FAO / WHO; 1971.
64. JMPR. 1973 Evaluations of some pesticide residues in food. Geneva: FAO / WHO; 1974.
65. JMPR. 1974 Evaluations of some pesticide residues in food. Rome: FAO / WHO; 1975.
66. JMPR. Pesticide residues in food – 1977 evaluations. The monographs. Rome: FAO / WHO; 1978.
67. JMPR. Pesticide residues in food – 1978 evaluations. The monographs. Rome: FAO / WHO; 1979.
68. JMPR. Pesticide residues in food – 1979 evaluations. The monographs. Rome: FAO / WHO; 1980.
69. JMPR. Pesticide residues in food – 1980 evaluations. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1981.
70. JMPR. Pesticide residues in food – 1981 evaluations. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1982.
71. JMPR. Pesticide residues in food – 1982 evaluations. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1983.

72. JMPR. Pesticide residues in food – 1983. The monographs. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1985.
73. JMPR. Pesticide residues in food – 1984. The monographs. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1985.
74. JMPR. Pesticide residues in food – 1985. Part II – Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1986.
75. JMPR. Pesticide residues in food – 1986. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1987.
76. JMPR. Pesticide residues in food – 1987. Part II – Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1988.
77. JMPR. Pesticide residues in food – 1988. Part II – Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1989.
78. JMPR. Pesticide residues in food – 1989 evaluations. Part II - Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Rome: FAO / WHO; 1990.
79. JMPR. Pesticide residues in food – 1990 evaluations. Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1991.
80. JMPR. Pesticide residues in food – 1991 evaluations. Part II – Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1992.
81. JMPR. Pesticide residues in food – 1992 evaluations. Part II – Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1993.
82. JMPR. Pesticide residues in food – 1993 evalations. Part II – Toxocology. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1994.
83. JMPR. Pesticide residues in food – 1994 evaluations. Part II – Toxicology. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1995.
84. JMPR. Pesticide residues in food – 1995. Part II – Toxocological and Environmental. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1996.
85. JMPR. Pesticide residues in food – 1996. Toxicological. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1997.
86. JMPR. Pesticide residues in food – 1997. Part II – Toxocological and Environmental. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1998.
87. JMPR. Pesticide residues in food – 1998. Part II – Toxocological. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 1999.
88. JMPR: Pesticide residues in food – 1999. Part II – Toxocological. Joint FAO / WHO Meeting on Pesticide Residues. Geneva: FAO / WHO; 2000.
89. Jonker D, Woutersen RA, Feron VJ. Toxicity of mixtures of nephrotoxicants with similar or dissimilar mode of action. Food Chem Toxicol 1996;34(11-12):1075-1082.
90. Jonker D, Woutersen RA, van Bladeren PJ, Til HP, Feron VJ. 4-Week oral toxicity study of a combination of eight chemicals in rats: Comparison with the toxicity of the individual compounds. Food Chem Toxicol 1990;28:623-631.
91. Jonker D, Woutersen RA, van Bladeren PJ, Til HP, Feron VJ. Subacute (4-wk) oral toxicity of a combination of four nephrotoxins in rats: Comparison with the toxicity of the individual compounds. Food Chem Toxicol 1993;31(2):125-136.
92. Joshi UM, Thornburg JE. Interactions between cimetidine, methylparathion, and parathion. J Toxicol Environ Health 1986;19(3):337-44.
93. Kakko I, Toimela T, Tähti H. Piperonyl butoxide potentiates the synaptosome AT-Pase inhibiting effect of pyrethrin. Chemosphere 2000;40:301-305.

94. Kholkute SD, Rodriguez J, Kaneene JB, Dukelow WR. Effects of a pesticide mixture and two herbicide mixtures on *in vitro* fertilization in the mouse. In Vitro Toxicol 1993;6(4):291-298.
95. Kligerman AD, Chapin RE, Erexson GL, Germolec DR, Kwanyuen P, Yang RSH. Analyses of cytogenetic damage in rodents following exposure to simulated groundwater contaminated with pesticides and a fertilizer. Mutat Res 1993;300(2);125-134.
96. Könemann WH, Pieters MN. Confusion of concepts in mixture toxicology. Food Chem Toxicol 1996;34(11-12):1025-1031.
97. Krechniak J, Englot B, Wrzesniowska K, Hac E. Interaction of lindane and carbaryl on hepatic microsomal enzymes in rats. Bull Environ Contam Toxicol 1994;52:927-934.
98. Krishnan K, Andersen ME, Clewell III, HJ, Yang RSH. Physiologically based pharmacokinetic modeling of chemical mixtures. In: Toxicology of Chemical Mixtures. Yang RSH, editor. Academic Press. New York; 1994. p. 399-437
99. Krishnan K, Paterson J, Williams DT. Health Risk Assessment of Drinking Water Contaminants in Canada: The Applicability of Mixture Risk Assessment Methods. Regul Toxicol Pharmacol 1997;26(2):179-187.
100. Kuntz DJ, Rao NGS, Berg IE, Khattree R, Chaturvedi AK. Toxicity of mixtures of parathion, toxaphene and/or 2,4-D in mice. J Appl Toxicol 1990;10(4):257-266.
101. Larsen JC et al. Combined actions and interactions of chemicals in mixtures. The toxicological effects of exposure to mixtures of industrial and environmental chemicals. In preparation.
102. Larsen JC. Personal communication.
103. Levnedsmiddelstyrelsen. Nordiske næringsstofanbefalinger 1996. LST-Nyt 1996;4.
104. Lewtas J, Nesnow S, Albert RE. A comparative potency method for cancer risk assessment: Clarification of the rationale, theoretical basis, and application to diesel particulate emmisions. Risk anal 1993;3:133-137.
105. Lewtas J. Development of a comparative potency method for cancer risk assessment of complex mixtures using short-term in vivo and in vitro bioassays. Toxicol Ind Health 1985;1:193-203.
106. Lodovici M, Aiolli S, Monserrat C, Dolara P, Medica A, Di Simplicio P. Effect of a mixture of 15 commonly used pesticides on DNA levels of 8-hydroxy-2-deoxyguanosine and xenobiotic metabolizing enzymes in rat liver. J Environ Pathol Toxicol Oncol 1994;13(3):163-8.
107. Lodovici M, Casalini C, Briani C, Dolara P. Oxidative liver DNA damage in rats treated with pesticide mixtures. Toxicology 1997;117(1):55-60.
108. Loewe S, Muischnek H. Über kombinationswirkungen. I. Mitteilung: Hilfsmittel der Fragestellung. Naunyn-Schmiedebergs Arch Pharmakol 1926;114;313-326.
109. Ma TH, Sandhu SS, Peng Y, Chen TD, Kim T. Synergistic and antagonistic effects on genotoxicity of chemicals commonly found in hazardous waste sites. Mutat Res 1992;270:71-77.
110. Marinovich M, Ghilardi F, Galli CL. Effect of pesticide mixtures on *in vitro* nervous cells: comparison with single pesticides. Toxicology 1996;108(3):201-206.
111. Marinovich M, Guizzetti M, Galli CL. Mixtures of benomyl, pirimiphos-methyl, dimethoate, diazinon and azinophos-methyl affect protein synthesis in HL-60 cells differently. Toxicology 1994;94(1-3):173-185.

112. McLachlan JA. Synergistic effect of environmental estrogens: report withdrawn. Science 1997;277(5325):462-463
113. Medinsky MA, Klaassen CD. Toxicokinetics. In: Casarett & Doull's Toxicology. The basic science of poisons. Klaassen CD, editor. McGraw-Hill; 1996.
114. Mileson BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT et al. Common mechanism of toxicity: a case study of organophosphorus pesticides. Toxicol Sci 1998;41(1):8-20.
115. Mills LJ, Robson DL, Malcolm AR. Interactive effects of aldrin, cyclohexylamine, 2,4-diaminotoluene and two phorbol esters on metabolic cooperation between V79 cells. Carcinogenesis 1991;12;1293-9.
116. Moriya M, Ohta T, Watanabe K, Miyazawa T, Kato K, Shirasu Y. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat Res 1983;116(3/4):185-216.
117. Motomura H, Narahashi T. Interaction of tetramethrin and deltamethrin at the single sodium channel in rat hippocampal neurons. Neurotoxicology 2001;22:329-339.
118. Mumtaz MM, De Rosa CT, Groten J, Feron VJ, Hansen H, Durkin PR. Estimation of toxicity of chemical mixtures through modeling of chemical Interaction. Environ Health Perspect 1998;106(Suppl 6):1353-1360.
119. Mumtaz MM, Durkin PR. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 1992;8(6):377-406.
120. Mumtaz MM. Risk assessment of chemical mixtures from a public health perspective. Toxicol Lett 1995;82-83:527-32.
121. NAP: Drinking water and health. Volume 9: Selected issues in risk assessment. Washington D.C. National Academy Press; 1989.
122. Narotsky MG, Weller EA, Chinchilli VM, Kavlock RJ. Nonadditive developmental toxicity in mixtures of trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a 5 X 5 X 5 design. Fundam Appl Toxicol 1995;27(2):203-216.
123. National Toxicology Program. Chemical Health and Safety Data. Website: (http://ntp-server.niehs.nih.gov/Main_Pages/Chem-HS.html)
124. Otto Meyer, personal communication
125. Oversigt over godkendte bekæmpelsesmidler. Orientering fra Miljøstyrelsen, nr. 1, 1992. In Danish.
126. Oversigt over godkendte bekæmpelsesmidler. Orientering fra Miljøstyrelsen, nr. 1, 1997. In Danish.
127. Pasquini R, Scassellati-Sforzolini G, Dolara P, Pampanella L, Villarini M, Caderni G et al. Assay of linuron and a pesticide mixture commonly found in the Italian diet, for promoting activity in rat liver carcinogenesis. Pharmacol Toxicol 1994;75(3-4):170-176.
128. Patel Y, Kushwan HS, Kushwah A, Sahini YP. Biochemical and neuro-behavioural changes in rats exposed to pesticides mixture. Indian Vet J 1998;75(8):744-746.
129. Pesticides Safety Directorate (PSD): Methodology for the toxicological assessment of exposures from combinations of cholinesterase inhibiting compounds. Medical and Toxicological Panel, Advisory Committee on Pesticides, Pesticides Safety Directorate, UK Ministry of Agriculture, Fisheries and Food, April 19, 1999. Draft document.

130. Pesticidrester i danske levnedsmidler 1997. Veterinær- og Fødevaredirektoratet, 1998. In Danish.
131. Pesticidrester i danske levnedsmidler 1998. Veterinær- og Fødevaredirektoratet, 1999. In Danish.
132. Pesticidrester i danske levnedsmidler 1999. Veterinær- og Fødevaredirektoratet, 2000. In Danish.
133. Porter WP, Green SM, Debbink NL, Carlson I. Groundwater pesticides: interactive effects of low concentrations of carbamates aldicarb and methomyl and the triazine metribuzin on thyroxine and somatotropin levels in white rats. J Toxicol Environ Health 1993;40(1):15-34.
134. Porter WP, Jaeger JW, Carlson IH. Endocrine, immune, and behavioral effects of aldicarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations. Toxicol Ind Health 1999;15(1-2):133-150.
135. Raizada RB, Scivastave MK, Kaushal RA, Singh RP, Gupta KP. Subchronic oral toxicity of a combination of insecticide (HCH) and herbicide (ISP) in male rats. J Appl Toxicol 2001;21(1):75-79.
136. Richardson JR, Chambers HW, Chambers JE. Analysis of the Additivity of *in vitro* Inhibition of Cholinesterase by Mixtures of Chlorpyrifos-oxon and Azinphos-methyl-oxon. Toxicol Appl Pharmacol 2001;172(2):128-139.
137. Safe SH. Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. Environ Health Perspect 1998;106(Suppl. 4):1051-1058.
138. Schoeny RS, Margosches E. Evaluating comparative potencies: Developing approaches to risk assessment of chemical mixtures. Toxicol Ind Health 1989;5:825-837.
139. Sedrowicz L, Witkowska D, Oledzka R. Effect of chlorfenvinphos, cypermethrin and their mixture on the intestinal transport of leucine and methionine. J Appl Toxicol 1996;16(6):483-489.
140. Seed J, Brown RP, Olin SS, Forna JA. Chemical Mixtures: Current Risk Assessment Methodologies and Future Directions. Regul Toxicol Pharmacol 1995;22(1):76-94.
141. Segal LM, Fedoroff S. Cholinesterase inhibition by organophosphorus and carbamate pesticides in aggregate cultures of neural cells from the foetal rat brain: the effects of metabolic activation and pesticide mixtures. Toxicol In Vitro 1989;3(2):123-128.
142. Selmanoglu G (Özmen), Akay MT. Biochemical study of the combined effects of endosulfan, dimethoate and carbaryl on albino rats. Pesticides 2001;16:77-84.
143. Selmanoglu G (Özmen), Akay MT. Histopathological effect of the pesticide combinations on liver, kidney and testis of male albino rats. Pesticides 2000;15:253-262.
144. Simmons J E. Chemical mixtures: challenge for toxicology and risk assessment. Toxicology 1995;105(2-3):111-119.
145. Srikanth NS, Seth PK. Alterations in xenobiotic metabolizing enzymes in brain and liver of rats coexposed to endosulfan and malathion. J Appl Toxicol 1990;10(3):157-160.
146. Stelzer A, Chan HM. The relative estrogenic activity of technical toxaphene mixture and two individual congeners. Toxicology 1999;138(2):69-80.
147. Su MQ, Kinoshita FK, Frawley JP. Comparative inhibition of aliesterases and cholinesterase in rats fed eighteen organophosphorus insecticides. Toxicol Appl Pharmacol 1971;20(2):241-249.

148. Svendsgaard DJ, Greco WR. Session summary: experimental designs, analyses and quantitative models. Toxicology 1995;105(2-3):157-160.
149. Svendsgaard DJ, Hertzberg RC. Statistical methods for the toxicological evaluation of the additivity assumption as used in the environmental protection agency chemical mixture risk assessment guidelines. In: Toxicology of Chemical Mixtures. Yang RSH, editor. Academic Press; New York; 1994. p. 599-642.
150. Taets C. The effects of herbicide interaction on chinese hamster ovary cells. J Nat Resour Life Sci Educ 1996;25(1):81-84.
151. Teuschler LK, Hertzberg RC. Current and future risk assessment guidelines, policy, and methods development for chemical mixtures. Toxicology 1995;105(2-3):137-144.
152. The e-Pesticide Manual, 11. ed., Version 1.1, 1999
153. The e-Pesticide Manual, 8. ed., 1987
154. Tordoir WF, Maron, M, He F, editors. Health Surveillance of pesticide workers. A manual for occupational health professionals. Toxicology 1994;91(1).
155. Toxicology Excellence For Risk Assessment, TERA, website: www.tera.org
156. Tully DB, Cox VT, Mumtaz MM, Davis VL, Chapin RE. Six high-priority organochlorine pesticides, either singly or in combination, are nonestrogenic in transfected HeLa cells. Reprod Toxicol 2000;14:95-102.
157. U.S. EPA. Guidance for health risk assessment of chemical mixtures. U.S. EPA, September 24, 1986
158. U.S. EPA. Guidance for identifying pesticide chemicals and other substances that have a common mechanism of toxicity. U.S. EPA, January 1999.
159. U.S. EPA. Proposed guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity. US EPA, June 22, 2000a.
160. U.S. EPA. Risk assessment guidance for superfund (RAGS): Volume I - Human health evaluation manual. Part D, Standardized planning, reporting and review of Superfund risk assessments. December, 2001. Website: www.epa.gov/superfund/programs/risk/ragsd/
161. U.S. EPA. Supplementary guidance for conducting health risk assessment of chemical mixtures. U.S. EPA, August, 2000b, EPA/630/R-00/002.
162. U.S. EPA. The grouping of a series of chloroacetanilide pesticides based on a common mechanism of toxicity. U.S. EPA, June 7, 2001a.
163. U.S. EPA. The grouping of a series of dithiocarbamate pesticides based on a common mechanism of toxicity. U.S. EPA, August 17, 2001b.
164. U.S. EPA. Thiocarbamates: A screening level cumulative dietary (food) risk assessment. U.S. EPA, August 17, 2001c.
165. Wang TC, Wu CL, Lin JH, Tarn CY, Lin SY. Sister chromatid exchanges and chromosome aberrations induced by pesticide combinations in Chinese hamster ovary cells. Bull Inst Zool, Academia Sinica 1987;26(4):317-29.
166. Wilkinson CF, Christoph GR, Julien E, Kelley JM, Kronenberg J, McCarthy J, et al. Assessing the risk of exposures to multiple chemicals with a common mechanism of toxicity: how to cumulate. Regul Toxicol Pharmacol 2000;31:30-43.
167. Williams MW, Fuyat HN, Frawley JP, Fitzhugh OG. *In vivo* effects of paired combinations of five organic phosphate insecticides. Agric Food Chem 1958;6(7):514-516.

168. Wu HX, Evreux-Gros C, Descotes J. Influence of Cimetidine on the Toxicity and Toxicokinetics of Diazinon in the Rat. Hum Exp Toxicol 1996;15(5):391-395.

Appendix 1: Studies on combined actions of pesticides

Compounds	Why these compounds?	Doses/concentrations	Exposure	Species / test	Effects studied/found	Interpretation	Comments	Reference
Endosulfan, dimethoate and carbaryl	Wide use	1- and 10-fold ADI	-	Rats	Did not cause significant effects in immune and hematological parameters of rats	-	Data from a previous study	Akay et al., 1999
Endosulfan, dimethoate and carbaryl	Wide use	100- and 1000-fold ADI	Orally, 3.5 month	Rats	Immune (IgG, IgM) and haematological (monocytes, granulocytes) parameters	Inertism (3 mixtures), antagonism (3 mixtures), coalism/ authors: At 1000*ADI: carbaryl had a slightly antagonistic effect with endosulfan and dimethoate.	Only endosulfan had an effect.	Akay et al., 1999
Endosulfan, dieldrin	Chemicals reported to have an (weak) estrogenic activity	0.001-10 µM	-	*In vitro* (MCF-7 focus assay)	Formation of foci	Inertism / Authors: no synergy found	Only endosulfan had an effect - and only at highest conc.	Arcaro et al., 1998
Toxaphene, dieldrin, chlordane, endosulfan	-	0.001-100000 nM	-	*In vitro*	Inhibition of 17beta-estradiol binding	? / Authors: synergism	Results withdrawn in 1997	Arnold et al., 1996; withdrawn by McLach-

								lan, 1997
Toxaphene, dieldrin, chlordane, alachlor	-	200-630 nM; 1 µM alachlor	-	*In vitro* (recombinant hER extracted from Sf9 insect cells infected with a baculovirus containing cDNA of the hER)	Inhibition of 17beta-estradiol binding	synergism, coalism / Authors: synergism	only dieldrin had an effect	Arnold et al., 1997
DDT+ dieldrin	Known endpoints	DDT: 1.0 µg/ml. Dieldrin: 3-7 µg/ml	-	*In vitro* (chinese hamster V79 cells)	Gap junction mediated intercellular communication	Bliss independence (dieldrin: 4-7 µg/ml), Bliss antagonism (dieldrin: 3 µg/ml) / authors: synergism	Their model is simple. Model for Bliss independence more appropriate.	Aylsworth et al., 1989
Aldicarb, metribuzin, methomyl	-	1-10000 ppb	90 days, drinkingwater, ad libitum	Rats (Sprague-Dawley, albino, male)	Slower speeds in maze-running. Changes in levels of choline and acetylcholine in r neostriatum and hippocampus	Aldicarb+metribuzin: ACh/Ch ratio in hippocampus: antagonism. Other parameters: ?	ACh/Ch ratio: only metribuzin had an effect. Many effects did not show maximum effect. Did not examine the effects of methomyl	Boyd et al., 1990

2 toxaphene congeners (T2, T12), toxaphene technical mixtures	Persistent, found in fish + human milk	0, 100, 1000 or 5000 ng/ml of toxaphene technical mixture, T2, T12 or 50:50 mixture of T2 and T12	48 h, treatment period corresponds to gestational day 10-12 (morphogenesis + organogenesis)	*In vitro* (rat embryos)	Dysmorphogenic activity. Target site is highly congener specific	?/ Authors: T2+T12: decreasing crown-rump and head lenght: synergism	Model to be used: Loewe additivity. But data are not adequate. Authors: 100 ng/ml ~ 1000*conc found in breast milk of Inuit women	Calciu C, et al, 1997
parathion, toxaphene, 2,4-D	Widely used	5, 50 mg/kg	oral, daily for 7 days	Mice (ICR, male)	Effects on the hepatic mixed-function oxygenase system. Hepatic cytochrome P-450 content	Inertism for mixtures with toxaphene and parathion+2,4-D / authors: antagonistic effect of toxaphene on parathion and on paraoxon	Only toxaphene had an effect	Chaturvedi, et al., 1991
Alachlor, aldrin, atrazine, 2,4-D, DDT, dieldrin, endosulfan, lindane, parathion, toxaphene	Widely used	0.01, 0.1, 1.0 and 10 ppm of each of the ten pesticides	a) Via drinkingwater, 90 days. b) oral, daily for 14 days	Mice (ICR, male)	Capable to induce the xenobiotic-metabolizing enzymes which possibly would not have been observed with individual pesticides at the doses and experimental conditions used in the study	?	Did not examine the effects of single compounds	Chaturvedi, 1993
Toxaphene (- not congeners)	Found in the Great Lakes ecosystem	0, 4, 20, 100 or 500 ppm	Corn oil	Rats (Sprague-Dawley)	Reproductive effects. Histological changes in liver, kidney, thyroid of adult rats. NOAEL = 4 ppm	?	Did not examine the effects of single congeners	Chu et al, 1988

Toxaphene - technical mixture	-	0, 0.2, 2, 5 mg/kg bw/day	13 weeks	Dogs (beagle, 7-8 months of age)	Effects on liver, thyroid. Accumulation in fat+liver	?	Did not examine the effects of single congeners	Chu et al., 1986
Toxaphene - technical mixture	-	0, 4, 20, 100, 500 ppm.	13 weeks	Rats (Sprague-Dawley)	Effects on liver, thyroid, kidney. Accumulation in fat+liver.	?	Did not examine the effects of single congeners	Chu et al., 1986
Dimethoate, omethoate, deltamethrin, benomyl	Commonly used pesticides	total in mixture: 41.5, 83 µ g/l	-	*In vitro* (human lymphocytes)	Sister-chromatid exchanges	Coalism / authors: potentiation	Neither compound was effective individually	Dolara et al., 1992
15 pesticides	Commonly found pesticides in foods of Italy	1-20 µg/ml	-	*In vitro* (human lymphocytes)	Cytogenetic effects. Found an increase in the number of nonsynchronous centromeric separations - mixture without benomyl did not cause this effect	? / Authors: the effect found is caused by benomyl	Did not examine the effects of single compounds.	Dolara et al., 1994
15 pesticides	Found in food at higher levels in Italy. Some of the compounds are previously found to be mutagenic	1) A concentration similar to the average exposure for an adult (1-148 µg/day, total: 716). 2) 0.1-20 µg/ml	-	*In vitro* 1) salmonella microsome assay, 2) human lymphocytes	1) No mutagenic activity. 2) Slightly but significant increase in sister-chromatid exchanges at 1 µg/ml	?	Did not examine the effects of single compounds	Dolara et al., 1993

15 pesticides	Found in food at higher levels in Italy. Some of the compounds are previously found to be mutagenic	1, 10 and 100 µg/kg (total dose) ~ 100*, 1000* and 10000* the estimated human exposure	Single exposure, in corn oil, by gavage	Rats (Wistar)	Sign of cellular toxicity	?	Did not examine the effects of single compounds	Dolara et al., 1993
Pyrethroids, organophosphorus pesticides	Pyrethroids - selected on basis of varying ease of esterase attack. OPs used together with pyrethroids in cotton	*In vivo* esterase inhibition: 0.13-64 mg/kg. Toxicity: 4-25 mg/kg of OP compounds, methylcarbamates or formamidine	Malathion or fenvalerate intraperitoneally 1 h after pyrethroid or malathion (also given intra peritoneal)	Mice	Esterase inhibition *in vivo*; toxicity (LD50)	? / Authors: synergism. Profenfos, sulprofos, DEF and EPN are potent inhibitors of mouse liver esterase which hydrolyses transpermethrin - they also synergise fenvalerate toxicity to mice	-	Gaughan, et al., 1980
Organophosphorous insecticides (12 different)	Same chemical group (OPs)	0.2-228 mM	-	*In vitro*, mutagenicity (yeast: Schizosaccharomyces pompe)	Toxicity + mutation frequency	3 mixtures of trichlorfon and malathion, parathion-methyl, azinphos-methyl: synergism / Authors: synergism	Only trichlorfon had an effect	Gilot-Delhalle, et al., 1983
Salute, Dursban, Hostathion, Nogos, Acetellic,	OP formulations	10 % of LD50	Corn oil, by gavage	Mice	Decreased AChE, hepatic dysfunction and disturbance of amino	?	Did not examine the effects of single compounds	Gomes et al., 1999

Selecron. Active compounds: dimethoate, chlorpyrifos, profenofos, pirimiphos methyl, triazophos, dimethoate					acids profile			
1) 19 OPs + 1 organochlorine. 2) As experiment 1 + a mixture of 40 pesticides of high volume production + 5 known carcinogens	1) Suspected carcinogens. 2) High volume production + carcinogens. All pesticides are authorized for use in Japan.	1) ADI, 100 * ADI of each compound. 2) ADI	1) 8 weeks. 2) 28 weeks	Rats (F344)	1) Increased number and area of liver lesions at 100*ADI - not at ADI. 2) Did not modulate carcinogenesis in any organ initiated by five known potent carcinogens in combinations	?	Did not examine the effects of single compounds. Too many compounds in the mixtures	Ito et al., 1996; Ito et al., 1995a
Initially: diethylnitrosamine. After 2 weeks: 1) 20 pesticides. 2) 40 high volume production pesticides	1) Carcinogenicity repeated or suspected. 2) high volume production.	1) ADI, 100*ADI. 2) multiorgan bioassay	Dietary	Rats (F344)	1) Did not enhance GST-P-positive foci at ADI. 100*ADI increased GST-P-postitive foci. 2) No effects	? / Authors: 1) synergism	Interpretation difficult with so many compounds	Ito et al., 1998
19 organophosphorus, 1 organochlorine	-	ADI, 100*ADI of each compound. Initially single ip. injection of diethyl-	2 week recovery from diethylnitrosamine injection to pesticide	Rats (F344, male)	100*ADI: lesion promoting potential - the mixture is possibly carcinogenic in the liver	?	Did not examine the effects of single compounds. Too many compounds in the mixtures	Ito et al., 1995b

		of diethylnitrosamine 200 mg/kg bw	exposure. Dietary pesticide exposure					
Carbofuran, oxamyl, propoxur	Same chemical group (carbamates)	10^{-6} M. Mixtures: 1:1	-	*In vitro* (plasma and erythrocytes from male rats)	AChE inhibition.	? / Authors: additivity	Their model is simple. Model for Loewe additivity more appropriate - but data are not adequate.	Iyaniwura, 1991
Aldicarb, carbofuran, oxamyl	Same chemical group (carbamates)	10^{-10}-10^{-5} M. Mixtures: 1:1:1	-	*In vitro* (rat plasma ChE)	AChE inhibition (plasma)	Loewe additivity / weak Loewe synergism. / Authors: potentiation	1 mixture, varying conc.	Iyaniwura, 1989
9-13 herbicides/ pesticides per mixture	Widely used	Pesticide mix: 0.1-0.6 mg/ml of each compound depending of the compound. Herbicide mix: 0.1 mg of each compound in 1 ml of ETOAC	-	*In vitro* (IVF medium. Oocytes contained in cumulus masses collected from superovulated B6D2F1 mice - sperm added)	Pesticide mix: effect on IVF rate. Increased incidence of abnormal embryos + degenerative oocytes. Herbicide mix: increased incidence of degenerative oocytes following culture (But no effect on fertilization)	?	Did not examine the effects of single compounds	Kholkute et al., 1993

Lindane, carbaryl	Frequently found in food and feeds	Lindane: 11, 22, 44 mg/kg/day. Carbaryl: 44, 150, 176 mg/kg/day.	Orally in soy bean oil for 3 days. Killed on day 4	Rats (albino, male)	Induction of microsomal enzymes	Antagonism at highest doses. Inertism at lowest doses	Only lindane had an effect	Krechniak et al., 1994
parathion, toxaphene, 2,4-D	widely used, different chemical classes	Parathion: 1, 2.5, 5 or 10 mg/kg. Toxaphene: 50, 100 or 200 mg/kg. 2,4-D: 50, 100 or 200 mg/kg	Oral intubation, in corn oil, daily up to 14 days. Animals were monitored for an additional period of 7 days	Mice (ICR, male)	effects on body weight, liver/body weight ratio, effects on serum glutamic pyruvic transaminase (SGPT), serum AChE and brain AChE	Effect on SGPT: 1) Toxaphene+2,4-D synergism day 15 (highest effect). Brain ChE: 2) PA+2,4-D: Bliss antagonism 3) PA+ toxaphene: inertism	1) only 2,4-D had an effect; 2) only parathion had an effect	Kuntz et al., 1990
15 pesticides	Commonly found in foods of central Italy	Total: 0.75-10 mg/kg	10 d, p.o., corn oil	Rat (Wistar, male)	DNA oxidative damage – levels of 8-OH-2-deoxyguanosine	? / Authors: The mixture induce free radical DNA damage at low doses. At higher doses: depression of cellular metabolism, inhibiting a furter expression of oxidative damage	Did not examine the effects of single compounds. The doses are 100* supposed human exposure (716 µg/l) - relative proportion of each compound ~pesticide residues in diet, Italy	Lodovici et al, 1994

15 pesticides. Mix1: dithiocarbamate+ benomyl. Mix2: peocymidone+ methidathion+ chlorpyrifos-ethyl+ parathion-methyl+ chlorpropham+ parathion+ vinclozolin+ chlorfenvinphos+ pirimiphos-ethyl. Mix3: thiabendazole+ fenarimol+ diphenylamine+ chlorothalonil	Commonly found in foods of central Italy	Depending of the compound: 0.001-0.207 mg/kg/day. 3 mixtures. Single compounds: thiabendazolel fenarimol, diphenylamine, chlorothalonil	10 d, corn oil	Rat (Wistar, male)	Mix1/mix2: No effects. Mix3: DNA oxidative damage – levels of 8-OH-2-deoxyguanosine relative to 2-deoxyguanosine in DNA	Mix 3: Loewe antagonism	Mix 3: same mechanism - only diphenylamine and chlorothalonil had an effect. Authors: 0.09 and 0.13 mg/kg/day ~ 1/100 the calculated human exposure through food in Italy.	Lodovici et al, 1997
Dimethoate, azinophos-methyl, diazinon, pirimiphos methyl, benomyl	Three mixtures of compounds found in groundwater and food - potential widespread exposure	10, 6, 4, 15, 15 µg/ml - based on median concentration found in current food in Italy	-	*In vitro* (human neuroblastoma cell line, SH-SY5Y)	Inhibition of protein synthesis and/or inhibition of AChE	1) Pirimiphos+ benomyl, 2) Dimethoate+ diazinon+ azinphos, 3) Dimethoate+ diazinon+ azinphos+ pirimiphos+ benomyl: synergism / Authors: mix more toxic than single compounds on protein synthesis - potentiation in some cases	Only one compound had an effect (benomyl in 1) and 3); azinophos in 2)). Model to be used for the other data: Loewe additivity. But data are not adequate	Marinovich et al, 1996

Mix1: pirimiphos methyl, benomyl. Mix2: dimethoate, azinophos-methyl, diazinon	Widely used OPs + benomyl. Known effect: Induce different cytotoxic effects on the human leukemia cell line HL-60	Mix1: 7.5-30 µg/ml. Mix2: 100:40:60 µg/ml	2, 4, 24, 48 h	*In vitro* (human leukemia cell line HL-60 cells)	Changes in protein synthesis. 3H-leucine incorporation into HL-60 cell protein	Mix1: 1:1: antagonism at 4h. At lower doses of pirimiphos methyl and at 24 h: inertism. Mix2: synergism	Mix1: only benomyl had an effect. Mix2: only azinphos-methyl had an effect	Marinovich et al, 1994
Tetramethrin, deltamethrin	Pyrethroids - type I (tetramethrin) and II (deltamethrin)	1-10 µM	-	*In vitro* (hippocampal neurons from 17 day-old embryos of a Sprague-Dawley rat)	Action on sodium channels. Deltamethrin-modified sodium channels opened longer than tetramehtrin-modified sodium channels. Mix (10:10 µM): shorter openings compared to deltamethrin-modified	? / Authors: displacement of type II pyrethroid by the type I pytrethroid from the same binding site or to the allosteric interaction of the two pyrethroids at separate sodium channel sites	Data not adequate for an analysis	Motomura and Narahashi, 2001
Linuron+15 pesticides	Found in italian food	Initially: 100 mg/kg diethylnitrosamine. 1 week later: 150 mg/kg/day linuron or 10 mg/kg/day pesticide	Diethylnitrosamine: ip., pesticides: by gavage. 3 days/week for 8 weeks	Rats (Sprague-Dawley, male)	Pesticide mix: increased number of preneoplastic foci - the area not increased. No increases in proliferation enzymatic markers	?	Did not examine the effects of single compounds	Pasquini et al., 1994

		mixture						
Monocrotophos, endosulfan, hexachloro-cyclobezene (HCH)	-	Daily: 0.44 mg mixture suspended in 0.5 ml coco-nut oil. It is not specified in what ratio the com-pounds are mixed.	4 groups: 7, 15, 30 or 60 days	Rats (al-bino)	No effects on enzyme activity. Spontaneous motor activity was reduced by 40-44 %. After >/= 15 days ex-posure: changes in behaviour, drastic re-duction in muscle tone balance activity, re-duced AChE activity	?	Did not examine the effects of sin-gle compounds. Unclear what the dose was	Patel et al., 1998
Aldicarb, metribuzin, methomyl	-	Aldicarb: 0, 1, 10 ppb. Metribuzin: 0, 1000, 10000 ppb. Methomyl: 0, 100, 1000 ppb	1) Females: 6 weeks. 2) Males: 16 weeks	Rats (Spra-gue-Dawley)	1) Metribuzin -> hy-perthyroid animals. Interaction of all three compunds increased thyroxine levels. 2) Metribuzin increased thyroxine. Altered so-matotropin levels. Mixture: implicated in learning impairment, other neurological functions, immune parameter changes, endocrine changes.	?	-	Porter et al., 1993

Hexachloro-cyclohexane (HCH), isoprotu-ron	Widely used pesticides. HCH resi-dues in food and in body tissues of occupational workers	HCH: 12.5, 25 or 50 mg/kg/day. Isoproturon: 22.5, 45, 90 mg/kg/day	90 days, orally, by gavage, in peanut oil,	Rats (Rattus norvigicus. Druckery strain, male)	Effects on haemato-logical parameters	WBC: Bliss antago-nism. Other parame-ters: ? Authors: no synergistic effects	The other haema-tological data were not adequate for evaluation	Raizada et al., 2001
Chlorpyrifos-oxon, azinphos-methyl-oxon	The active metabolites of chlorpyri-fos and azi-nophos-methyl - most widely used OPs. Same mechanism (inhibition of ChE). Display dif-ferent po-tencies to-ward ChE	Conc. of each individual compound was chosen so the ChE iunhibtion was 10-90 %	-	*In vitro* (blood+brai ns from Sprague-Dawley rats, male)	Brain and serum AChE activity	Brain AChE: Loewe additivity. Serum AChE: unclear. Au-thors: brain AChE: dose additivity. Se-rum AChE: non lin-ear response. > addi-tivity	Their model of dose additivity ~ Loewe additivity	Richardson et al., 2001
Chlorfenvinphos, cypermethrin	Used alone or in mix-ture in agri-culture	5 % LD50 of each com-pound	Intestinal perfusion, 6/week by gavage, 2 weeks in soy bean oil	Rats (Wis-tar, male)	Effects on the intesti-nal transport of leucine and methionine / Leu-cine absorbed during perfusion and retained in the liver	Bliss independence	High dose	Sedrowicz et al., 1996

chlorfenvinphos, cypermethrin	Used alone or in mixture in agriculture	5 % LD50 of each compound	Jejunal slices	*In vitro*	-	*In vitro*: ? / Authors: mixture caused a larger decrease of leucine in the liver compared to single compounds	-	Sedrowicz et al., 1996
Mix1: malathion+ fenitrothion. Mix2: malathion+ carbofuran. Mix3: carbofuran+ fenitrothion. Mix4: triallate+ carbofuran. Mix 5: triallate+ fenitrothion	-	10 µL aliquots of 95 % ethanol solutions of the pesticides to cultures	-	*In vitro* (neural cells from the foetal rat brain, Wistar)	AChE inhibition.	Mix1: synergism. Mix2,4,5: inertism. Mix3: weak antagonism. Authors: Mix1: potentiation. Mix2: ~ carbofuran. Mix3 ~ antagonism. Mix4/5: ~ carbofuran/fenitrothion	Mix1,2,4,5: Only carbofuran and fenitrothion had an effect. Mix3: qualitative interpretation	Segal and Fedoroff, 1989
Endosulfan, dimethoate and carbaryl	widely used in Turkey	endosulfan: 6.12 mg/kg/day. Dimethoate: 20.4 mg/kg/day. Carbaryl: 10.1 mg/kg/day	3.5 months, by gavage, dissolved in sunflower oil, mix of 2 or 3 agents	Rats (Wistar albino, male)	Changes in serum AST, ALT, glucose, urea nitrogen, and others	Inertism (2), coalism (3), antagonism	Only dimethoate had an effect. Authors: doses are 1000*ADI of each compound.	Selmanoglu and Akay, 2001
Endosulfan, dimethoate, carbaryl	Widely used in Turkey	10, 100, 1000* ADI of each compound	3.5 months, dissolved in sunflower oil	Rats (Swiss albino, male)	Effects on liver, kidney, testis	?	Did not examine the effects of single compounds	Selmanoglu and Akay, 2000
Endosulfan,	Widely used	Endosulfan: 3	21 days.	Rats (Wis-	Endosulfan blocked	? / Authors: endosul-	Dose of endosul-	Srikanth et

malathion	in India. Different metabolism	mg/kg bw. Malathion: 30 mg/kg bw	Malathion orally in peanut oil. Endosulfan i.p. suspended in propylene glycol	tar, albino, male)	the GSH and carboxylesterase routes of malathion detoxifcation	fans effect on malation: potentiation	fan is found in a previous study to be minimum dose causing neurotoxic effects. Malathion dose based on litterature.	al., 1990
Toxaphene, technical mixture, T2, T12	Most abundant persistent organic pollutat in Arctic + Great Lakes	Toxaphene: 0.1, 1.0, 10, 100 and 1000 µM. T2, T12: 0.001, 0.01, 0.1 and 10 µM	-	*In vitro* (MCF-7 E3 human breast cancer cell model)	T2 and T12: lower estrogenic activities than toxaphene. Effects of mixtures on relative proliferation (PE) ~ single compounds	? / Authors: additivity	-	Stelzer and Chan, 1999
19 organophosphorus pesticides	Common mechanism (inhibition of esterases). OP insecticides	Various doses: 0-50 ppm of each of the 18 pesticides. Malathion: ?	1 week, dietary, dissolved in corn oil. Malathion: i.p.	Rats (Holtzman, female)	Authors: all the OP's can potentiate the toxicity of malathion when appropriate levels are fed in the diet. Close correlation between the amount of increase in acute tox. of malathion and the amount of inhibition of aliesterase activity of liver.	? / Authors: potentiation	Did not examine the effects of single compounds. Acute tox. increased from 2 ppm and up	Su et al., 1971

Atrazine, simazine, cyanazine. Single compounds and mixtures of 2 or 3 compounds	Found in large amounts in water in Illinois	1) US.EPA maximum contamination level (MCL). 2) highest contamination level found in Illinois water supplies	48 h	*In vitro* (Chinese hamster ovary cells)	Atrazine and atrazine+cyanazine showed whole cell clastogenicity at both levels, and atrazine+ simazine at MCL. No effects of the other mixtures	?	Doses: U.S. EPA MCL + contaminated levels. Data not adequate for an analysis	Taets, 1996
4,4'-DDT, 4,4'-DDD, 4,4'-DDE, aldrin, dieldrin, endrin	Mode of action (classified to modulate transcriptional activitation of an estrogen-responsive reporter gene in transfected HeLa cells)	0.001-10 µM. Single compounds and 3 equimolar mixtures of two pesticides	-	*In vitro* (HeLa cells)	No evidence of estrogenicity	-	-	Tully et al., 2000
12 combinations of 20 pesticides	The 12 combinations have recently been applied for registration to commercial-	Various conc. Mix1: Captafol: 0.05-3.10 µg/ml. Polyoxin: 0.0004-0.25 µg/ml	-	*In vitro* (Chinese hamster ovary cells)	Sister-chromatid exchanges induction. Chromosome aberations induction	Captafol+polyoxin: antagonism. Other mixtures: ?. / Authors: effects ~ effect of one of the compound in the mixture	Mix1: Only captafol had an effect. Other data not adequate for quantitative analysis	Wang et al., 1987

	ize in China							
EPN, systox, malathion, methyl parathion, parathion	Same chemical group (OPs)	Tolerance and "safe" levels (highest no-effect level)	6 weeks, food added pesticides diluted in corn oil. Food and water ad libitum	Dogs, unknown ages, 6-10 kg, 1 male + 1 female/group	Depression of cholinesterase in plasma or red blood cells	EPN+ Systox (in plasma): synergism. EPN+ malathion (in red blood cells): synergism. Other data: ? / Authors: additive effect in many combinations. Dose> tolerance level: potentiation between EPN (2 ppm) and malathion (100 ppm) or systox (20 ppm).	EPN+ Systox: qualitative interpretation. EPN+ malathion: quantitative interpretation. Other data: model: Loewe additivity - but data not adequate	Williams et al., 1958

Appendix 2: Studies on combined actions of pesticides and non-pesticides

Compounds	Why these compounds?	Doses	Exposure	Species / test	Effects studied/found	Interpretation	Comments	Reference
Chlordane, lead	Chlordane used in Iraq until 1984 as a soil insecticide - soil contaminant. Lead - major soil contaminant in urban areas in Baghdad	Chlordane: 75, 275 mg/kg bw. Lead oxide: 50 mg/kg bw. Given alone and in combination	Orally. 35 days, chlordane dissolved in corn oil. Mice sacrificed at the 2., 3., 4. or 5. Week	Mice (Swiss, male)	Changes in testis weight, diameter of the seminiferous tubules, number of Sertoli cells, spermatogonia, spermatids, primary+ secondary spermatocytes	Bliss independence and Bliss synergism / authors: not clear whether the effect is synergistic or additive	-	Al-Omar et al., 2000
1) DDT+ oleic acid. 2) DDT+ 12-O-tetradecanoylphorbol-13-acetate (TPA)	known endpoints	1) A. oleic acid: 6.0 µg/ml; DDT: 0.5-3.0 µg/ml. B. DDT: 2.0 µg/ml; oleic acid: 1.5-12.0 µg/ml. 2) TPA: 0.025 ng/ml; DDT: 500-4000 ng/ml.	-	*In vitro* (chinese hamster V79 cells)	Gap junction mediated intercellular communication - inhibition of metabolic cooperation	1) Bliss synergism but Bliss independence at lowest DDT conc. (0.5 µg/ml+oleic acid: 6.0 µg/ml). 2) Bliss synergism but Bliss independence at lowest	Their model is simple. Model for Bliss independence more appropriate.	Aylsworth et al., 1989

						DDT conc. (500-2000 ng/ml)		
Chlordane, benzo[a]pyrene, 12-O-tetradecanoylphorbol-13-acetate (TPA)	carcinogenic compounds	chlordane: 0-40 µg/ml. Benzo[a]pyrene: 11 µg/ml. TPA: 0.1 µg/ml	-	*In vitro* (Syrian hamster embryo cells (SHE), V79 cells)	Effects of chlordane on SHE cell transformation - weak transforming activity. TPA (0.1 µg/ml) + chlordane (5-20 µg/ml): increased effect	? / Authors: potentiation	-	Bessi et al., 1995
Phosphorothioate, pyrethrin, piperonyl butoxide, petroleum distillates	Case study, a co-worker sprayed the work area for roaches and book mites	-	Approx. 3.5 h	Human, female, 38 year, employed as a file clerk	Symptoms consistent with acute AChE inhibitor poisoning + an upper respiratory tract irritant	?	Case study	Callender et al., 1994
Methoxychlor (an estrogenic pesticide), 17-beta-estradiol	-	17-beta-estradiol: 2, 5 or 25 ng/day. Methoxychlor: 4, 8, 16 mg/kg bw/day. Separately or in combination	3 daily intraperitoneal. Animals sacrificed on day 4	Mice (ND4 Swiss Webster, ovariectomised at 36 d of age)	Effect on reproductive-tract	? / Authors: effects of mixtures either minimal or showed no additional signs of stimulation	Did not examine the effects of single compounds at all doses	Eroschenko and Johnson, 2000

25 common groundwater contaminants (only one pesticide - aroclor)	Frequently found in polluted groundwater	Designed to mimic a worst-case scenario	In drinking water, 14 or 90 days	Mice (D6C3F1, female)	Immune function changes and altered resistance to challenge with an infectious agent at highest dose	?	Did not examine the effects of single compounds	Germolec et al., 1989
Halogenated hydrocarbon insecticides and polychlorinated biphenyls (PCBs)	Found in human adipose tissue, serum and milk	Low dose (L), 2*L, 10*L, 100*L. L=0.045-1.53 mg/kg of each compound	Intraperitoneal injection. Administered on day 1+3 - killed on day 6. Compounds dissolved in corn oil	Rats (Wistar, male, immature, 1 month old)	Induction of several hepatic drug-metabolising enzymes. Mild alterations in thyroid architecture, etc.	?	Did not examine the effects of single compounds	Gyorkos et al., 1985
1) Aldicarb, atrazine, dibromochloropropane, 1,2-dichloropropane, ethylene dibromide, simazine, ammonium nitrate. 2) Alachlor, atrazine, cyanazine, metolachlor, metribuzin, ammonium nitrate	1) Representative of groundwater contamination in California. 2) Simulated groundwater in Iowa	1*, 10*, 100* median concentration of each pesticide component as determined in the groundwater	In drinking water	Mice (Swiss CD-1)	Reproductive toxicity. No effects found	?	Did not examine the effects of single compounds	Heindel et al., 1994

1) Aldicarb, atrazine, dibromochloropropane, 1,2-dichloropropane, ethylene dibromide, simazine, ammonium nitrate. 2) Alachlor, atrazine, cyanazine, metolachlor, metribuzin, ammonium nitrate	1) Representative of groundwater contamination in California. 2) Simulated groundwater in Iowa	1*, 10*, 100* median concentration of each pesticide component as determined in the groundwater	In drinking water	Rats (Sprague-Dawley)	Developmental toxicity. No effects found	?	Did not examine the effects of single compounds	Heindel et al., 1994
Fenarimol, trichloroethylene	-	Fenarimol: 150 mg/kg bw. Trichloroethylene: 457 mg/kg bw	Corn oil, ip., single dose. Killed 30 h after treatment	Mice (Swiss albino CD2, male)	Frequency of micronuclei in mouse bone marrow	?	Data not adequate for quantitative analysis - they do not show maximum effect	Hrelia et al., 1994
chlorpyrifos-oxon, 4 polycyclic aromatic hydrocarbons	Chlorpyrifos-oxon: the active metabolite of chlorpyrifos. chlorpyrifos often used ind private homes in USA. PAH found in house dust	PAHs: 2-50 µM. Chlorpyrifos-oxon: 1, 40, 180 nM	-	*In vitro* (AChE from electric eel and human)	AChE inhibition.	Loewe additivity (4 mixtures)	-	Jett et al., 1999
Cimetidine, methylparathion,	Same mechanism of action	0.01 ml/g bw of methylparathion	Dissolved in peanut oil. Pre-	Mice (ICI)	Pretreatment with cimetidine de-	? / Authors: antagonism	Model to be used: Bliss independ-	Joshi and Thorn-

parathion	(methylparathion + parathion need to be degraded to excert toxic effect - to oxon-analogs by microsomal mixed-function oxidases). Cimetidine inhibits hepatic microsomal oxidation metabolism of a number of drugs.	and parathion, 0.02 ml/g bw of cimetidine. Pretreatment of mice with cimetidine: 25-200 mg/kg	treatment with cimetidine ip 30 min prior to treatment with the other compounds		creased the tox of methylparathion but not of parathion. Inhibit AChE (cerebral cortex); survival.		ence - but data not adequate.	burg, 1986
Pyrethrin, piperonyl butoxide (PBO)	Pyrethrin: widely used. PBO: well known synergist used to intensify the effects of pyrethrins	Pyrethrin: 0.1-1000 µM. PBO: 0.4-4000 µM	-	*In vitro* (cerebral synaptosomes of rat brain (Sprague-Dawley, male))	Decreased ATPase activity. Pyrethrin:PBO in 1:4 most efficient mixture.	? / Authors: synergism	-	Kakko et al., 2000
California: aldicarb, atrazine, dibromochloropropane, 1,2-dichloropropane, ethylene dibromide, simazine, ammonium nitrate	Found in contaminated groundwater in California	1*, 10*, 100*levels found in groundwater. Range: 0.01-9 ppb pesticides. 10000 ppb ammonium ntrate	In drinking water, 71 days	Rats (Fischer 344, male)	Conc.-related increase in SCEs at all conc.	? / authors: 1,2-dibromo-3-chloropropane and to a lesser extent 1,2-dichloropropane are responsible for the effect	Did not examine the effects of single compounds	Kligerman et al., 1993

1) California: aldicarb, atrazine, dibromochloropropane, 1,2-dichloropropane, ethylene dibromide, simazine, ammonium nitrate. 2) Iowa: alachlor, atrazine, cyanazine, metribuzin, metolachlor, ammonium nitrate	Found in contaminated groundwater in California and Iowa	1*, 10*, 100*levels found in groundwater. Range: 0.01-9 ppb pesticides. 10000 ppb ammonium ntrate	In drinking water, mice: 91 days	Mice (B6C3F1, female)	Conc.-related increase in SCEs at 100*conc.	? / authors: 1,2-dibromo-3-chloropropane and to a lesser extent 1,2-dichloropropane are responsible for the effect	Did not examine the effects of single compounds	Kligerman et al., 1993
Lead tetraacetate, arsenic trioxide, dieldrin, tetrachloroethylene (TCE)	-	Mix. in ratios: 1:1, 1:2, 2:1, 1:1:1. Stock solution around or below minimum effective dose	-	*In vitro* (Tradescantia-micronucleus assay)	MCN frequencies	? / Authors: lead tetraacetate+arsenic trioxide: antagonistic. TCE+dieldrin: synergistic	Data not adequate for an analysis	Ma et al., 1992
Aldrin, cyclohexylamine, 2,4-diaminotoluene, phorbol-12-myristate-13-acetate (PMA), phorbol-12,13-dibutyrate (PDBu)	Affects metabolic cooperation at different concentration levels - different mechanisms	Conc. which are found to inhibit metabolic cooperation	-	*In vitro* (V79 cells/metabolic cooperation assay)	Metabolic cooperation between V79 cells	? / Authors: PMA+ PDBu: additive at conc below the maximally effective conc of either. Aldrin+ cyclohexylamine:	Their model ~ toxic equivalency. Authors: Additive= same mechanism. Summative= different mechanism of action.	Mills et al., 1991

						summation. PMA+ aldrin or cyclo-hexylamine: synergism. 2,4-diaminotolu-ene+ PMA or aldrin or cyclohexyl-amine: inter-action		
Trichloroethyle-ne (TCE), di(2-ethylhe-xyl)phtalate (DEHP), hep-tachlor (HEPT)	Found at hazard-ous waste sites. Different mechanisms	TCE: 0-320 mg/kg/d. DEHP: 0-780 mg/kg/d. HEPT: 0-8.0 mg/kg/d. 5x5x5 design	In corn oil, by gavage, gesta-tion days 6-15	Rats (F-344)	Maternal weight gain during GD 6-8. Full-litter resorption. De-creased pup weights. Postna-tal loss	? / Authors, overall con-clusion: TCE+DEHP synergism, DEHP+HEPT and TCE+HEPTa ntagonism	Data not adequate for an analysis	Narotsky et al., 1995
Aldicarb, atrazine, nitrate	Common groundwater contaminants in USA	Aldicarb and atrazine: 3, 10 ppb (µg/l). Nit-rate: 10, 28 ppb (mg/l)	22-103 days. Voluntary con-sumption of drinking water	Mice (Swiss Webster + wild deer)	Endocrine im-mune and behav-ioral effects	? / Authors: nitrate + a pesticide: endocrine, immune and behavior changes at low dose (MCL) >< single com-	Low doses in the study are MCLs - such levels are found in ground-water in US	Porter et al., 1999

						pounds		
Cimetidine, diazinon	Widely used	Cimetidine: 80 mg/kg, ip.. Diazinon: 50 mg/kg ip., acute tox: 270 mg/kg	Intraperitoneal injection of cimetidine at 1 and 24 h prior to diazinon dosing	Rats (Wistar, male)	Changed activities of AChE and carboxylesterase (especially in brain and plasma)	Inhibit AChE (brain), carboxylesterase (brain): (Weak) Bliss synergism, synergism. AChE (RBC), carboxylesterase (plasma, liver): (Weak) Bliss antagonism. / Authors: potentiation	carboxylesterase (brain): only diazinon had an effect	Wu et al., 1996

Appendix 3: Compounds found in fruit, vegetables, and corn

Compounds are grouped by chemical structure - mainly based upon 6)

Compound 1), 2), 3)	Use 4), 5)	Chemical name 4), 5)	Designed mode of action
Carbamate insecticides			
Anticholinesterase carbamates and procarbamates			
Carbaryl	I	1-naphthyl methylcarbamate	Inhibit acetylcholi-nesterase 9)
Pirimicarb	I	2-(dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate	Inhibit acetylcholi-nesterase 9)
Mecarbam	A/I	Ethyl (mercaptoace-tyl)methylcarbamate S-ester with O,O-diethyl phosphorodithioate	Inhibit acetylcholi-nesterase 9)
Dithiocarbamates (analysed as a sum of 6 dithiocarbamates)			
Organic phosphorus pesticides - cholinesteraseinhibitors			
Dimethoxy compounds of category IV			
Fenitrothion	I	O,O-dimethyl O-(3-methyl-4-nitrophenyl)-phosphorothioate 6)	Inhibit acetylcholi-nesterase 9)
Fenthion	I	O,O-dimethyl O-(4-(methylmercapto)-3-methylphenyl)thiophosphate 6)	Inhibit acetylcholi-nesterase 9)
Malathion	A/I	O,O-dimethyl-S-(1,2-dicar-bethoxyethyl)phosphorodithioate 6)	Inhibit acetylcholi-nesterase 9)
Parathion-methyl	A/I	O,O-dimethyl O-(p-nitrophenyl) phosphorothioate	Inhibit acetylcholi-nesterase 9)
Azinphos-methyl	A/I	O,O-dimethyl-S-[4-oxo-1,2,3-benzotriazine-3(4H-yl) methyl]phosphorothioate 6)	Inhibit acetylcholi-nesterase 9)
Dimethoate	A/I	O,O-dimethyl S-2-(methylamino)-2-oxoethyl phosphorodithioate	Inhibit acetylcholi-nesterase 9)
Monocrotophos	A/I	E isomer of O,O-dimethyl-O-(1-methyl-3-oxo-1-propenyl) phos-phate	Inhibit acetylcholi-nesterase 9)
Methidathion	I	O,O-dimethyl S-(2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazolin-3-ylmethyl) phosphorodithioate	Inhibit acetylcholi-nesterase 9)

Phenthoate	A/I	O,O-dimethyl S-alfa-ethoxycarbonylbenzyl phosphoro-dithioate	Inhibit acetylcholi-nesterase 9)
Pirimiphos-methyl	A/I/N	O-[2-(diethylamino)-6-methyl-4-pyrimidinyl] O,O-dimethyl phos-phorothioate	Inhibit acetylcholi-nesterase 9)
Thiometon	A/I	O,O-dimethyl S-ethylmercaptoethyl dithiophos-phate 6)	Inhibit acetylcholi-nesterase 9)
Chlorpyrifos-methyl	I	O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Inhibit acetylcholi-nesterase 13)
Tolclofos-methyl	F	O-(2,6-dichloro-4-methylphenyl) O,O-dimethyl phosphorothioate	Inhibit of phospholipid biosynthesis 13)
Etrimfos	I	O-(6-ethoxy-2-ethyl-4pyrimidinyl) O,O-dimethyl phosphorothioate	Inhibit acetylcholi-nesterase 13)
Phosmet	A/I	O,O-dimethyl phosphorothioate S-ester with N-(mercaptomethyl)phthalimide	Inhibit acetylcholi-nesterase 9)
Diethoxy compounds of category IV			
Chlorpyrifos	I	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Inhibit acetylcholi-nesterase 9)
Parathion	A/I/V	O,O-diethyl O-(p-nitrophenyl) phosphorothioate	Inhibit acetylcholi-nesterase 9)
Diazinon	A/I	O,O-diethyl O-(2-isopropyl-6-methyl-4-pirimidinyl) phos-phorothioate	Inhibit acetylcholi-nesterase 9)
Dioxathion	I	O,O,O',O'-tetraethyl-S,S'-1,4-dioxane-2,3-diyl di(phosphorodithioate)	Inhibit acetylcholi-nesterase 9)
Phosalone	A/I	O,O-diethyl-S-(6-chloro-2-oxobenzoxazolin-3-yl-methyl) phosphorodithioate 6)	Inhibit acetylcholi-nesterase 9)
Ethion	A/I	S,S'-methylene O,O,O',O',-tetraethyl phosphorodithioate	Inhibit acetylcholi-nesterase 9)
Quinalphos	I	O,O-diethyl O-2-quinoxylinyl) phosphorothioate	Inhibit acetylcholi-nesterase 9)
Pyrazophos	F	Ethyl-2-hydroxy-5-methylpyrazol[1,5-a]pyrimidin-6-carboxylate O-ester with O,O-diethyl phosphorothioate	Unknown 9)
Mixed substitute Compounds of category IV			
Prothiofos	A/I	O(2,4-dichlorophenyl) O-ethyl S-propyl phosphorodithioate	Inhibit acetylcholi-nesterase 13)
Chlorinated hydrocarbon insecticides			
DDT and its analoges			

DDT	I	Technical 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (a complex chemical mixture where pp'-DDT is most dominating)	Acts via the nerveous system - acts on axonal transmission 9)
Methoxychlor	I	1,1,1-trichloro-2,2-bis-(p-methoxyphenyl)ethane (p,p'-dimethoxy analog of p,p'-DDT)	Acts via the nerveous system 9)
Dicofol	A	4,4'-dichloro-X-(trichloromethyl)benzhydrol or 2,2,2-trichloro-1,1-bis(4-chlorophenyl)-ethanol	Acts via the nerveous system 9)
Chlorobenzilate	A 8)	Ethyl 2-hydroxy-2,2-bis(4-chlorophenyl)acetate 7)	Acts via the nerveous system 9)
Chloropropylate	A 8)	1-methylethyl 4-chloro-alpha-(4-chlorophenyl)-alpha-hydroxybenzenacetate 7)	Acts via the nerveous system 9)
Bromopropylate	A	Isopropyl-4,4'-dibromobenzilat	Acts via the nerveous system 9)
Metabolite of DDT			
DDE, o, p'-	Metabo-lite of I (DDT)		Acts via the nerveous system 9)
Benzene hexachloride and lindane			
Alfa-HCH, benzene hexachloride, BHC	I	Alfa-1,2,3,4,5,6-hexachlorocyclohexane	Acts via the nerveous system 9)
Beta-HCH, benzene hexachloride, BHC	I	Beta-1,2,3,4,5,6-hexachlorocyclohexane	Acts via the nerveous system 9)
Lindane, gamma-HCH, benzene hexachloride, BHC	I	Gamma-1,2,3,4,5,6-hexachlorcyclohexane (a gamma isomer of BHC, i.e. benzene hexachloride)	Acts via the nerveous system 9)
Cyclodiene and related compounds			
Heptachlor	I	1,4,5,6,7,8,8-heptachloro-3a,7,7a-tetrahydro-4,7-methanindene	Acts via the nerveous system 9)
Dieldrin	I	Endo,exo-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4:5,8-dimethanonaphthalen	Acts via the nerveous system 9)
Endosulfan	A/I	1,4,5,6,7,7-hexachloro-5-norbornen-2,3-dimethyl cyclic sulfite	Acts via the nerveous system 9)
Pesticides derived from plants and other organisms			
Pyrethrum and related compounds			

Deltamethrin	I	[IR-[IX(S*),3X])-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromethylenyl)-2,2-dimethylcyclopropancarboxylate	Prevents the sodium channels from functioning, so that no transmission of nerveimpulses can take place 6)
Fenvalerate	I	Cyano(3-phenoxyphenyl)methyl 4-chloro-X-(1-methylethyl)benzenacetate	Prevents the sodium channels from functioning, so that no transmission of nerveimpulses can take place 6)
Permethrin	I	(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropancarboxylate	Prevents the sodium channels from functioning, so that no transmission of nerveimpulses can take place 6)
Cypermetrin	I	Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichlorethenyl)-2,2-dimethylcyclopropancarboxylate	Prevents the sodium channels from functioning, so that no transmission of nerveimpulses can take place 6)
Fenpropathrin/fenproponat	I	Cyano(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropancarboxylate	Prevents the sodium channels from functioning, so that no transmission of nerveimpulses can take place 6)
Bifenthrin	I/A	[1X,3X(Z)]-(±)-(2-methyl[1,1'-biphenyl]-3-yl)methyl3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropancarboxylate	Prevents the sodium channels from functioning, so that no transmission of nerveimpulses can take place 6)
Herbicides			
Organic phosphorus herbicides			
Glyphosate	H	N-(phosphonomethyl)glycine	Amino acid synthesis - EPSP synthase 10)
Metabolite of glyphosate			
AMPA	Metabolite of H	Aminomethylphosphoric acid	
Carbamate herbicides			
Chloropropham	H	Isopropyl m-chlorcarbanilate	Inhibit photosynthetic electron transport 9)
Fungicides and related compounds			
Chloroalkyl thio fungicides			
Captan	F	N-[(trichloromethyl)thiol]-4-cyclohexene-1,2-dicarboximide	Non-specific combination with thio groups in the fungal cell 9)

Folpet	F	2-[(trichloromethyl)thio]-1H-isoindol-1,3(2H)-dion	Non-specific combination with thio groups in the fungal cell 9)
Dichlofluanid	F	N-[dichlorofluoromethyl)thio]-N',N'-dimethyl-N-phenylsulfamide	Non-specific combination with thio groups in the fungal cell 9)
Tolyfluanid	F	N-[(dichlorofluoromethyl)thio]-N',N'-dimethyl-N-p-tolylsulfamide	Non-specific combination with thio groups in the fungal cell 9)
Other aromatic hydrocarbons			
Diphenyl	F	1,1'-biphenyl	Effect on the fungal membrane at the site of potassium transport 9)
Chlorothalonil	F	Tetrachloroisophthalonitrile	Non-specific combination with thio groups in the fungal cell 9)
Hexachlorobenzene	F 6)	Hexachlorobenzene	Unknown 9)
Pentachlorobenzene		1,2,3,4,5-pentachlorobenzene	
Pentachloranisole		Pentachloromethoxybenzene 7)	
Anilino and nitrobenzenoid fungicides			
Dichloran	F 6)	2,6-dichloro-4-nitroanilin 6)	Inhibit protein synthesis 9)
Quintozen	F	Pentachloronitrobenzene	Interfere with chitin synthesis 9). Inhibition of iodine pump, enhancement of hepatic thyroid hormone metabolism and excretion 11)
Technazen	F	1,2,4,5-tetrachloro-3-nitrobenzene	Unknown, but t. is closely related to quintozen which inhibit chitin synthesis 9)
Benzimidazole 13)			
Carbendazim	F	Methyl 1H-benzimidazol-2-yl carbamate	Photosynthesis - D-1 quinone-binding protein 10). Mitosehæmmer – eukaryotiske cellers mikrotubuli påvirkes 12)
Thiabendazol	F	2-(4-thiazolyl)benzimidazol	Inhibit mitochondrial electron transport - but it seems unlikely that this is the primary site of action 9). photosynthesis - D-1 quinone-binding protein 10)

Phenylamide (Acylalanine type) 13)			
Metalaxyl	F	N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanin methyl ester	Inhibits protein synthesis in fungi, by interference with the synthesis of ribosomal RNA 13)
Dicarboximide 13)			
Iprodione	F	3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide	Inhibits germination of spores and growth of fungal mycelium 13)
Procymidone	F	3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3,1,0]hexane-2,4-dione	Inhibits triglyceride synthesis in fungi 13)
Vinclozolin	F	3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione	Inhibits germination of spores 13)
Azole fungicides 8), 13)			
Imazalil	F	1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole	
Triadimefon	F	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone	Inhibits ergosterol biosynthesis (steroid demethylation) 13)
Triadimenol	F	1,2,4-triazole-1-ethanol	Inhibits ergosterol biosynthesis (steroid demethylation) and other enzymic processes in photopathogenic fungi 13)
Fungicide not otherwise classified			
Diphenylamine	F	N-phenylbenzenamine	
Plant growth regulators			
Quaternary ammonium compounds			
Mepiquat	P	1,1-dimethylpiperidiniumion	Inhibits the biosynthesis of gibberelic acid. Plant growth regulator. 13)
Chlormequat	P	(2-chloroethyl)-trimethylammoniumion	Inhibits the biosynthesis of gibberelic acid. Plant growth regulator which inhibits cell elongation, hence shortening and strenghtening the stem and producing a sturdier plant. 13)
Micellaneous pesticides			
Syntetic acaricides			
Chlorfenson	A	p-chlorophenyl-p-chloro-	

		benzenesulfonate	
Tetrasul	A	p-chlorophenyl 2,4,5-trichlorophenyl sulfide	Unknown 9)
Fenson	A	4-chlorophenyl benzenesulfonate	Unknown 9)
Tetradifon	A	4-chlorophenyl 2,4,5-trichlorophenyl sulfone	Unknown 9)
Phenols = metabolites			
Ortho-phenylphenol	Metabo-lite/ F	Ortho-phenylphenol or 2-phenylphenol	

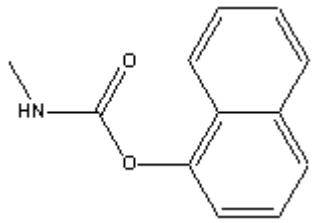
Abbreviations used in the table:
H = Herbicide
A = Acaricide
F = Fungicide
I = Insecticide
N = Nematocide
P = Plant growth regulators

References used in appendix 3:

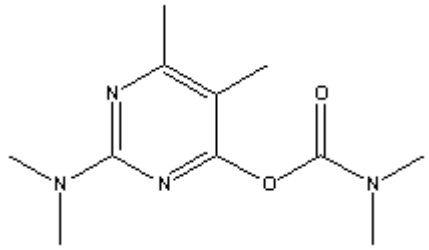
1) Pesticidrester i danske levnedsmidler 1997. (Veterinær- og Fødevaredirektoratet, 1998. In Danish).
2) Pesticidrester i danske levnedsmidler 1998. (Veterinær- og Fødevaredirektoratet, 1999. In Danish).
3) Pesticidrester i danske levnedsmidler 1999. (Veterinær- og Fødevaredirektoratet, 2000. In Danish).
4) Oversigt over godkendte bekæmpelsesmidler. (Orientering fra Miljøstyrelsen, nr. 1, 1992. In Danish).
5) Oversigt over godkendte bekæmpelsesmidler. (Orientering fra Miljøstyrelsen, nr. 1, 1997. In Danish).
6) Hayes W Jr. and Laws, ER Jr. (Editors): Handbook of pesticide toxicology. Volume 1-3 (Academic Press, 1991)
7) Chemfinder, website: (www.chemfinder.com)
8) Compendium of pesticide common names. (www.hclrss.demon.co.uk/)
9) Corbett, J R: The Biochemical Mode of Action of Pesticides. (Academic Press, 1974)
10) Duke, S. O.: Overview of herbicide mechanisms of action. (Environmental Health Perspectives, 1990, 87, 263-271)
11) Hurley PM: Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. (Environ Health Perspect, 1998, 106, 8, 437-45)
12) Miljøstyrelsen, Bekæmpelsesmiddelkontoret. Grundvurdering af Derosal indeholdende Carbendazim som aktivstof. J.nr. M 741-0242, TBL/MKA/SM/sm/11, Den 4 januar 1995
13) The e-Pesticide Manual, 11. ed., 1999

Appendix 4: Structure of the pesticides

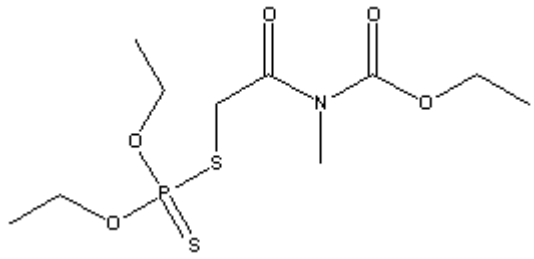
Carbamate insecticides
Anticholinesterase carbamates and procarbamates



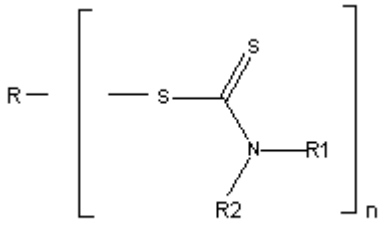
Carbaryl



Pirimicarb



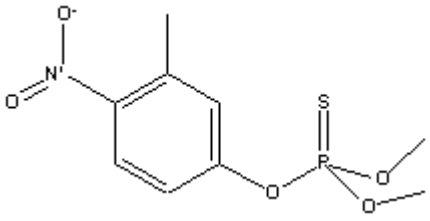
Mecarbam



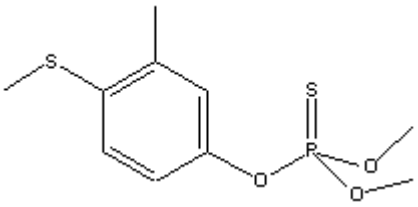
R: Na^+, Fe^{3+}, S-C(S)-N-$(CH_3)_2$ and others
R1: H, -CH_3 or -CH_2CH_3
Dithiocarbamates (analysed as a sum of 6 dithiocarbamates)

Organic phosphorus pesticides - cholinesteraseinhibitors

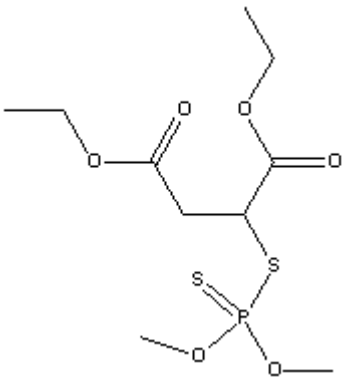
Dimethoxy compounds of category IV ($(CH_3–O)_2P(R)X$, R=O or S)



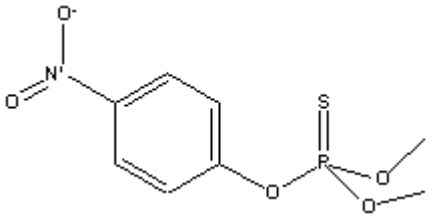
Fenitrothion



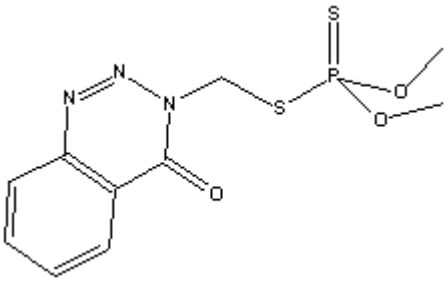
Fenthion



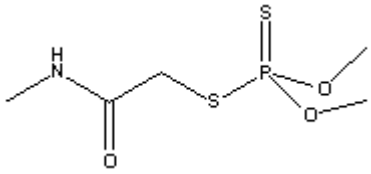
Malathion



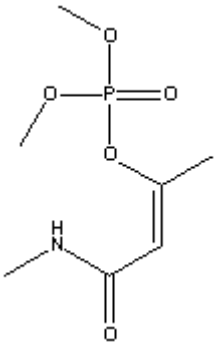
Parathion-methyl



Azinphos-methyl

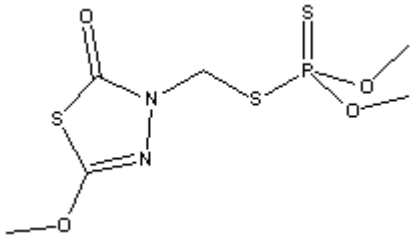


Dimethoate

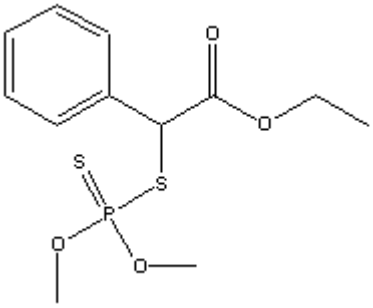


Monocrotophos

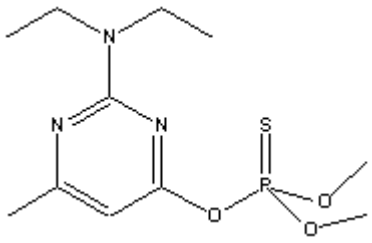
APPENDIX 4



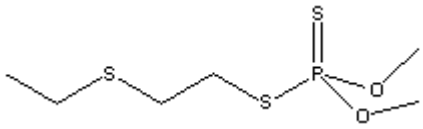
Methidathion



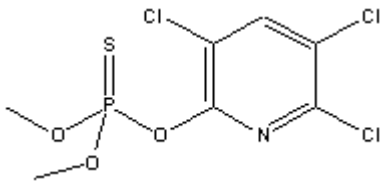
Phenthoate



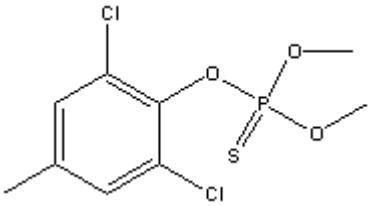
Pirimiphos-methyl



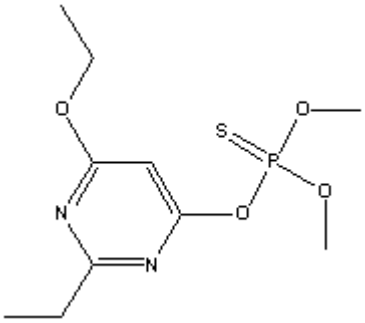
Thiometon



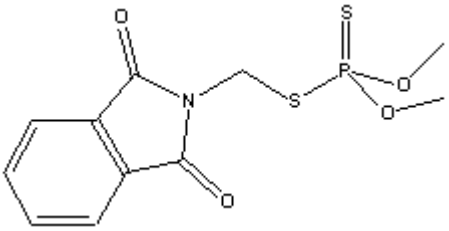
Chlorpyrifos-methyl



Tolclofos-methyl

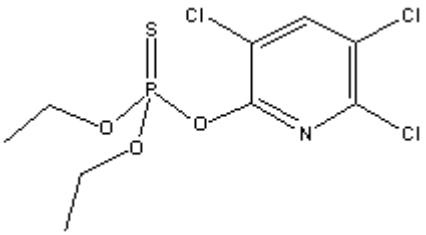


Etrimfos

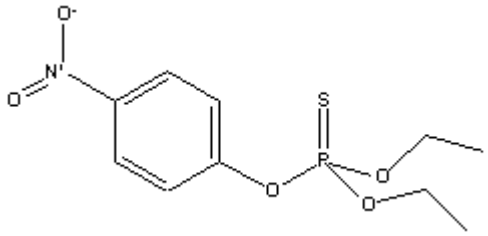


Phosmet

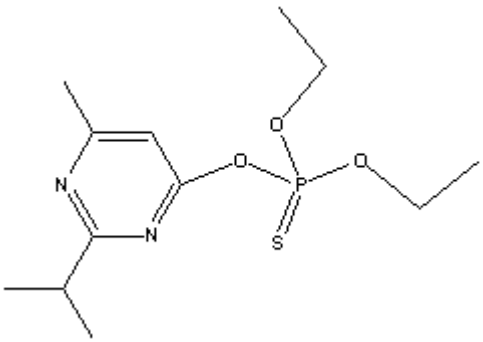
Diethoxy compounds of category IV ($(C_2H_5–O)_2P(R)X$, R=O or S)



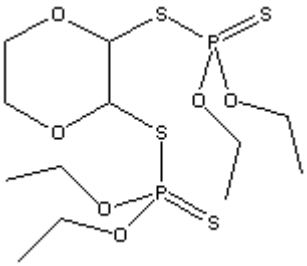
Chlorpyrifos



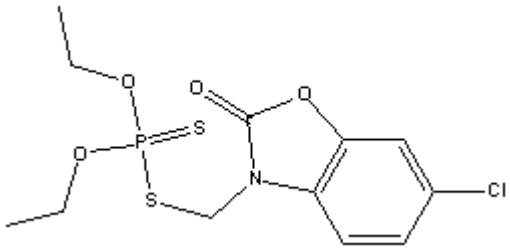
Parathion



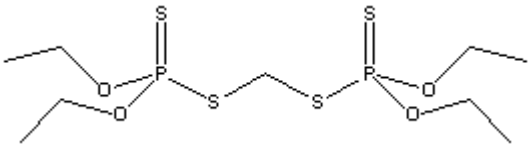
Diazinon



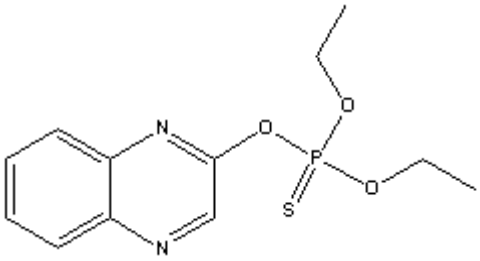
Dioxathion



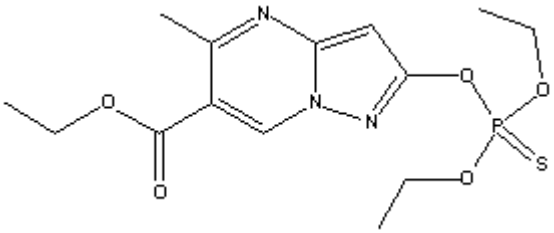
Phosalone



Ethion

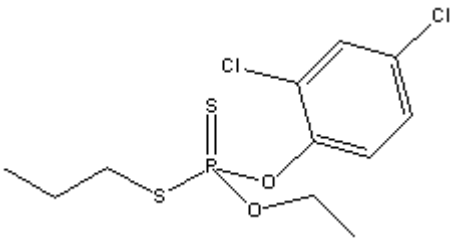


Quinalphos



Pyrazophos

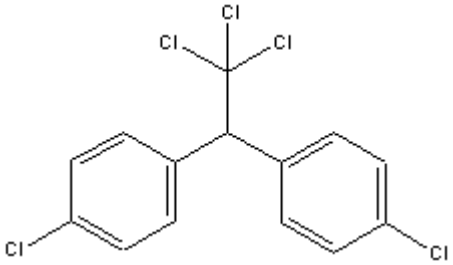
Mixed substitute Compounds of category IV



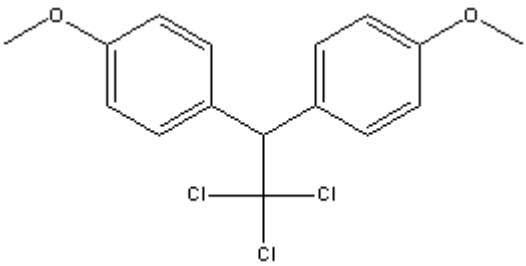
Prothiofos

APPENDIX 4

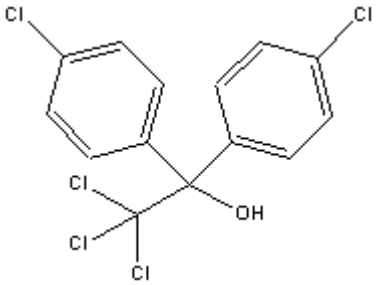
Chlorinated hydrocarbon insecticides
DDT and its analoges



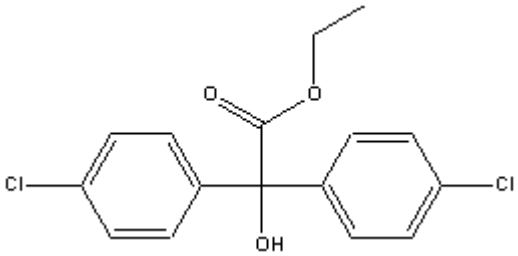
DDT



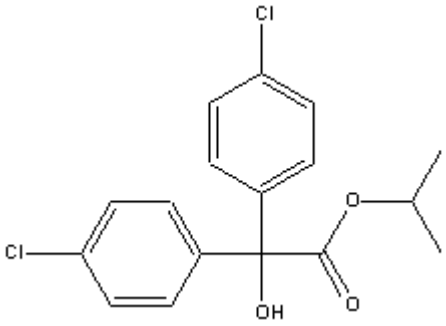
Methoxychlor



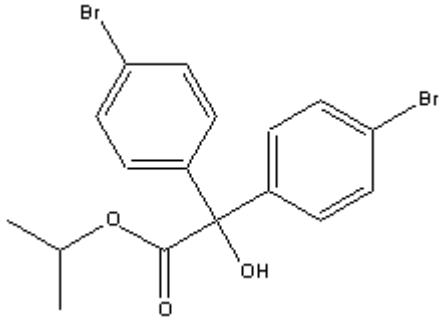
Dicofol



Chlorobenzilate

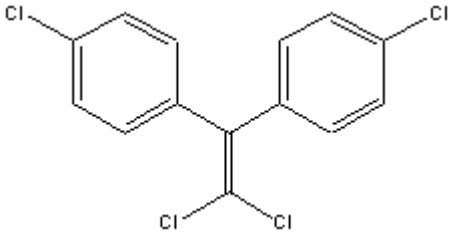


Chloropropylate



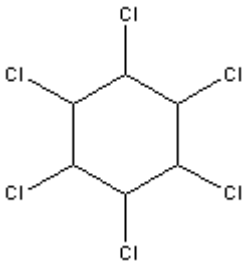
Bromopropylate

Metabolite of DDT



DDE, o, p'-

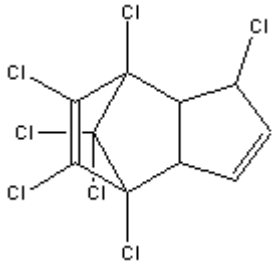
Benzene hexachloride and lindane



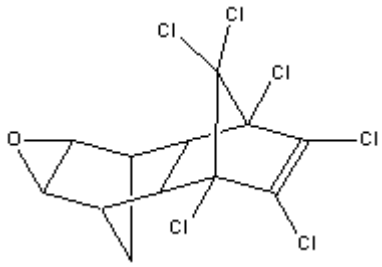
alfa-HCH, benzene hexachloride, BHC
beta-HCH, benzene hexachloride, BHC
Lindane, gamma-HCH, benzene hexachloride, BHC

APPENDIX 4

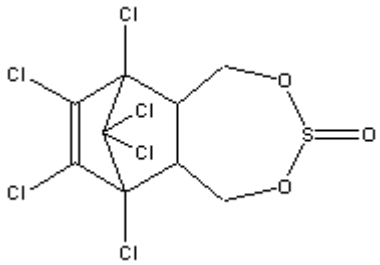
Cyclodiene and related compounds



Heptachlor



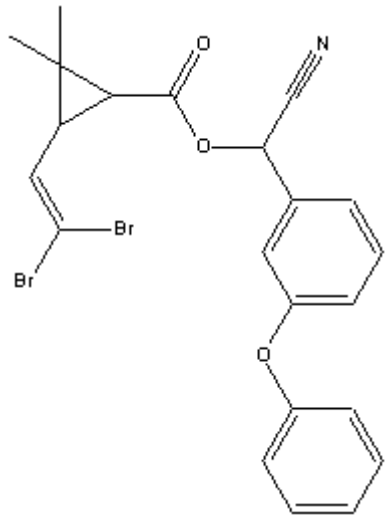
Dieldrin



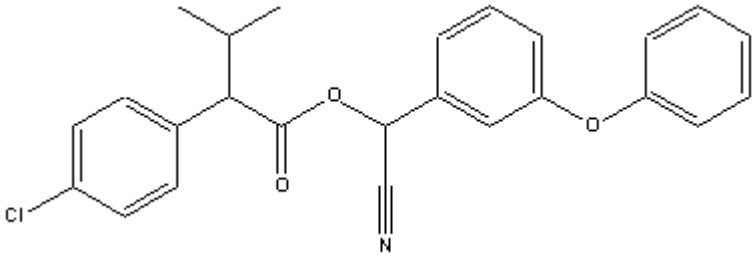
Endosulfan

APPENDIX 4

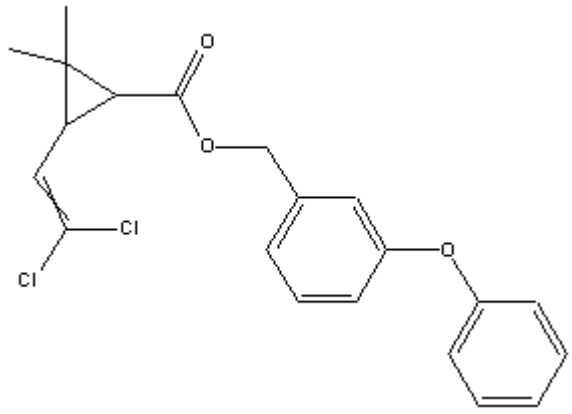
Pesticides derived from plants and other organisms
Pyrethrum and related compounds



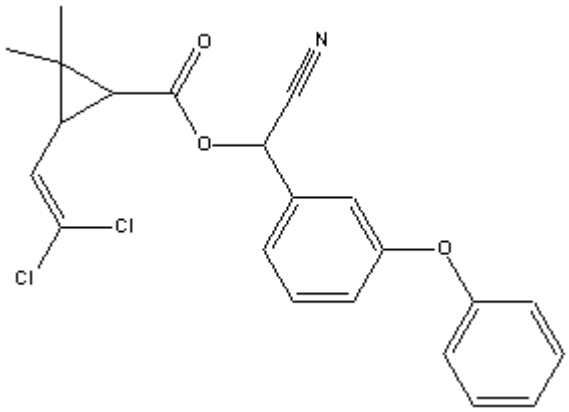
Deltamethrin



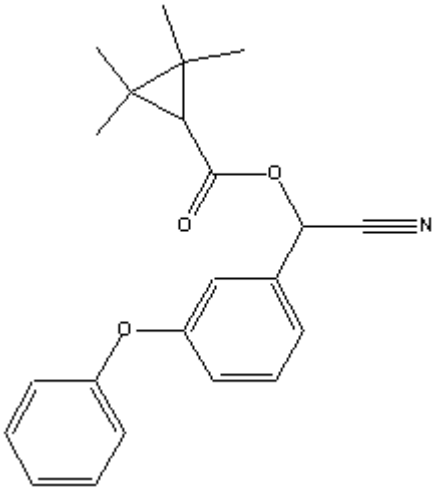
Fenvalerate (a pyrethroid but not based on a cyclopropane ring structure)



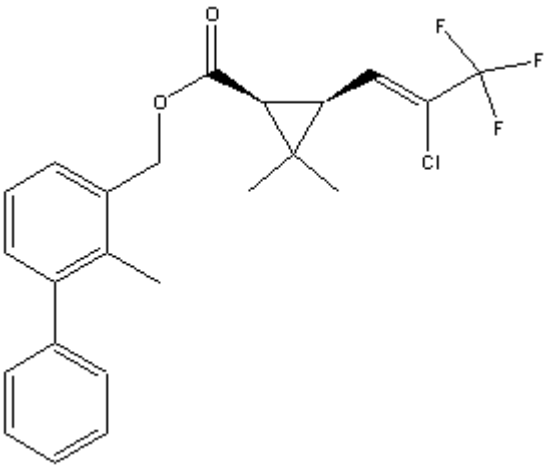
Permethrin



Cypermetrin



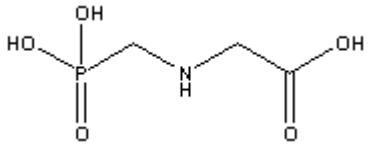
Fenpropathrin/fenproponat



Bifenthrin

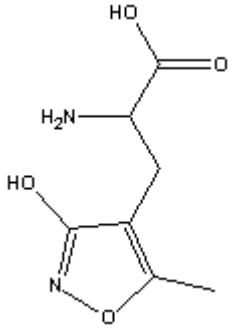
Herbicides

Organic phosphorus herbicides



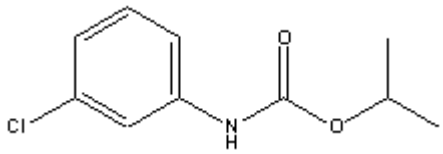
Glyphosate

Metabolite of glyphosate



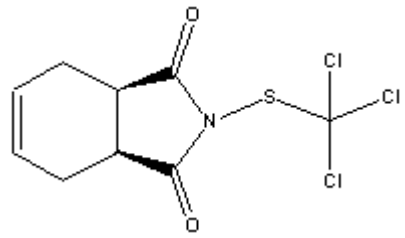
AMPA

Carbamate herbicides

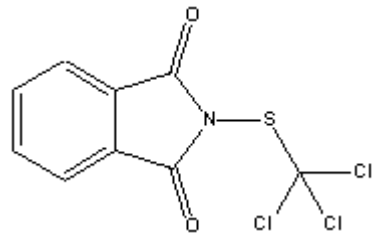


Chlorpropham

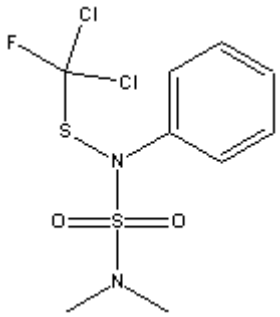
Fungicides and related compounds
Chloroalkyl thio fungicides (RSCCl)



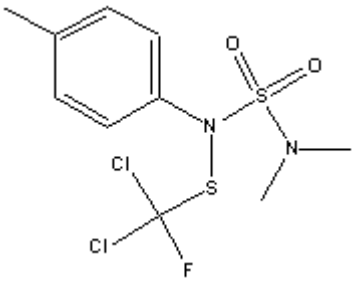
Captan



Folpet

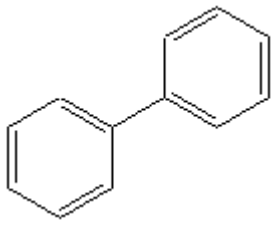


Dichlofluanid

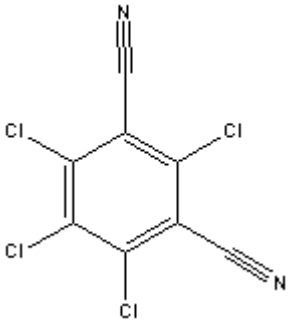


Tolyfluanid

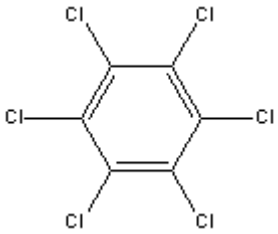
Other aromatic hydrocarbons



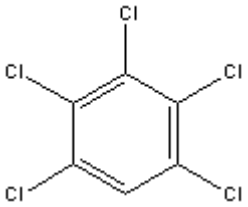
Diphenyl



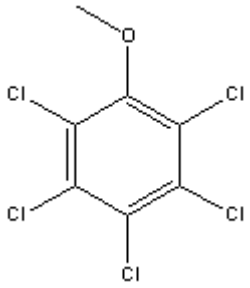
Chlorthalonil



Hexachlorobenzene

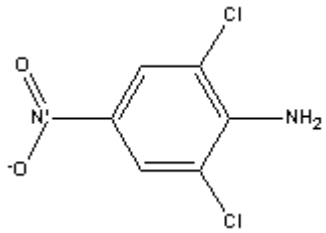


Pentachlorobenzene

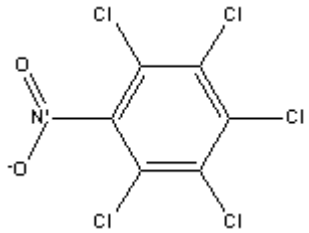


Pentachloranisole

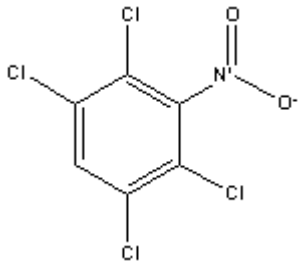
Anilino and nitrobenzenoid fungicides



Dichloran

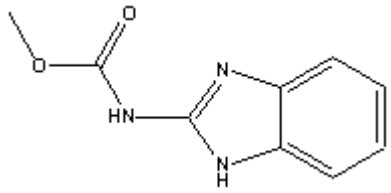


Quintozen

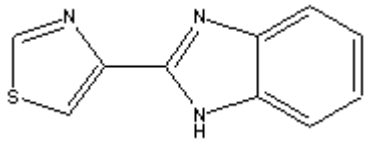


Technazen

Benzimidazole

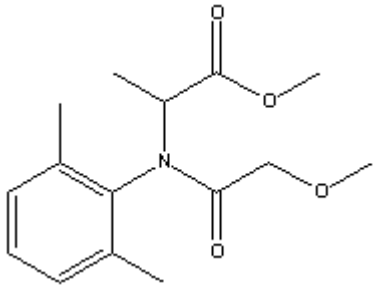


Carbendazim



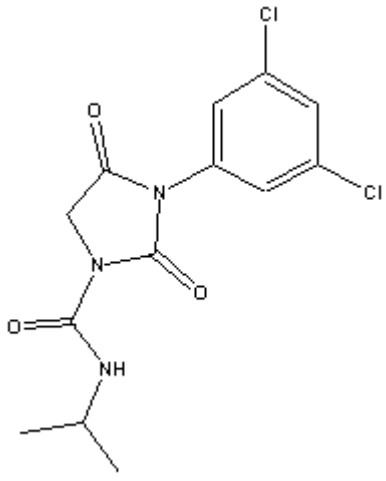
Thiabendazol

Phenylamide (acylalanine type)

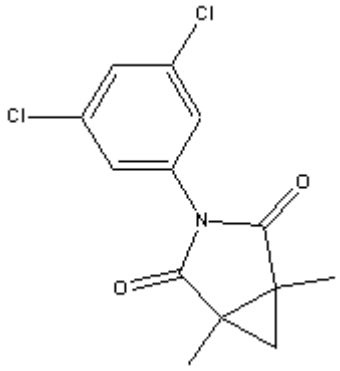


Metalaxyl

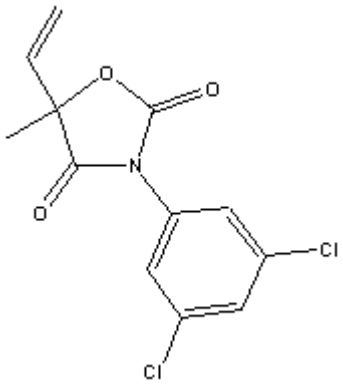
Dicarboximide



Iprodione

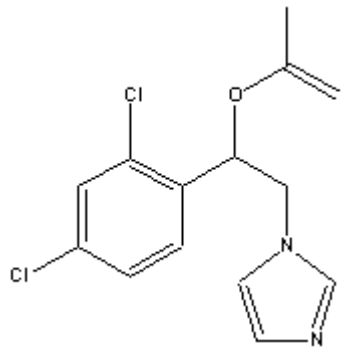


Procymidone

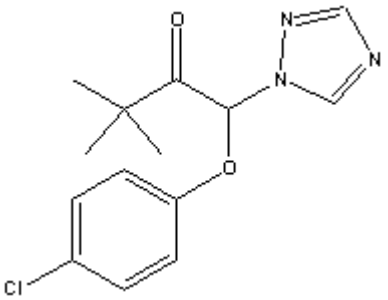


Vinclozolin

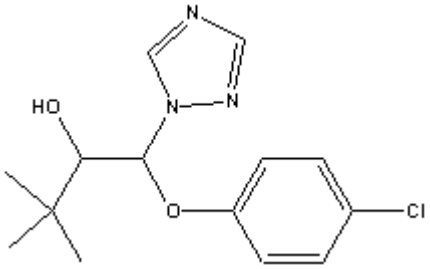
Azole fungicides



Imazalil

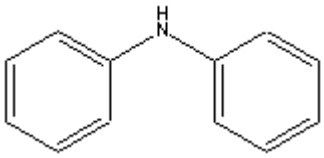


Triadimefon



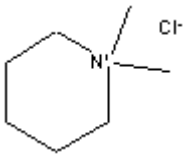
Triadimenol

Fungicides not otherwise classified

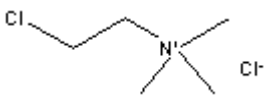


Diphenylamine

Plant growth regulators
Quaternary ammonium compounds

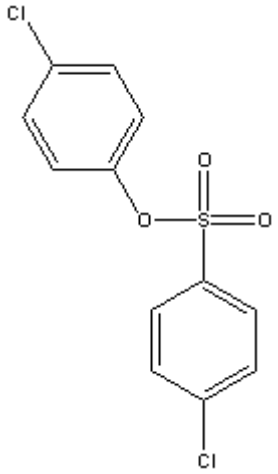


Mepiquat

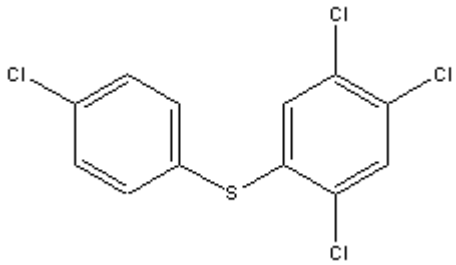


Chlormequat

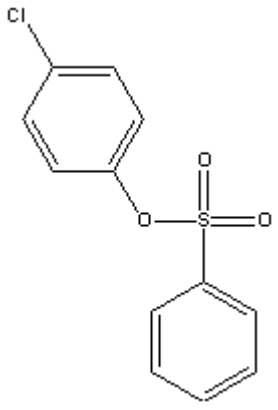
Micellaneous pesticides
Syntetic acaricides



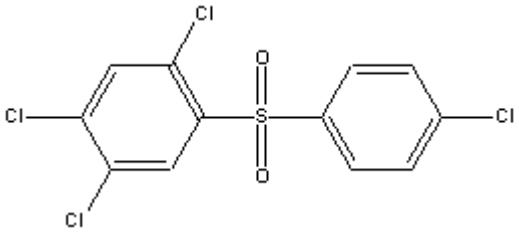
Chlorfenson



Tetrasul

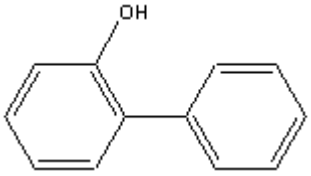


Fenson



Tetradifon

Phenoles = metabolites



Ortho-phenylphenol

Appendix 5: Toxicological data on single compounds – pesticides found in food in Denmark

CARBAMATE INSECTICIDES
Anticholinesterase carbamates and procarbamates

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Carbaryl	carcinogenicity, 1	mice	both	2 years, repeated oral	0, 100, 1000 or 8000 ppm	NOAEL not identified for neoplastic lesion. For non-neoplastic lesions: NOAEL = 100 ppm = 14.7 mg/kg bw/day	vascular tumours at highest dose. Other effects: liver tumours (females), kidney tumours (males), vascular tumours at the lowest doses (males). Conclusion: carcinogenic in mice.	liver, kidney, vascular	JMPR 1996
	carcinogenicity (and toxicity), 2	rats	-	2 years, repeated oral	0, 250, 1500 or 7500 ppm	-	tumors (thyroid (males), liver (females), bladder(both)) at 7500 ppm - 350 mg/kg bw/day, which exceeded the MTD. Conclusion: carcinogenic in rats only at levels that exceed the MTD	thyroid, liver, urinary bladder	JMPR 1996
	(carcinogenicity and) toxicity, 2	rats	-	2 years, repeated oral	0, 250, 1500 or 7500 ppm	NOAEL = 10 mg/kg bw/day = 250 ppm	inhibition of erythrocyte and brain acetylcholinesterase activity and reduced body weight at 1500 ppm.	nervous system	JMPR 1996
	systemic toxicity, 1	rats	both	1 year, repeated oral (gavage)	-	NOAEL = 2 mg/kg bw/day	effects on thyroid and male and female reproductive organs and/or function at doses >= 5 mg/kg bw/day	reproductive organs, thyroid	JMPR 1996
	systemic toxicity, 2	dogs	-	5 weeks and 1 year	20-125 ppm and 125-1250 ppm	NOAEL = 3.1 mg/kg bw/day (highest dose tested)	effects on liver weight. Inhibition of acetylcholinesterase activity in erythrocytes and brain at 400 ppm	liver, nervous system	JMPR 1996
	systemic toxicity, 3	human	-	6 weeks	0.06 or 0.13 mg/kg bw	NOAEL = 0.06 mg/kg bw/day	increased ration of amino acid nitrogen to creatinine in urine at 0.13 mg/kg bw/day	-	JMPR 1996
	systemic toxicity, 4	dogs	-	1 year, repeated oral (gavage)	0.45, 1.8 or 7.2 mg/kg bw/day	NOAEL = 1.8 mg/kg bw/day	slight effects on kidney at 7.2 mg/kg bw/day	kidney	JMPR 1996
	systemic toxicity, 5	rats	both	Short-term (1-7 days)	-	LD50 = 225-721 mg/kg bw	-	-	JMPR 1996
	systemic toxicity, 6	cats	-	-	-	LD50 = 150 mg/kg bw	-	-	JMPR 1996
ADI: 0-0,003 mg/kg bw. (LOAEL = 15 mg/kg bw/day in the carcinogenicitystudy in mice. Safety factor = 5000 - including a safety factor of 50 for presence of vascular tumours in male mice.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Carbofuran	carcinogenicity, 1	mice	both	repeated oral, 2 years	0, 20, 125 or 500 ppm = 0, 2.8, 18 or 70 mg/kg bw/day	NOAEL = 2.8 mg/kg bw/day = 20 ppm	brain acetylcholinesterase inhibition at 125 ppm. No evidence of carcinogenicity	nervous system	JMPR 1996
	carcinogenicity, 2	rats	both	repeated oral, 2 years	0, 10, 20 or 100 ppm	NOAEL = 1 mg/kg bw/day = 20 ppm	reduced body-weight gain and cholinesterase inhibition in erythrocytes and brain. No evidence of carcinogenicity	nervous system	JMPR 1996
	repro-tox, 1	rats	-	repeated oral, gavage	up to 1.2 mg/kg bw/day	NOAEL = 1.2 mg/kg bw/day	no evidence of teratogenicity	-	JMPR 1996
	repro-tox, 2	rats	-	repeated oral, feeding	-	NOAEL = 1.5 mg/kg bw/day	maternal toxicity	-	JMPR 1996
	repro-tox, 3	rats	24 mated female	repeated oral	0, 20, 75 or 300 ppm = 0, 1.7, 5 or 20 mg/kg bw/day	NOAEL = 1.7 mg/kg bw/day	reduced body-weight gain of dams and pups. Reduced pup survival and slight developmendal delay at doses >= 75 ppm	-	JMPR 1996
	repro-tox, 4	rats	-	-	-	NOAEL = 1.6 mg/kg bw/day	parental and pup toxicity	-	JMPR 1996
	repro-tox, 5	rabbit	-	repeated oral	-	NOAEL = 0.6 mg/kg bw/day	maternal toxicity, no evidence of teratogenicity	-	JMPR 1996
	mutagenicity	in vitro/in vivo	-	-	-	-	+/- conclusion: not genotoxic	-	JMPR 1996
	systemic toxicity	dogs	4 males	repeated oral	0-5 ppm	NOAEL = 0.22 mg/kg bw/day = 5 ppm	on effects on clinical signs, body weight, food consumption, plasma or erythrocyte cholinesterase activity	nervous system	JMPR 1996
	systemic toxicity	rats	both	-	-	LD50 = 6-18 mg/kg bw	typical acetylcholinesterase inhibition: salivation, cramp trembling, sedation	nervous system	JMPR 1996
ADI: 0-0,02 mg/kg bw based on the NOAEL for erythrocyte acetylcholinesterase inhibition of 0.22 mg/jg bw/day in a four-week study in dog (most sensitive species). Safety factor: 100									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Aldicarb	carcinogenicity	rats, mice					-		JMPR 1992
	repro-tox	rats		dietary, 3-generation	0, 0.2, 0.3 or 0.7 mg/kg bw/day	NOAEL = 0.7 mg/kg bw/day	reproductive toxicity. Not terato-genic + no maternally toxicity	-	JMPR 1992
	genotoxicity	in vitro/in vivo	-				-		JMPR 1992
	neurotoxicity						no delayed neurotoxicity		JMPR 1992
	systemic toxic-ity, 1	mice	5/sex	oral, 7 days	0, 0.1, 0.3, 0.6 or 1.2 mg/kg bw/day	NOAEL = 0.6 mg/kg bw/day	mortality at 1.2 mg/kg bw/day		JMPR 1992
	systemic toxic-ity, 2	rat	10/sex	oral, 93 days	0, 0.02, 0.1 or 0.5 mg/kg bw/day	NOAEL = 0.1 mg/kg bw/day	mortality, growth depression		JMPR 1992
	systemic toxic-ity, 3	dog	5/sex	oral, 52 weeks, Limiting feeding time: 2 h/day	0, 0.027, 0.054, 0.131 or 0.241mg/kg bw/day = 0, 1, 2, 5 or 10 ppm	NOAEL = 2 ppm = 0.054 mg/kg bw/day	changes in plasma enzyme activity		JMPR 1992
	systemic toxic-ity, 3	dog	5/sex	oral, 52 weeks, Limiting feeding time: 2 h/day	0, 0.027, 0.054, 0.131 or 0.241mg/kg bw/day = 0, 1, 2, 5 or 10 ppm	NOAEL = 1 ppm = 0.027mg/kg bw/day	plasma cholinesterase inhibition	nervous system	JMPR 1992
	systemic toxic-ity, 4	human	44 ma-le/14 female	single oral dose	0, 0.01, 0.025, 0.05 or 0.075 mg/kg bw/day	NOAEL = 0.025 mg/kg bw/day	inhibition of erythrocyte acetyl cholinesterase	nervous system	JMPR 1992
	systemic toxic-ity, 5	rats	both	oral		LD50 = 0.62-1.23 mg/kg bw	-		JMPR 1992
	systemic toxic-ity, 6	mice	both	oral		LD50 = 0.382-1.5 mg/kg bw	-		JMPR 1992
	systemic toxic-ity, 7	guinea-pig	-	oral		LD50 = 1.0 mg/kg bw			JMPR 1992
	systemic toxic-ity, 8	rabbit		oral		LD50 = 1.3 mg/kg bw			JMPR 1992
ADI: 0-0,003 mg/kg bw based on NOAEL for depression of erythrocyte cholinesterase activity in human volunteers. Safety factor: 10									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Pirimicarb	carcinogenicity						-		JMPR 1982
	mutagenicity	in vitro/in vivo	-				-		JMPR 1982
	systemic toxicity	mice	60/sex/group	96 weeks	0, 200, 400 or 1600 ppm	-	decreased bodyweight at 1600 ppm. Mortallity decreased for high dose females.	-	JMPR 1982, 1981, 1978
	systemic toxicity	monkey	4/sex	17 weeks followed by 8 weeks recovery	0, 2, 7 or 25 mg/kg	NOAEL = 2 mg/kg bw/day	cholinesterase inhibition in erythrocytes	nervous system	JMPR 1982, 1981, 1978
	systemic toxicity	rat	20 female	8 weeks	0, 100, 175, 250 or 750 ppm	NOAEL = 175 ppm = 9 mg/kg bw/day	reduction of body weight gain at 750 ppm. Lower food consumption	-	JMPR 1982, 1981, 1978
	systemic toxicity	dog (beagle)	-			NOAEL = 1.8 mg/kg bw/day	-		JMPR 1982, 1981, 1978
	systemic toxicity	dog and monkey	-				cholinesterase inhibition in plasma at 25 mg/kg bw and above	nervous system	JMPR 1982, 1981, 1978
ADI: 0-0,02 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STU-DY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Mecarbam	neurotoxicity (and toxicity)	hens	both	single oral dose, 21 days study. Some of the animals got another dose - sacrificed on day 22	0 or 175 mg/kg bw at a dose volume of 2.5 mg/kg bw	-	no delayed neurotoxicity		JMPR 1986
	(neurotoxicity and) toxicity	hens	both	single oral dose, 21 days study. Some of the animals got another dose - sacrificed on day 22	0 or 175 mg/kg bw at a dose volume of 2.5 mg/kg bw	-	cholinergic signs of acute toxicity. Small increase in mortality at 175 mg/kg bw. De-creased bodyweight and food consumption	-	JMPR 1986
	systemic toxicity	hens				LD50 = 171 mg/kg bw			JMPR 1986
	systemic toxicity	rat		dietary, 104 weeks	0, 1, 5 or 50 mg/kg	NOAEL = 5 ppm = 0.21 mg/kg bw/day	cholinesterase inhibition	nervous system	JMPR 1986
	systemic toxicity	dog				NOAEL = 10 ppm = 0.35 mg/kg bw/day	-		JMPR 1986
ADI: 0-0,002 mg/kg bw based on the metabolism and delayed neurotoxicity studies									

APPENDIX 5

ORGANIC PHOSPHORUS PESTICIDES - ACETYLCHOLINESTERASE-INHIBITORS

Dimethoxy compounds of category IV

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Fenitrothion	carcinogenicity						-		JMPR 1974
	repro-tox study	rat	20-24 mated females	daily gavage; day 6 to 15 of gestation; animals were sacri-ficed on day 20 of gestation	0, 3, 8 or 25 mg/kg bw in corn oil	NOAEL = 8 mg/kg bw/day for dams	Signs of toxicity (tremors, rhinorrea and rough haircoat, etc.). Reduced body weight gain. No evidence of developmental toxicity	-	JMPR 1988
	repro-tox study	rabbit	insemi-nated females	daily gavage; day 7 to 19 of gestation; animals were sacri-ficed on day 29 of gestation	0, 3, 10 or 30 mg/kr bw in corn oil	NOAEL = 10 mg/kg bw/day for dams	lower bodyweight gain. Maternal toxicity at 30 mg/kg. No effects on foetal growth or development	-	JMPR 1988
	repro-tox study	rats					no adverse effects at doses below those toxic to parents	-	
	neurotoxicity	hens		2 * single dose	500 mg/kg. Protected against acute anticholon-esterase effect by atro-pine and PAM	-	5 of 16 hens died after the first treatment. Symp-toms of cholinesterase inhibition	nervous system	JMPR 1977
	neurotoxicity	hens		2 * single dose	35 mg/kg. Protected against intoxication by atropine and PAM	-	5 of 12 hens died		JMPR 1977
	mutagenicity	rats		in diet, 4-generation	0, 10, 40 or 80 ppm	-	-		JMPR 1974
	systemic toxic-ity, 1	rats	15/sex	in diet	0, 10, 30 or 150 ppm	-	depression of cholinesterase activity in the brain, red cells and plasma at 150 ppm. Depression in females at 30 ppm. At 10 ppm depression of plasma cholinesterase activity in females.	nervous system	JMPR 1974
	systemic toxic-ity, 2	rats	8/sex	34 weeks, in diet	0, 10, 50 or 250 ppm	-	lower relative weight of the spleen at 125 ppm. At 250 ppm in females histological changes of the liver and spleen. decrease of brain cholinesterase activity at 250 ppm. Dose dependent decrease in cholinesterase activities in plasma and erythrocytes	liver, spleen, nervous system	JMPR 1974

	systemic toxic-ity, 3	dogs	4/sex	90 days (5 ppm) and 1 year (10 ppm), in diet	0, 5 or 10 ppm for 90 days	-	inhibition of acetylcholinesterase activity in plasma. No difference in blood and brain choli-nesterase activities	nervous system	JMPR 1974
	systemic toxic-ity, 4	dogs	6/sex	2 years, in diet	0, 30, 100 or 200 ppm	-	inhibition of cholinesterase activity in plasma. Inhibition of erythrocyte enzyme activity was not affected at 30 ppm. Brain cholinesterase activity depressed at 200 ppm	nervous system	JMPR 1974
	systemic toxic-ity, 5	rats	15/sex	92 weeks	0, 2.5, 5 or 10 ppm	-	inhibition of cholinesterase activity in plasma and the red blood cells. Brain cholinesterase activity was not affected	nervous system	JMPR 1974
	systemic toxic-ity, 6	man	2/sex + con-trols: 5/sex	21 + 3 + 18 +21 days	0.1 or 0.3 mg/kg bw for 21 days, then 0.5 mg/kg bw for 3 days, 18 days recovery and finally 0,2 mg/kg bw for 21 days	-	inhibition of plasma cholinesterase activity. No cholinesterase inhibition at 0.2 mg/kg for 21 days	nervous system	JMPR 1974
	systemic toxic-ity, 7	rats				NOAEL = 10 ppm = 0.5 mg/kg bw/day	brain acetylcholinesterase inhibition and reproduc-tion	nervous system	JMPR 1988
	systemic toxic-ity, 8	dogs (beagle)	6/sex	oral, 12 months	0, 5, 10 or 50 ppm	NOAEL = 50 ppm = 1.25 mg/kg bw/day	reduced plasma cholinesterase at 50 ppm. Erythro-cyte cholinesterase in high dose males	nervous system	JMPR 1988
	systemic toxic-ity, 9	man		oral, 4 times at 24 h interval	0.04-0.08 mg/kg bw	NOAEL = 0.08 mg/kg bw/day	a rise in red cell cholinesterase activity	nervous system	JMPR 1969
	systemic toxic-ity, 10	mice				LD50 = 110-1336 mg/kg bw	-		JMPR 1969
	systemic toxic-ity, 11	rats				LD50 = 33-740 mg/kg bw	-		JMPR 1969
	systemic toxic-ity, 12	guinea-pigs	-			LD50 = 110-1850 mg/kg bw	-		JMPR 1969
ADI: 0-0,005 mg/kg bw									

CHEMICAL	TYPE OF STU-DY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Fenthion	carcinogenicity (and toxicity), 1	mouse	60/sex	2 years	0, 0.1, 1.0, 5.0 or 25 ppm = 0, 0.03, 0.40, 1.95 or 9.42 mg/kg bw/day	NOAEL = 1.95 mg/kg bw/day = 5 ppm	-		JMPR 1995
	(carcinogenicity and) toxicity, 1	mouse	60/sex	2 years	0, 0.1, 1.0, 5.0 or 25 ppm = 0, 0.03, 0.40, 1.95 or 9.42 mg/kg bw/day	NOAEL = 1.95 mg/kg bw/day = 5 ppm	depression of brain acetylcholinesterase activity	nervous system	JMPR 1995
	carcinogenicity (and toxicity), 2	rat	50/sex/dose. Controls: 100/sex	2 years	0, 3, 15 or 75 ppm	-	-		JMPR 1995
	(carcinogenicity and) toxicity, 2	rat	50/sex/dose. Controls: 100/sex	2 years	0, 3, 15 or 75 ppm	NOAEL = 0.14 mg/kg bw/day = 3 ppm	inhibtion of erythrocyte acetylcholi-nesterase. Brain acetylcholinesterase was not determined	nervous system	JMPR 1995
	repro-tox, 1	rat		diet, two-generation	0, 1, 2, 14 or 100 ppm = 0, 0.08, 0.16, 1.16 or 8.3 mg/kg bw/day	NOAEL = 0.16 mg/kg bw/day = 2 ppm	maternal toxicity based on consistent inhibition of brain and erythrocyte acetyl-cholinesterase activity	nervous system	JMPR 1995
	repro-tox, 1	rat		diet, two-generation	0, 1, 2, 14 or 100 ppm = 0, 0.08, 0.16, 1.16 or 8.3 mg/kg bw/day	NOAEL = 14 ppm = 1.2 mg/kg bw/day	decreased fertility, implantation sites, litter size, pup viability and growth		JMPR 1995
	repro-tox, 2	rat	33 mated females	oral by gavage	0, 1, 4.2 or 18 mg/kg bw/day	NOAEL = 18 mg/kg bw/day (highest dose tested)	no signs of embryo- and fetotoxicity and teratogenicity were seen		JMPR 1995
	repro-tox, 3	rabbit	17 inseminated females	daily dose on day 6-18 of gestation. Ga-vage	0, 1, 2.75, 7.5 mg/kg bw/day	a) NOAEL = 7.5 mg/kg bw/day (highest dose tested). b) NOAEL = 1 mg/kg bw/day	a) no signs of embryo- and fetotoxicity and teratogenicity was seen on any dose level. b) maternal toxicity based on inhibi-tion of brain and erythrocyte acetylcholi-nesterase activity	nervous system	JMPR 1995
	mutagenicity	in vitro/in vivo	-				weakly genotoxic		
	neurotoxicity	hens					not delayed neuropathy		
	systemic toxicity	mouse, rat, guinea-pig, rabbit	-	oral, 1-7 days		LD50 = 50-500 mg/kg bw			JMPR 1995

	systemic toxicity	rat		single oral	0, 1, 50 (males), 75 (females), 150 (males) or 225 (females) mg/kg bw	NOAEL = 1 mg/kg bw	inhibition of brain acetylcholinesterase activity and neurobehavioural effects	nervous system	JMPR 1997
	systemic toxicity	dog	4/sex	repeated dietary, 2 years	0, 3, 10 or 30 ppm (weeks 0-64), 50 (weeks 65-67) or 60 (weeks 68-104) = 0, 0.09, 0.32 or 1.28 mg/kg bw/day	NOAEL = 0.09 mg/kg bw/day = 3 ppm	inhibition of brain and erythrocyte acetyl-cholinesterase at 10 ppm	nervous system	JMPR 1995
	systemic toxicity	human	4males/group	oral, 25 days	0, 0.02 or 0.07 mg/kg bw/day	NOAEL = 0.07 mg/kg bw (highest dose tested)	no inhibition of erythrocyte acetylcholi-nesterase activity	nervous system	JMPR 1995
	ocular toxicity	rat					toxic to the eyes at 100 ppm = 5.2 mg/kg bw/day	-	JMPR 1995
ADI: 0-0,007 mg/kg bw based on NOAEL = 0.07 mg/kg bw/day for inhibition of erythrocyte acetylcholinesterase activity in a 25 day study in human volunteers, safety factor: 10									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Malathion	Carcinogenicity and toxicity, 1	mice		Dietary, 18 months	0, 100, 800, 8000 or 16000 ppm	NOAEL = 800 ppm = 140 mg/kg bw/day	Inhibition of brain acetylcholinesterase activity, liver adenomas	nervous system, liver	JMPR 1997
	Carcinogenicity (and toxicity), 2	rats		Dietary, 2 years	0, 100, 1000 or 5000 ppm	-	-		JMPR 1997
	(Carcinogenicity and) toxicity, 3	rats		Dietary, 2 years	0, 100, 1000 or 5000 ppm	NOAEL = 100 ppm = 5 mg/kg bw/day	Inhibition of erythrocyte acetylcholinesterase activity, reduced body weight	nervous system	JMPR 1997
	Carcinogenicity (and toxicity), 2	rats		Dietary, 2 years	0, 100/50, 500, 6000 or 12000 ppm		-		JMPR 1997
	(Carcinogenicity and) toxicity, 3	rats		Dietary, 2 years	0, 100/50, 500, 6000 or 12000 ppm	NOAEL = 500 ppm = 29 mg/kg bw/day	Decreased survival and body weight gain, changes in haematological parameters, decreased brain acetylcholinesterase activity, increased gamma-glutamyl transpeptidase activity, increased liver, kidney and thyroid/parathyroid weights, changes in the olfactory epithelium	blood, liver, kidney, thyroid/parathyroid, nervous system	JMPR 1997
	Repro-tox, 1	rats		Gavage, days 6-15 of gestation	0, 200, 400 or 800 mg/kg bw/day	NOAEL = 400 mg/kg bw/day	Maternal toxicity. No fetal toxicity observed	-	JMPR 1997
	Repro-tox, 2	rabbits		Orally, days 6-18 of gestation	0, 25, 50 or 100 mg/kg bw/day	NOAEL = 25 mg/kg bw/day	Maternal toxicity.		JMPR 1997
	Repro-tox, 2	rabbits		Orally, days 6-18 of gestation	0, 25, 50 or 100 mg/kg bw/day	NOAEL = 100 mg/kg bw/day	fetal toxicity. No teratogenicity		JMPR 1997
	Repro-tox, 3	rats		Dietary, 2 generation	0, 550, 1700, 5000 or 7500 ppm	NOAEL = 7500 = 600 mg/kg bw/day	Reproductive toxicity		JMPR 1997

	Repro-tox, 3	rats		Dietary, 2 generation	0, 550, 1700, 5000 or 7500 ppm	NOAEL = 1700 = 130 mg/kg bw/day	Developmental toxicity – reduced pup weights	-	JMPR 1997
	Mutagenicity	In vitro/in vivo	-				+/- conclusion: not genotoxic		JMPR 1997
	Neurotoxicity	rats		Single dose	0, 500, 1000 or 2000 mg/kg bw	-	Clinical signs at all doses.		JMPR 1997
	Neurotoxicity	rats		Dietary, 13 weeks	0, 50, 5000 or 20000 ppm	NOAEL = 5000 ppm = 350 mg/kg bw/day	Inhibition of brain acetylcholinesterase activity	nervous system	JMPR 1997
	neurotoxicity	hens					No delayed neurotoxicity		JMPR 1997
	Systemic toxicity	rats		Dietary, 30 days	0, 50, 100, 500, 10000 or 20000 ppm	NOAEL = 500 ppm = 52 mg/kg bw/day	Increased liver weight, histopathological changes in the liver (periportal hepatocyte hypertrophy)	liver	JMPR 1997
	Systemic toxicity	rats		Dietary, 90 days	0, 100, 500, 5000, 10000 or 20000 ppm	NOAEL = 500 ppm = 34 mg/kg bw/day	Decreased mean corpuscular volume, mean corpuscular haemoglobin, increased liver weights and relative kidney weights, chronic nephropathy in males. De-creased mean cell volume, hepatocyte hypertrophy, increased relative kidney weight in females	-	JMPR 1997
	Systemic toxicity	Dogs		Gelatine capsules, 28 days	0, 125, 250 or 500 mg/kg bw/day	-	Clinical signs at all doses		JMPR 1997
	Systemic toxicity	dogs		Capsules, 1 year	0, 62.5, 125 or 250 ppm	NOAEL = 125 mg/kg bw/day	Reduced body weight, changes in haematological and clinical chemical parameters	-	JMPR 1997
	Systemic toxicity	rodents		Orally		LD50 = 1000-10000 mg/kg bw. LD50 = 100-220 mg/kg bw/day for the metabolite malaoxon	-		JMPR 1997
	Systemic toxicity	humans		Orally, 47 days	0, 8, 16 or 24 mg/day	NOAEL = 16 mg/day = 0.27 mg/kg bw/day	Inhibition of plasma and erythrocyte cholinesterase activity. Old study, not used to estimate ADI	nervous system	JMPR 1997
ADI = 0-0.3 mg/kg bw based on NOAEL = 29 mg/kg bw/day in the 2-year study of toxicity and carcinogenicity in rats. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Parathion-methyl	carcinogenicity and toxicity	mice		dietary			not carcinogenic		JMPR 1995
	carcinogenicity and toxicity	mice		dietary		NOAEL = 7 ppm = 1.6 mg/kg bw/day	decrease in erythrocyte, plasma and brain acetyl-cholinesterase activity	nervous system	JMPR 1995
	carcinogenicity and toxicity	rats		dietary	0, 2, 10 or 50 ppm	-	not carcinogenic		JMPR 1995
	carcinogenicity and toxicity	rats		dietary	0, 2, 10 or 50 ppm	NOAEL = 2 ppm = 0.1 mg/kg bw/day	reduction in brain acetylcholinesterase activity	nervous system	JMPR 1995
	repro-tox, 1	rats		gavage, gestation day 6-15	0, 0.3, 1 or 3 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	maternal + developmental toxicity - increased number of deaths, ataxia, dyspnoea in dams + delayed ossification in fetuses	-	JMPR 1995
	repro-tox, 2	rats	inseminated females	gavage, gestation day 6-15	0, 0.3, 1 or 3 mg/kg bw/day	NOAEL = 0.3 mg/kg bw/day	decrease in maternal body weight gain. Increased incidence of stunted fetuses	-	JMPR 1995
	repro-tox, 3	rabbits	inseminated females	gavage, gestation day 6-18	0, 0.3, 1 or 3 mg/kg bw/day	NOAEL = 3 mg/kg bw/day	developmental toxicity - no effects found	-	JMPR 1995
	repro-tox, 3	rabbits	inseminated females	gavage, gestation day 6-18	0, 0.3, 1 or 3 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	maternal toxicity - decrease in erythrocyte + plasma cholinesterase activity in dams	nervous system	JMPR 1995
	repro-tox, 4	rats		dietary	0, 2, 10 or 50 ppm	NOAEL = 2 ppm = 0.1 mg/kg bw/day	decrease in pup survival		JMPR 1995
	repro-tox, 5	rats		dietary	0, 0.5, 5 or 25 ppm	NOAEL = 5 ppm = 0.25 mg/kg bw/day	decrease in maternal body weight		JMPR 1995
	mutagenicity	in vitro/in vivo	-				+/-		JMPR 1995
	systemic toxicity	rats		dietary, 90 day	0, 2.5, 25 or 75 ppm	NOAEL = 2.5 ppm = 0.12 mg/kg bw/day	decrease in plasma, erythrocyte and brain acetyl-cholinesterase activity	nervous system	JMPR 1995
	systemic toxicity	dogs		dietary, 1 year	0, 0.03, 0.1 or 0.3 mg/kg bw/day	NOAEL = 0.3 mg/kg bw/day (highest dose tested)	no effects found		JMPR 1995
	ocular	rats		dietary, 1 year	0, 0.5, 2.5, 12 or 50 ppm	-	no ocular toxicity		JMPR 1995

	neurotoxicity	rats		dietary, 1 year	0, 0.5, 2.5, 12 or 50 ppm	NOAEL = 2.5 ppm = 0.1 mg/kg bw/day	degenerative changes in the sciatic nerve and its extensions consistent with demyelination	nervous system	JMPR 1995
	observation in humans	human				NOAEL = 0.3 mg/ kg bw/day	depression of erythrocyte acetylcholinesterase activity	nervous system	JMPR 1995
ADI: 0-0,003 mg/kg bw based on NOAEL = 5 ppm = 0.25 mg/kg bw/day in 2-year study in rats for retinal degeneration, sciatic nerve demyelination, reduced body weight, anaemia, decreased brain acetylcholinesterase activity. safety factor: 100									
Acute RfD = 0.03 mg/kg bw based on NOAEL = 19 mg/kg bw ~ 0.3 mg/kg bw/day in humans - absence of inhibition of erythrocyte acetylcholinesterase activity									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Azinphos-methyl	carcinogenicity (and toxicity)	rats		dietary, 12/24 months	0, 5, 15 or 45 ppm	-	-		JMPR 1991
	(carcinogenicity and) toxicity	rats		dietary,	0, 5, 15 or 45 ppm	NOAEL = 15 ppm = 0.86 mg/kg bw/day	inhibition of brain acetylcholinesterase	nervous system	JMPR 1991
	carcinogenicity (and toxicity)	mice		dietary, 2 years	0, 5, 20 or 40 ppm	-	-		JMPR 1991
	(carcinogenicity and) toxicity	mice		dietary, 2 years	0, 5, 20 or 40 ppm	NOAEL = 5 ppm = 0.88 mg/kg bw/day	inhibition of acetylcholinesterase in plasma, erythrocytes and brain	nervous system	JMPR 1991
	repro-tox	rats		dietary, 2 genera-tion	0, 5, 15 or 45 ppm	NOAEL = 5 ppm = 0.48 mg/kg bw/day	repro-tox		JMPR 1991
	neurotoxicity	hens					-		JMPR 1991
	mutagenicity	in vitro/in vivo	-				+/- conclusion: not genotoxic		JMPR 1991
	systemic toxicity	dogs		dietary, 52 weeks	0, 5, 25 or 125 ppm	NOAEL = 25 ppm = 0.74 mg/kg bw/day	reduced body-weight gain, inhibition of acetylcholinesterase activity	nervous system	JMPR 1991
	systemic toxicity	humans		30 days	0-0.3 mg/kg bw/day	NOAEL = 0.3 mg/kg bw/day	no effects on plasma or erythrocyte choli-nesterase activity	-	JMPR 1991
ADI = 0-0.005 mg/kg bw based on NOAEL = 0.48 mg/kg bw/day in the rat repro-tox study. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Dimethoate	carcinogenicity (and toxicity)	mice		dietary, 18 months	0, 25, 100 or 200 ppm	-	-		JMPR 1996
	(carcinogenicity and) toxicity	mice		dietary, 18 months	0, 25, 100 or 200 ppm	-	inhibition of acetylcholinesterase activity in brain and erythrocytes at all doses	nervous system	JMPR 1996
	carcinogenicity	rats		dietary, 2 years	0, 5, 25 or 100 ppm	-	-		JMPR 1996
	repro-tox, 1				0, 1, 15 or 65 ppm	NOAEL = 15 ppm = 1.2 mg/kg bw/day	reproductive toxicity		JMPR 1996
	repro-tox, 1				0, 1, 15 or 65 ppm	NOAEL = 1 ppm = 0.08 mg/kg bw/day	maternal toxicity		JMPR 1996
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 3, 6 or 18 mg/kg bw/day	-	no teratogenic effects		JMPR 1996
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 3, 6 or 18 mg/kg bw/day	NOAEL = 6 mg/kg bw/day	maternal toxicity		JMPR 1996
	repro-tox, 3	rabbits		gavage, days 7-19 of gestation	0, 10, 20 or 40 mg/kg bw/day	-	no teratogenic effects		JMPR 1996
	repro-tox, 3	rabbits		gavage, days 7-19 of gestation	0, 10, 20 or 40 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	maternal toxicity		JMPR 1996
	mutagenicity	in vitro/in vito	-				+/- conclusion: not genotoxic		JMPR 1996
	neurotoxicity	hens		single dose, orally or subcutaneous injection	55 mg/kg bw		no delayed neurotoxicity		JMPR 1996
	systemic toxicity	dogs		dietary, 12 months	0, 5, 20 or 125 ppm	NOAEL = 5 ppm = 0.2 mg/kg bw/day	inhibition of acetylcholinesterase activity in brain and erythrocytes	nervous system	JMPR 1996
	systemic toxicity	rats		dietary, life-span	0, 1, 5, 25 or 100 ppm	NOAEL = 1 ppm = 0.04 mg/kg bw/day	inhibition of acetylcholinesterase activity in brain and erythrocytes	nervous system	JMPR 1996
	systemic toxicity	rats				LD50 = 310 mg/kg bw			JMPR 1996
	systemic toxicity	mice				LD50 = 150 mg/kg bw			JMPR 1996
	systemic toxicity	humans		orally, 39 days	0.2 mg/kg bw/day	NOAEL = 0.2 mg/kg bw/day	no inhibition of blood cholinesterase activity found	-	JMPR 1996
	eye test	rabbits					irritating		JMPR 1996
ADI = 0-0.002 mg/kg bw (sum of dimethoate and omethoate expressed as dimethoate) based on NOAEL = 1.2 mg/kg bw/day in the rat repro-tox study. Safety factor: 500.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Monocrotophos	carcinogenicity (and toxicity), 1	mice		dietary, 2 year	0, 2, 5 or 10 ppm	-	-		JMPR 1993/1991
	(carcinogenicity and) toxicity, 1	mice		dietary, 2 year	0, 2, 5 or 10 ppm	-	inhibition of brain acetylcholinesterase	nervous system	JMPR 1993/1991
	carcinogenicity (and toxicity), 2	rats		dietary, 2 year	0, 0.01, 0.03, 0.1, 1 or 10 ppm	-	-		JMPR 1993/1991
	(carcinogenicity and) toxicity, 2	rats		dietary, 2 year	0, 0.01, 0.03, 0.1, 1 or 10 ppm	NOAEL = 0.1 ppm = 0.005 mg/kg bw/day	inhibition of brain acetylcholinesterase	nervous system	JMPR 1993/1991
	repro-tox, 1	rats		gavage, days 6-15 of gestation	0, 0.1, 0.3, 1 or 2 mg/kg bw/day	NOAEL = 2 mg/kg bw/day	no teratogenicity, embryo/fetotoxicity	-	JMPR 1993
	repro-tox, 1	rats		gavage, days 6-15 of gestation	0, 0.1, 0.3, 1 or 2 mg/kg bw/day	NOAEL = 0.3 mg/kg bw/day	maternal toxicity		JMPR 1993
	repro-tox, 2	rabbits		stomach tube, days 6-18 of gestation	0, 0.1, 1, 3 or 6 mg/kg bw/day	NOAEL = 6 mg/kg bw/day	embryo/fetotoxicity. No teratogenicity in the study	-	JMPR 1993
	repro-tox, 2	rabbits		stomach tube, days 6-18 of gestation	0, 0.1, 1, 3 or 6 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	maternal toxicity		JMPR 1993
	mutagenicity	in vitro/in vivo	-		-		+		JMPR 1993
	neurotoxicity	hens					no delayed neurotoxicity		JMPR 1991
	systemic toxicity	humans	male	orally, 30 days	0.0059 mg/kg bw/day	NOAEL = 0.006 mg/kg bw/day	no inhibition of erythrocyte cholinesterase. But + inhibition of plasma cholinesterase	nervous system	JMPR 1993
ADI = 0-0.0006 mg/kg bw based on NOAEL = 0.006 mg/kg bw/day in the 30-day human study (absence erythrocyte acetylcholinester ase inhibition). Safety factor: 10.									
Acute RfD: 0.002 mg/kg bw/day (JMPR 1995)									

APPENDIX 5

CHEMICAL	TYPE OF STU-DY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Methidathion	Carcinogenicity (and toxicity), 1	mice		Dietary, 23 months	0, 3, 10, 50 or 100 ppm	NOAEL = 10 ppm = 1.4 mg/kg bw/day	Hepatocellular tumours (adenomas, carcinomas) in males. Meeting concluded: no carcinogenic hazard in humans	liver	JMPR 1992
	(carcinogenicity and) toxicity, 1	mice		Dietary, 23 months	0, 3, 10, 50 or 100 ppm	NOAEL = 50 ppm = 7 mg/kg bw/day	Inhibition of erythrocyte acetylcholi-nesterase activity	nervous system	JMPR 1992
	(carcinogenicity and) toxicity, 1	mice		Dietary, 23 months	0, 3, 10, 50 or 100 ppm	NOAEL = 100 ppm = 13.7 mg/kg bw/day	Inhibition of brain acetylcholi-nesterase activity	nervous system	JMPR 1992
	Carcinogenicity (and toxicity), 2	rats		Dietary, 104 weeks	0, 4, 40 or 100 ppm		-		JMPR 1992
	(carcinogenicity and) toxicity, 2	rats		Dietary, 104 weeks	0, 4, 40 or 100 ppm	NOAEL = 4 ppm = 0.16 mg/kg bw/day	Inhibition of erythrocyte and brain acetylcholinesterase activity	nervous system	JMPR 1992
	Repro-tox, 1	rats		Dietary, 2 genera-tion	0, 5, 25 or 50 ppm	NOAEL = 5 ppm = 0.43 mg/kg bw/day	Reduced mating incidence in the F1 generation. Decreased progeny body weights		JMPR 1992
	Repro-tox, 2	rats		Gavage, days 6-15 of gestation	0, 0.25, 1 or 2.5 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	Maternal toxicity – mortality, de-creased body-weights, food intake, clinical signs		JMPR 1992
	Repro-tox, 2	rats		Gavage, days 6-15 of gestation	0, 0.25, 1 or 2.5 mg/kg bw/day	NOAEL = 2.5 mg/kg bw/day	Embryofetal toxicity – no effects found		JMPR 1992
	Repro-tox, 3	Rabbits		Gavage, days 7-19 of gestation	0, 2, 6 or 12 mg/kg bw/day	NOAEL = 6 mg/kg bw/day	Maternal toxicity		JMPR 1992
	Repro-tox, 3	Rabbits		Gavage, days 7-19 of gestation	0, 2, 6 or 12 mg/kg bw/day	NOAEL = 12 mg/kg bw/day	No embryofetal developmental toxicity and teratogenicity found		JMPR 1992
	Mutagenicity	In vitro/in vivo					-		JMPR 1992
	neurotoxicity	hens					No delayed neurotoxicity		JMPR 1992
	Systemic toxicity	rats		Single oral dose		NOAEL = 1 mg/kg bw	Inhibition of brain acetylcholi-nesterase activity	nervous system	JMPR 1997
	Systemic toxicity	dogs		Capsules, 90 days	0, 0.5, 4, 45 or 140 ppm	NOAEL = 4 ppm = 0.16 mg/kg bw	Liver effects (cholestasis, increased liver enzymatic activity in serum). At 140 ppm: inhibition of brain acetyl-cholinesterase activity	liver, nervous system	JMPR 1992
	Systemic toxicity	dogs		Capsules, 12 months	0, 0.5, 2, 4, 40 or 140 ppm	NOAEL = 4 ppm = 0.16 mg/kg bw	Liver effects (cholestasis, increased liver enzymatic activity in serum). At 140 ppm: inhibition of brain acetylcholinesterase activity	liver, nervous system	JMPR 1992

	Systemic toxicity	humans				NOAEL = 0.11 mg/kg bw	Inhibition of erythrocyte acetylcholi-nesterase activity	nervous system	JMPR 1992
ADI = 0-0.001 mg/kg bw based of NOAEL = 0.1 mg/kg bw/day in the dog studies. Safety factor: 100.									
Acute RfD = 0.01 mg/kg bw based on NOAEL = 0.11 mg/kg bw/day in the human study. Safety factor: 10. (JMPR 1997)									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Phenthoate	carcinogenicity (and toxicity)	rats		dietary, 116 weeks	0, 20, 100 or 500 ppm	-	-		JMPR 1984
	(carcinogenicity and) toxicity	rats		dietary, 116 weeks	0, 20, 100 or 500 ppm	-	inhibition of cholinesterase activity at all doselevels	nervous system	JMPR 1984
	carcinogenicity (and toxicity)	mice		dietary, 18 months	0, 500 or 1000 mg/kg	-	-		JMPR 1980
	(carcinogenicity and) toxicity	mice		dietary, 18 months	0, 500 or 1000 mg/kg	-	no effects found		JMPR 1980
	repro-tox	rats		dietary, 3-generation	0, 10, 30 or 100 mg/kg	-	no effects on reproduction and tera-togenicity		JMPR 1980
	mutagenicity	mice		intraperitonal injection	0, 150 or 300 mg/kg bw	-	-		JMPR 1980
	neurotoxicity	hens					no delayed neuropathy		JMPR 1980
	systemic toxicity	dogs		dietary, 2 years	0, 10, 30 or 100 mg/kg	NOAEL = 10 ppm = 0.29 mg/kg bw/day	inhibition of cholinesterase activity (erythrocyte)	nervous system	JMPR 1984/1980
	systemic toxicity	rats		dietary, 3 months	0, 5, 10, 30, 100, 300 or 1000 mg/kg	NOAEL = 10 ppm = 1.0 mg/kg bw/day	inhibition of cholinesterase activity (erythrocyte)	nervous system	JMPR 1984/1980
	systemic toxicity	mice		dietary, 3 months	0, 5, 10, 30, 100, 300 or 1000 mg/kg	NOAEL = 30 ppm = 4.5 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1984/1980
	systemic toxicity	mice, rats, rabbits	-	orally		LD50 = 245-840 mg/kg bw	-		JMPR 1980
ADI = 0-0.003 mg/kg bw									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Pirimiphos-methyl	Carcinogenicity (and toxicity), 1	mice		Dietary, 80 weeks	0, 5, 250 or 500 ppm	-	-		JMPR 1992
	(carcinogenicity and) toxicity, 1	mice		Dietary, 80 weeks	0, 5, 250 or 500 ppm	NOAEL = 5 ppm = 0.5 mg/kg bw/day	Inhibition of blood cholinesterase	nervous system	JMPR 1992
	Carcinogenicity (and toxicity), 2	Rats		Dietary, 2 years	0, 10, 50 or 300 ppm	-	-		JMPR 1992
	(carcinogenicity and) toxicity, 2	Rats		Dietary, 2 years	0, 10, 50 or 300 ppm	NOAEL = 10 ppm = 0.5 mg/kg bw/day	Inhibition of brain acetylcholinesterase	nervous system	JMPR 1992
	Repro-tox, 1	rats		Dietary, 4 generation	0, 20 or 200 ppm – doses uncertain	-	Dose-related reduction in pregnancy rats + reduced mating performance at 200 ppm.		JMPR 1992
	Repro-tox, 2	rats		Dietary, 3 generation	0, 5, 10 or 100 ppm	NOAEL = 100 ppm	No reproductive effects found		JMPR 1992
	Repro-tox, 3	rats		Gavage, corn oil, days 7-16 of gestation	0, 1.5, 15 or 150 mg/kg bw/day	NOAEL = 15 mg/kg bw/day	Maternal toxicity and embryotoxicity (based on reduced pes scores)		JMPR 1992
	Repro-tox, 3	rats		Gavage, corn oil, days 7-16 of gestation	0, 1.5, 15 or 150 mg/kg bw/day	NOAEL <= 150 mg/kg bw/day	Teratogenicity		JMPR 1992
	Repro-tox, 4	rabbits		Dietary, days 1-28 of gestation	0, 1 or 16 mg/kg bw/day	NOAEL = 16 mg/kg bw/day	Fetotoxicity and teratogenicity		JMPR 1992
	Mutagenicity	In vitro/in vivi	-				+/- conclusion: not genotoxic		JMPR 1992
	Neurotoxicity	hens					No delayed neurotoxicity		JMPR 1992
	Systemic toxicity	Rats, 4 studies	-	Dietary, up to 3 months	0-1000 ppm	NOAEL = 10 ppm = 0.5 mg/kg bw/day	Inhibition of erythrocyte cholinesterase + brain acetylcholinesterase	nervous system	JMPR 1992
	Systemic toxicity	Dogs, 2 studies	-	Capsules, 13 weeks and 2 years	0, 2, 10 or 25 mg/kg bw/day and 0, 0.5, 2 or 10 mg/kg bw/day	NOAEL = 2 mg/kg bw/day	Inhibition of brain acetylcholinesterase	nervous system	JMPR 1992
	Systemic toxicity	Humans, 2 studies	-	28 and 56 days	0-0.25 mg/kg bw/day	NOAEL = 0.25 mg/kg bw/day	No inhibition of erythrocyte cholinesterase		JMPR 1992
ADI = 0-0.03 mg/kg bw based on NOAEL = 0.25 mg/kg bw/day in the human studies. Safety factor: 10									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Thiometon	carcinogenicity (and toxicity)	rats		dietary, 2 years	0, 1, 2.5, 6.25 or 300 ppm; the first 6 weeks: 0, 0.2, 1, 2 or 20 ppm	-	-		JMPR 1979
	(carcinogenicity and) toxicity	rats		dietary, 2 years	0, 1, 2.5, 6.25 or 300 ppm; the first 6 weeks: 0, 0.2, 1, 2 or 20 ppm	NOAEL = 2.5 ppm = 0.12 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1979
	repro-tox	rats		dietary, 3-generation	0, 1, 2.5 or 6.25 ppm		not teratogenic. 6.25 ppm had a marginal effect on reproduction		JMPR 1979
	systemic toxicity	dogs		dietary, 2 years	0, 6, 12 or 48 ppm	NOAEL = 6 ppm = 0.15 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1979
	mutagenicity	in vitro					+		Moriya et al., 1983
ADI = 0-0.003 mg/kg bw									

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CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlorpyrifos-methyl	Carcinogenicity (and toxicity), 1	rats		Dietary, 2 years	0, 0.05, 0.1, 1 or 50 mg/kg bw/day	-	-		JMPR 1992
	(carcinogenicity and) toxicity, 1	rats		Dietary, 2 years	0, 0.05, 0.1, 1 or 50 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	Vacuolation of the zona fasciculata		JMPR 1992
	Repro-tox	rabbits		Stomach catheter, days 6-18 of gesta-tion	0, 4, 8 or 16 mg/kg bw/day	NOAEL = 16 mg/kg bw/day	No teratogenicity		JMPR 1991
	Mutagenicity	In vitro/in vivo	-				+/- conclusion: not genotoxic		JMPR 1991
	neurotoxicity	hens					No delayed neurotoxicity		JMPR 1991
	Systemic toxicity	mice		Dietary, 78 weeks	0, 1, 5, 50 or 500 ppm	NOAEL = 50 ppm = 3.9 mg/kg bw/day	Inhibition of acetylcholinesterase. Centrilobular hepatocellular fatty change, cortical cellular swelling of the adrenals	nervous system, liver	JMPR 1992/1991
	Systemic toxicity	mice		Dietary, 28 days	0, 1, 5, 10, 1000 or 10000 ppm	NOAEL = 10 ppm = 1.5 mg/kg bw/day	Inhibition of brain cholinesterase. Alteration in the adrenal glands.	nervous system	JMPR 1991
	Systemic toxicity	rats		Dietary, 13-weeks	0, 0.1, 1, 10 or 250 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	Histological alteration in the adrenals	nervous system	JMPR 1991
	Systemic toxicity	dogs		Dietary, 13-weeks	0, 0.1, 10 or 50 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	Inhibition of brain cholinesterase inhibition, increased liver weight, reduced body weight gain	nervous system	JMPR 1991
	Systemic toxicity	humans	man	Gelatine capsules, 4 weeks	0, 0.03 or 0.1 mg/kg bw/day	NOAEL = 0.1 mg/kg bw/day	No effects found		JMPR 1992
ADI = 0-0.01 mg/kg bw based on NOAEL = 0.1 mg/kg bw/day in the study in humans. Safety factor: 10. (Supported by NOAEL = 0.1 mg/kg bw/day in the study in rats. Safety factor: 100)									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Tolclofos-methyl	carcinogenicity (and toxicity)	mice		dietary, 104 weeks	0, 10, 50, 250 or 1000 ppm	-	-		JMPR 1994
	(carcinogenicity and) toxicity	mice		dietary, 104 weeks	0, 10, 50, 250 or 1000 ppm	NOAEL = 50 ppm = 6.5 mg/kg bw/day	reduced brain cholinesterase activity and increased kidney weights	nervous system	JMPR 1994
	carcinogenicity (and toxicity)	rats		dietary, 122-129 weeks	0, 100, 300 or 1000 ppm	-	-		JMPR 1994
	(carcinogenicity and) toxicity	rats		dietary, 122-129 weeks	0, 100, 300 or 1000 ppm	NOAEL = 1000 ppm = 41 mg/kg bw/day	no effects found		JMPR 1994
	repro-tox	rabbits		orally	0, 300, 1000 or 3000 ppm	NOAEL = 300 mg/kg bw/day	maternal toxicity. No evidence of teratogenicity		JMPR 1994
	repro-tox	rats		gavage	0, 100, 300 or 1000 mg/kg bw/day	NOAEL = 300 mg/kg bw/day	recuced body-weigth gain in dams. No teratogenic effects.		JMPR 1994
	mutagenicity	in vitro/in vivo	-				not genotoxic		JMPR 1994
	neurotox	chikens					no delayed neuropathy		JMPR 1994
	systemic toxicity	dogs		dietary, 52 weeks	0, 80, 400 or 2000 ppm	NOAEL = 400 ppm = 11 mg/kg bw/day	increased liver weight (with hepatocytic hypertro-phy), reduced body weight gain and slight anaemia		JMPR 1994
	systemic toxicity	mice		dietary, 9 months	0, 10, 30, 100 or 3000 ppm	NOAEL = 100 ppm = 12 mg/kg bw/day	inhibition of brain cholinesterase activity + effect on body weight	nervous system	JMPR 1994
	systemic toxicity	rats		dietary, 32-34 days	0, 200, 1000, 5000 or 20000 ppm	NOAEL = 1000 ppm = 79 mg/kg bw/day	inhibition of brain cholinesterase activity + in-creased relative kidney weight	nervous system	JMPR 1994
	systemic toxicity	rats		dietary, 13 weeks	0, 100, 1000 or 10000 ppm	NOAEL = 1000 ppm = 66 mg/kg bw/day	effects on body, liver and kidney weights		JMPR 1994
	systemic toxicity	rats		dietary, 28 weeks	0, 300, 1000, 3000 or 10000 ppm	NOAEL = 1000 ppm = 65 mg/kg bw/day	histopathological changes in the livers of females	liver	JMPR 1994
ADI: 0-0,07 mg/kg bw based on NOAEL = 50 ppm = 6.5 mg/kg bw/dayin the 104 week study of toxicity and carcinogenicity in mice. Safety factor: 100									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Etrimfos	repro-tox	rats		dietary, 3-generation	0, 3, 9 or 28 mg/kg		no effects on reproduction and teratogenicity		JMPR 1980
	repro-tox	rabbits		dietary, days 6-18 of gestation	0, 25, 50 or 100 mg/kg bw		no effects on reproduction and teratogenicity		JMPR 1980
	mutagenicity	in vitro/in vivo	-				-		JMPR 1980
	neurotoxicity	chickens		gelatine capsules, 42 days	100, 200, 400, 500, 750 or 1000 mg/kg bw	-	no delayed neurotoxic response		JMPR 1980
	systemic toxicity	rats		dietary, 2 years	0, 6, 12 or 24 mg/kg	NOAEL = 6 mg/kg = 0.3 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1980/1982
	systemic toxicity	dogs		dietary, 106 weeks	0, 4, 10 or 25 mg/kg	NOAEL = 10 mg/kg = 0.25 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1980/1982
	systemic toxicity	mice, rats		orally		LD50 = 470-2354 mg/kg bw	-		JMPR 1980
ADI: 0-0,003 mg/kg bw									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Phosmet	carcinogenicity (and toxicity), 1	mice		2 years	0, 5, 25 or 100 ppm		-		JMPR 1994
	(carcinogenicity and) toxicity, 1	mice		2 years	0, 5, 25 or 100 ppm	NOAEL = 25 ppm = 4 mg/kg bw/day	hepatotoxicity + brain cholinesterase inhibition	liver, nervous system	JMPR 1994
	carcinogenicity (and toxicity), 2	rats		2 years	0, 20, 40 or 200 ppm		-		JMPR 1994
	(carcinogenicity and) toxicity, 2	rats		2 years	0, 20, 40 or 200 ppm	NOAEL = 40 ppm = 1.8 mg/kg bw/day	fatty changes in the liver, reduced brain cholinesterase activity in females	liver, nervous system	JMPR 1994
	repro-tox, 1	rats		dietary, 2 generation	0, 20, 80 or 300 ppm	NOAEL = 20 ppm = 1.3 mg/kg bw/day	maternal toxicity - reduced mating and fertility		JMPR 1994
	repro-tox, 1	rats		dietary, 2 generation	0, 20, 80 or 300 ppm	NOAEL = 80 ppm = 5 mg/kg bw/day	developmental toxicity		JMPR 1994
	repro-tox, 2	rats		gavage, corn oil, days 7-16 of gestation	0, 5, 10 or 15 mg/kg bw/day	NOAEL = 5 mg/kg bw/day	maternal toxicity - reduced body-weight gain		JMPR 1994
	repro-tox, 2	rats		gavage, corn oil, days 7-16 of gestation	0, 5, 10 or 15 mg/kg bw/day	NOAEL = 15 mg/kg bw/day	No evidence of fetotoxicity and teratogenicity		JMPR 1994
	repro-tox, 3	rabbits		gavage, corn oil, days 7-19 of gestation	0, 2, 5 or 15 mg/kg bw/day	NOAEL = 5 mg/kg bw/day	maternal toxicity - reduced body-weight gain		JMPR 1994
	repro-tox, 3	rabbits		gavage, corn oil, days 7-19 of gestation	0, 2, 5 or 15 mg/kg bw/day	NOAEL = 2 mg/kg bw/day	fetotoxicity - minor skeletal anomalies		JMPR 1994
	mutagenicity	in vitro/in vivo					+/- conclusion: not genotoxic		JMPR 1998/1994
	neurotoxicity	chickens					no delayed neurotoxicity		JMPR 1998
	systemic toxicity	mice		dietary, 4 weeks	0, 5, 15, 50, 150 or 500 ppm	NOAEL = 50 ppm = 7.5 mg/kg bw/day	reduced food intake, reduced body-weight gain, reduced liver and kidney weights		JMPR 1994
	systemic toxicity	rats		dietary, 14 weeks	0, 20, 100 or 500 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day	inhibition of brain cholinesterase activity	nervous system	JMPR 1994
	systemic toxicity	mice				LD50 = 20-50 mg/kg bw			JMPR 1994
	systemic toxicity	rats				LD50 = 100-300 mg/kg bw	-		JMPR 1994
ADI for humans: 0-0,01 mg/kg bw based on NOAEL = 1.3 mg/kg bw/day in the repro-tox study in rats. Safety factor: 100.									
Acute RfD: 0.02 mg/kg bw based on NOAEL = 2 mg/kg bw/day in repro-tox study in rabbits. Safety factor: 100									

APPENDIX 5

Diethoxy compounds of category IV

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlorpyrifos	carcinogenicity (and toxicity)	mice		79 weeks			not carcinogenic		
	(carcinogenicity and) toxicity	mice		79 weeks		NOAEL = 0,7 mg/kg bw/day (5 ppm)	inhibition of erythrocyte and brain cholinesterase activity	nervous system	JMPR 1999
	repro-tox	rats		2-generation		NOAEL = 1 mg/kg bw/day	neonatal toxicity (reduces pup body weight and survival		JMPR 1999
	repro-tox	rats				NOAEL = 1 mg/kg bw/day	fetal and perinatal toxicity at maternally toxic doses		JMPR 1999
	repro-tox	rats			5 mg/kg bw/day		no effect on cognitive function in rat pups		JMPR 1999
	mutagenicity	in vitro/in vivo	-				not genotoxic		JMPR 1999
	carcinogenicity						not carcinogenic in rats and mice		JMPR 1999
	neurotoxicity	hens		13 weeks	high acute dose (up to 150 mg/kg bw)	-	significant neuropathy target esterase inhibition and mild delayed neuropathy	nervous system	JMPR 1999
	neurotoxicity	rats		repeated dose, 13 weeks	up to 15 mg/kg bw/day	-	no treatment-related neurological lesions or effects on cognition and no inhibition of neuropathy target esterase activity, although significant inhibition of erythrocyte, brain and peripheral tissue cholinesterase activity was seen	nervous system	JMPR 1999
	systemic toxicity, 1	dogs		2 years		NOAEL = 1 mg/kg bw/day	inhibition of brain cholinesterase activity	nervous system	JMPR 1999
	systemic toxicity, 1	dogs		2 years		NOAEL = 3 mg/kg bw/day	clinical signs: cholinergic signs and decreased body weight and/or food consumption		JMPR 1999
	systemic toxicity, 2	rats		13 weeks		NOAEL = 1 mg/kg bw/day	clinical signs: cholinergic signs and decreased body weight and/or food consumption		JMPR 1999
	systemic toxicity, 3	rats		2 year	0, 0.01, 0.03, 0.1, 1 or 3 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	inhibition of brain cholinesterase activity	nervous system	JMPR 1999
	systemic toxicity, 3	rats		2 year	0, 0.01, 0.03, 0.1, 1 or 3 mg/kg bw/day	NOAEL = 0,1 mg/kg bw/day	inhibition of erythrocyte acethylcolinesterase activity	nervous system	JMPR 1999

	systemic toxicity, 4	humans	both	9 days	single oral dose of up to 1 mg/kg bw or 0.1 mg/kg bw/day	NOAEL = 0,1 mg/kg bw/day	no inhibition of erythrocyte acetylcholinesterase		JMPR 1999
	systemic toxicity, 5	humans	both (6/sex)	single dose	2 mg/kg bw		Inhibition of erythrocyte acetylcholinesterase in one female	nervous system	JMPR 1999
	systemic toxicity, 6	rats	both			LD50 = 96-475 mg/kg bw	female rats more sensitive to the acute effects than males		JMPR 1999
	systemic toxicity, 7	mice	both			LD50 = 100-150 mg/kg bw			JMPR 1999
ADI: 0-0,01 mg/kg bw based on the following studies: rat, 2-year dietary; rat, reproduction; mouse, developmental; dog, 2-year dietary; human, 9-day oral.									
Acute RfD: 0.1 mg/kg bw based on human, single dose									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Parathion	carcinogenicity (and toxicity)	rats		2 year, dietary	0, 0.5, 5 or 50 ppm	-	-		JMPR 1995
	(carcinogenicity and) toxicity	rats		2 year, dietary	0, 0.5, 5 or 50 ppm	NOAEL = 5 ppm = 0.25 mg/kg bw/day for systemic toxicity	decreased brain, plasma and erythrocyte cholinesterase activity, retinal atrophy, increased severity of degenerative changes in the sciatic nerve.	nervous system	JMPR 1995
	carcinogenicity (and toxicity)	rats		2 year, dietary	0, 2, 8 or 32 ppm	-	-		JMPR 1995
	(Carcinogenicity and) toxicity	rats		2 year, dietary	0, 2, 8 or 32 ppm	NOAEL = 8 mmg = 0.4 mg/kg bw/day	Decrease in brain acetyl cholinesterase activity and retinal atrophy.	nervous system	
	repro-tox, 1	Rats		gavage, gestation day 6-19	0, 0.25, 1 or 1.5 mg/kg bw/day	NOAEL = 1.5 mg/kg bw/day	developmental toxicity		JMPR 1995
	repro-tox, 1	rats		gavage, gestation day 6-19	0, 0.25, 1 or 1.5 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	maternal toxicity - increased mortality		JMPR 1995
	repro-tox, 2	rats		gavage, gestation day 6-15	0, 0.1, 0.3 or 1 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	developmental toxicity		JMPR 1995
	repro-tox, 2	rats		gavage, gestation day 6-15	0, 0.1, 0.3 or 1 mg/kg bw/day	NOAEL = 0.3 mg/kg bw/day	maternal toxicity - increased mortality + clinical signs		JMPR 1995
	repro-tox, 3	rabbits		gavage, gestation day 7-19	1, 4 or 16 mg/kg bw/day	NOAEL = 16 mg/kg bw/day	developmental toxicity		JMPR 1995
	repro-tox, 3	rabbits		gavage, gestation day 7-19	1, 4 or 16 mg/kg bw/day	NOAEL = 4 mg/kg bw/day	maternal toxicity - decreased body weight gain		JMPR 1995
	repro-tox, 4	rabbits		gavage, gestation day 6-18	0, 0.03, 0.1 or 0.3 mg/kg bw/day	NOAEL = 0.3 mg/kg bw/day	maternal and developmental toxicity		JMPR 1995
	repro-tox, 5	rats		Dietary	0, 0.5, 5 or 25 ppm	NOAEL = 25 ppm = 12 mg/kg bw/day	reproductive toxicity		JMPR 1995
	repro-tox, 5	rats		Dietary	0, 0.5, 5 or 25 ppm	NOAEL = 5 ppm = 0.25 mg/kg bw/day	maternal toxicity - tremors		JMPR 1995
	repro-tox, 6	rats		Dietary	0, 1, 10 or 20 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day	reproductive toxicity		JMPR 1995
	repro-tox, 6	rats		Dietary	0, 1, 10 or 20 ppm	NOAEL = 10 ppm = 1 mg/kg bw/day	perinatal toxicity - reduced body weights		JMPR 1995
	repro-tox, 6	rats		Dietary	0, 1, 10 or 20 ppm	NOAEL = 1 ppm = 0.05 mg/kg bw/day	maternal toxicity - decreased brain acetylcholinesterase activity	nervous system	JMPR 1995

	systemic toxicity	rats		90 days	0, 2.5, 25 or 75 ppm	NOAEL = 2.5 ppm = 0.2 mg/kg bw/day	depression of brain acetylcholi-nesterase	nervous system	JMPR 1995
	systemic toxicity	rats		oral, short term (1-7 days		LD50 = 2 mg/kg bw/day			JMPR 1995
	systemic toxicity	human	5 men	5 days	7.5 mg/day	NOAEL = 7.5 mg/day	effect on erythrocyte acetylcholi-nesterase	nervous system	JMPR 1995
	neurotoxicity	hens					not associated with organophospho-rous-induced delayed neurotoxicity		JMPR 1995
	ocular toxicity	dog		6 months	0, 0.002, 0.008 or 0.8 mg/kg bw/day		no effects on ocular		JMPR 1995
	ocular toxicity	rats		3 months	0, 0.04, 0.4 or 4 mg/kg bw/day		no effects on ocular		JMPR 1995
ADI: 0-0,004 mg/kg bw based on NOAEL = 0.4 mg/kg bw/day in a two year study in rats for retinal atrophy and inhibition of brain acetylcholinesterase. Safety factor: 100 Acute RfD = 0.01 mg/kg bw/day based on NOAEL = 7.5 mg/kg bw/day = 0.1 mg/kg bw/day in humans. Safety factor = 10									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Diazinon	carcinogenicity, 1	mice		dietary, 103 weeks	0, 100 or 200 weeks		-		JMPR 1993
	carcinogenicity, 2	rats		dietary, 103 weeks	0, 400 or 800 ppm		-		JMPR 1993
	carcinogenicity (and toxicity), 3	rats		dietary, 99 weeks	0, 0.1, 1.5, 125 or 250 ppm		-		JMPR 1993
	(carcinogenicity and) toxicity, 3	rats		dietary, 99 weeks	0, 0.1, 1.5, 125 or 250 ppm	NOAEL = 1.5 ppm = 0.07 mg/kg bw/day	inhibition of erythrocyte and brain cholinesterase	nervous system	JMPR 1993
	repro-tox, 1	rats		dietary, multi-generation	0, 10, 100 or 500 ppm	NOAEL = 10 ppm = 0.5 mg/kg bw/day	reduced parental body-weight gain in F1 generation; reduced survival rate + reduced body weight of F1 pups		JMPR 1993
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 10, 20 or 100 mg/kg bw/day	NOAEL = 20 mg/kg bw/day	maternal toxicity (weight loss - reduced food consumption) + fetotoxicity (retarded ossification, increased incidence of rudimentary ribs). No teratogenicity		JMPR 1993
	repro-tox, 3	rabbits		gavage, days 6-18 of gestation	0, 7, 25 or 100 mg/kg bw/day	NOAEL = 25 mg/kg bw/day	maternal toxicity - increased mortality, reduced body-weight gain. No teratogenicity		JMPR 1993
	neurotoxicity	hens			13 or 28 mg/kg bw/day	-	no evidence of delayed neurotoxicity		JMPR 1993
	mutagenicity	in vitro/in vivo	-				-		JMPR 1993
	systemic toxicity	rats		dietary, 90 days	0, 0.5, 5, 250 or 2500 ppm	NOAEL = 5 ppm = 0.4 mg/kg bw/day	inhibition of erythrocyte and brain cholinesterase	nervous system	JMPR 1993
	systemic toxicity	dogs, 2 studies	-	dietary, 90 days or 52 days	0, 0.1, 0.5, 150 or 300 ppm	NOAEL = 0.5 ppm = 0.02 mg/kg bw/day	inhibition of erythrocyte and brain cholinesterase	nervous system	JMPR 1993
	systemic toxicity	humans	male	capsules, 34-36 days	0.025 mg/kg bw/day	NOAEL = 0.025 mg/kg bw/day	no effects		JMPR 1993
ADI: 0-0.002 mg/kg bw based on NOAEL = 0.025 mg/kg bw/day in the study in humans. Safety factor: 10.									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Dioxathion	repro-tox	rats		dietary, 79 days before mating	0, 3 or 10 ppm		no effects found		JMPR 1968
	systemic toxicity	rats		dietary, 13 weeks	0, 1, 3, 10, 100 or 500 ppm	NOAEL = 3 ppm = 0.15 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1968
	systemic toxicity	dogs		dietary, 90 days, 5 days/week	0-0.075 mg/kg bw/day	NOAEL = 0.075 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1968
	systemic toxicity	human		orally, 28 days	0.075 mg/kg bw/day	NOAEL = 0.075 mg/kg bw/day	no effects found		JMPR 1968
ADI: 0-0.0015 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Phorate	carcinogenicity and toxicity	rats		2 years		NOAEL = 1 ppm = 0.05 mg/kg bw/day	-		JMPR 1996/1994
	repro-tox	rabbits			0, 0.15, 0.5, 0.9, 1.2 mg/kg bw/day	NOAEL = 0.15 mg/kg bw/day	maternal mortality, decreased body weight. No embryo- and fetotoxicity found		JMPR 1996/1994
	Mutagenicity	In vitro/in vivo	-				not genotoxic		JMPR 1996/1994
	toxicity	mice		13 weeks	0, 1, 3, 6 ppm	NOAEL = 1 ppm = 0.18 mg/kg bw/day	inhibition of cholinesterase activity	nervous system	JMPR 1996/1994
	toxicity	dogs		1 year	0, 0.005, 0.01, 0.01, 0.25 mg/kg bw/day	NOAEL = 0.05 mg/kg bw/day	decreased body weight, inhibition of cholinesterase activity in erythrocyte and brain, clinical signs consistent with cholinergic toxicity	nervous system	JMPR 1996/1994
ADI: 0-0.0005 mg/kg bw based on NOAEL = 0.05 mg/kg bw/day in the one-year toxicity study in dogs and a carcinogenicity and toxicity study in rats. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Phosalone	(carcinogenicity and) toxicity, 1	rats		Dietary, 2 years	0, 5, 50 or 1000/500 ppm	NOAEL = 50 ppm = 1.8 mg/kg bw/day	Inhibition of acetylcholinesterase activity	nervous system	JMPR 1997
	Carcinogenicity (and toxicity), 2	mice		Dietary, lifetime	0, 15, 50 or 150 ppm	-	-		JMPR 1993
	(carcinogenicity and) toxicity, 2	mice		Dietary, lifetime	0, 15, 50 or 150 ppm	NOAEL = 150 ppm = 23 mg/kg bw/day	Inhibition of brain cholinesterase activity – but an inhibition of plasma and red blood cell cholinesterase were seen at this level	nervous system	JMPR 1993
	Repro-tox,1	rats		Dietary, multigen-eration	0, 10, 50 or 400 ppm	NOAEL = 50 ppm = 2.5 mg/kg bw/day	Retarded pup growth + inhibition of plasma and eryth-rocyte cholinesterase	nervous system	JMPR 1993
	Repro-tox, 2	rats		Gavage, days 6-15 of gestation	0, 2, 10 or 20 ppm	NOAEL = 10 mg/kg bw/day	Maternal and fetotoxicity		JMPR 1993
	Repro-tox, 3	rabbits		Gavage, days 6-18 of gestation	0, 1, 10 or 20 ppm	NOAEL = 10 mg/kg bw/day	Maternal and fetotoxicity. No teratogenicity		JMPR 1993
	Mutagenicity	In vitro/in vivo	-				-		JMPR 1993
	Neurotoxicity						No delayed neurotoxicity		JMPR 1993
	Several studies	Dogs		Dietary, 107 weeks	0, 100, 200 or 1000 ppm	NOAEL = 200 ppm = 5 mg/kg bw/day	Clinical signs, body-weight loss		JMPR 1993
	Systemic toxicity	rats		Gavage, 5 weeks	0, 7.5 or 15 mg/kg bw/day	NOAEL = 7.5 mg/kg bw/day	Inhibition of brain cholinesterase activity	nervous system	JMPR 1993
	Systemic toxicity	rats		Dietary, 8 weeks	0, 10, 100, 300, 600 or 1200 ppm	NOAEL = 10 ppm = 0.87 mg/kg bw/day	Inhibition of brain cholinesterase activity	nervous system	JMPR 1993
	Systemic toxicity	dogs		Dietary, 1 month	0, 12.5, 25 or 37.5 ppm	NOAEL = 37.5 ppm = 0.81 mg/kg bw/day	Inhibition of plasma cholinesterase activity (neither brain or erythrocyte cholinesterase activity was de-pressed)	nervous system	JMPR 1993
	Systemic toxicity	dogs		Dietary, 2 years	0, 100, 200 or 1000 ppm	NOAEL = 200 ppm = 5 mg/kg bw/day	Inhibition of brain cholinesterase activity, reduced body weight, increased alanine aminotransferase	nervous system	JMPR 1993
	Systemic toxicity	dogs		Dietary, 1 year	0, 5, 25 or 300 ppm	NOAEL = 25 ppm = 0.89 mg/kg bw/day	Inhibition of brain cholinesterase activity	nervous system	JMPR 1993
ADI = 0-0.02 mg/kg bw based on NOAEL = 1.8 mg/kg bw/day in the study of carcinogenicity and toxicity in rats									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Ethion	carcinogenicity (and toxicity)	mice		dietary, 105 weeks	0, 0.75, 1.5 or 8 ppm		-		JMPR 1986
	(carcinogenicity and) toxicity	mice		dietary, 105 weeks	0, 0.75, 1.5 or 8 ppm	NOAEL = 1.5 ppm = 0.24 mg/kg bw/day	reduced serrum cholinesterase activity	nervous system	JMPR 1986
	carcinogenicity (and toxicity)	rats		dietary, 24 months	0, 2, 4 or 40 ppm		-		JMPR 1986
	(carcinogenicity and) toxicity	rats		dietary, 24 months	0, 2, 4 or 40 ppm	NOAEL = 4 ppm = 0.22 mg/kg bw/day	reduced serrum cholinesterase activity	nervous system	JMPR 1986
	repro-tox	rats		gavage, corn oil, days 6-15 of gestation	0, 0.2, 0.6 or 2.5 mg/kg bw/day	NOAEL = 0.2 mg/kg bw	embryotoxicity/fetotoxicity - decreased fetal body weight, delayed ossification		JMPR 1990/1986
	mutagenicity	in vitro/in vivo	-				-		JMPR 1986
	neurotoxicity	hens					no clinical signs of delayed neurotoxicity	-	JMPR 1990
	systemic toxicity	dogs		dietary, 90 days	0, 0.5, 2.5, 25 or 300 ppm	NOAEL = 2.5 ppm = 0.07 mg/kg bw	inhibition of brain cholinesterase activity	nervous system	JMPR 1990
	systemic toxicity	human				NOAEL = 0.15 mg/kg bw	inhibition of plasma cholinesterase activity	nervous system	JMPR 1990
ADI: 0-0.002 mg/kg bw based on the rat repro-tox study. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/NOEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Quinalphos	carcinogenicity						not undergone a complete evaluation and determination		IRIS website
	carcinogenicity (and toxicity)	mice		18 months		NOEL = 0.75 mg/kg/day	oncogenicity		IRIS website
	(carcinogenicity and) toxicity	mice		18 months		NOEL = 0.03 mg/kg/day	cholinesterase inhibition	nervous system	IRIS website
	repro-tox, 1	rats, rabbits					not teratogenic		The e-Pesticide Manual, 11. ed., 1999
	repro-tox, 2	rabbits				NOEL = 4 mg/kg/day	fetotoxic - increased resorption/litter		IRIS website
	repro-tox, 2	rabbits				NOEL = 4 mg/kg/day	maternal toxicity - decreased weight gain, ataxia, weakness, diarrhea		IRIS website
	repro-tox, 3	rats				NOEL = 0.5 mg/kg bw/day	fetotoxic, maternal toxicity; maternal and pup inhibition of cholinesterase	nervous system	IRIS website
	mutagenicity						not mutagenic		The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	rats		2 years		NOEL = 3 mg/kg diet = 0.15-0.3 mg/kg bw/day	cholinesterase inhibition	nervous system	The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	rats, mice, dogs					cholinesterase inhibition	nervous system	The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	dogs	4/sex/group	dietary, 2 years	0, 0.0025, 0.0125 or 0.05 mg/kg/day	NOEL = 0.05 mg/kg bw/day	no systemic toxicity	no	IRIS website
	systemic toxicity	dogs	4/sex/group	dietary, 2 years	0, 0.0025, 0.0125 or 0.05 mg/kg/day	NOEL = 0.0125 mg/kg bw/day	inhibition of plasma cholinesterase	nervous system	IRIS website
	systemic toxicity	rats	male	oral		LD50 = 71 mg/kg			The e-Pesticide Manual, 11. ed., 1999
	eye test	rabbits					not irritating		The e-Pesticide Manual, 11. ed., 1999
ADI = 0.0015 mg/kg bw based on a 2-year study in rats. Safety factor: 100. (Otto Meyer)									
RfD = 0.0005 mg/kg bw/day based on NOEL = 0.05 mg/kg bw/day in the 2 year study in dogs. Safety factor: 100. (EPA)									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Pyrazophos	carcinogenicity						-		JMPR 1992
	Repro-tox, 1	rats		Dietary, 3 generation	0, 5, 10 or 50 ppm	NOAEL = 5 ppm = 0.45 mg/kg bw/day	Increased thymus weight, increased lymphocyte counts. Not reproductive	thymus, blood	JMPR 1992
	Repro-tox, 2	rats		Dietary, 2 generation	0, 20, 20 or 200 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day	Reduced body-weight gain in pups of both generations, reduced lactation index of F1 generation, slight inhibition of brain cholinesterase in parental females and pups of F2 generation	nervous system	JMPR 1992
	Repro-tox, 3	Rats		Gavage, days 7, 10, 16 of gestation	0, 0.5, 1.6 or 5 mg/kg bw/day	NOAEL = 5 mg/kg bw/day	Maternal and embryofetal toxicity		JMPR 1992
	Repro-tox, 4	rabbits		Gavage, days 6-18 of gestation	0, 10, 32 or 100 mg/kg bw/day	NOAEL = 100 mg/kg bw/day	Maternal and embryofetal toxicity		JMPR 1992
	mutagenicity	In vitro/in vivo	-				-		JMPR 1992
	neurotoxicity	hens					No delayed neurotoxicity		JMPR 1992
	Systemic toxicity	mice		Dietary, 28 days	0, 1, 5, 25 or 125 ppm	NOAEL = 25 ppm = 4.7 mg/kg bw/day	Inhibition of brain cholinesterase	nervous system	JMPR 1992
	Systemic toxicity	Rats		Dietary, 13 weeks	0, 2.5, 50 or 1000 ppm	NOAEL = 2.5 ppm = 0.21 mg/kg bw/day	Inhibition of brain cholinesterase	nervous system	JMPR 1992
	Systemic toxicity	Rats		Dietary, 52 weeks	0, 2, 20 or 200 ppm	NOAEL = 20 ppm = 1.4 mg/kg bw/day	Inhibition of brain cholinesterase	nervous system	JMPR 1992
	Systemic toxicity	dogs		Dietary, 92 weeks	0, 0.5, 2, 5 or 10/125/320 ppm	NOAEL = 5 ppm = 0.4 mg/kg bw/day	Inhibition of erythrocyte cholinesterase (brain cholinesterase was not determined)	nervous system	JMPR 1992
	Systemic toxicity	dogs		Dietary, 6 months	0, 1.2, 18 or 320 ppm	NOAEL = 1.2 ppm = 0.09 mg/kg bw/day	Marginal inhibition of brain cholinesterase	nervous system	JMPR 1992
	Systemic toxicity	dogs		Dietary, 2 years	0, 2, 5 or 320 ppm	NOAEL = 5 ppm = 0.4 mg/kg bw/day	Inhibition of erythrocyte cholinesterase, reduced body weight gain, histopathological abnormalities in the kidneys	nervous system, kidneys	JMPR 1992
	Systemic toxicity	rats		Dietary, 2 years	0, 2, 80 or 320 ppm	NOAEL = 2 ppm = 0.1 mg/kg bw/day	Hemangiomas in mesenteric lymph nodes in males. Marginal inhibition of brain acetyl cholinesterase	lymph nodes, nervous system	JMPR 1992
	Systemic toxicity	rats		Dietary, 2 years	0, 5, 8, 10 or 50 ppm	NOAEL = 50 ppm = 2.5 mg/kg bw/day	Adverse effects including inhibition of brain acetyl cholinesterase	nervous system	JMPR 1992
ADI = 0-0.004 mg/kg bw/day based on NOAEL = 0.4 mg/kg bw/day in the 2-year study in dogs and the 3-generation repro-tox study in rats. Safety factor 100.									

APPENDIX 5

Mixed substitute Compounds of category IV

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Prothiofos	systemic toxicity	rats		2 years		NOEL = 5 mg/kg diet			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	mice		2 years		NOEL = 1 mg/kg diet			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	dogs		2 years		NOEL = 0.4 mg/kg diet			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	rats		oral		LD50 = 1390-1569 mg/kg			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	mice		oral		LD50 = 2200 mg/kg			The e-Pesticide Manual, 11. ed., 1999
	eye test	rabbits					not irritating		The e-Pesticide Manual, 11. ed., 1999
ADI = 0.0001 mg/kg bw									

APPENDIX 5

CHLORINATED HYDROCARBON INSECTICIDES

DDT and its analoges

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
DDT	carcinogenicity	human					-		JMPR 1984
	carcinogenicity	rats, hamsters, monkeys	-		< 50 mg/kg/day		-		JMPR 1984
	carcinogenicity	monkeys		gavage, 11 years	10 mg/kg/cay		-		JMPR 1984
	carcinogenicity	rats	females		125, 250 or 500	125 ppm = 6.25 mg/kg bw/day	no tumorigenicity at this level. Hepatomas at higher doses		JMPR 1984
	reproductive and terato-genic toxicity	human			< 10 mg/kg/day	NOAEL = 10 mg/kg/day	no effect		JMPR 1984
	systemic	human			0.25 mg/kg/day	NOAEL = 0.25 mg/kg/day	no effect		JMPR 1984
									JMPR 1984
	accumulation	human					DDT and its metabolites accumulate in human body fat	-	
ADI: 0-0,02 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Methoxychlor	carcinogencity	rats, mice			< 3500 ppm		-		JMPR 1977
	teratogenicity	rats					not teratogenic		JMPR 1977
	toxicity	rats				NOAEL = 10 mg/kg bw			JMPR 1977
ADI: 0-0,1 mg/kg bw based on carcinogenicity studies in rats and mice (NOAEL = 3500 ppm)									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Dicofol	Carcinogenicity	mice		time-weighted average concentration, 78 weeks	260 or 530 ppm for males; 120 or 240 ppm for females	-	increased incidence of liver adenomas and adenomas/carcinomas combined at both conc. in male. Not carcinogenic in female mice. => not carcinogenic harzard for humans	-	JMPR 1992
	carcinogenicity	rats		time-weighted average concentration, 78 weeks	470 or 940 ppm for males; 380 or 760 ppm for females	-	not carcinogenic		JMPR 1992
	repro-tox, 1	rats		dietary	5, 25, 125 or 250 ppm	NOAEL = 25 ppm = 2.1 mg/kg bw/day	reproductive parameters		JMPR 1992
	repro-tox, 1	rats		dietary	5, 25, 125 or 250 ppm	NOAEL = 5 ppm = 0.5 mg/kg bw/day	ovarian stromal cell hypertrophy and hepatocellular changes	-	JMPR 1992
	repro-tox, 2	rats		gavage	0, 0.25, 2.5 or 25 mg/kg bw/day	NOAEL = 0.25 mg/kg bw/day	maternal toxicity		JMPR 1992
	repro-tox, 2	rats		gavage	0, 0.25, 2.5 or 25 mg/kg bw/day	NOAEL = 25 mg/kg bw/day	embryofoetal toxicity		JMPR 1992
	repro-tox, 3	rabbits		gavage	0, 0.4, 4 or 40 mg/kg bw/day	NOAEL = 0.4 mg/kg bw/day	maternal toxicity - based on histopathological changes in the liver. No teratogenic effects	-	JMPR 1992
	repro-tox, 3	rabbits		gavage	0, 0.4, 4 or 40 mg/kg bw/day	NOAEL = 4 mg/kg bw/day	embryofoetal toxicity - increased incidence of abortion	-	JMPR 1992
	Mutagenicity						not genotoxic		JMPR 1992
	Systemic toxicity	mice		dietary, 13 week	0, 10, 125, 250, 500 or 1000 ppm	NOAEL = 10 ppm = 2.1 mg/kg bw/day	reduced body weight, liver enlargement, increased hepatic mixed function oxidase (MFO) activity	-	JMPR 1992
	Systemic toxicity	mice		dietary, 13 week	0, 250, 500 or 750 ppm	no NOAEL found	liver histopathology incl. centrilobular hypertrophy and eosinophilia of heptocytes	-	JMPR 1992
	Systemic toxicity	rats		dietary, 2 year	0, 5, 50 or 254 ppm	NOAEL = 5 ppm = 0.22 mg/kg bw/day	histopathological changes in liver and vacuolation of adrenal cortical cells	-	JMPR 1992
	Systemic toxicity	rats		dietary, 13 week	0, 1, 10, 100, 500 or 1500 ppm	NOAEL = 1 ppm = 0.07 mg/kg bw/day	thyroid follicular epithelial hyperthrophy	-	JMPR 1992
	Systemic toxicity	rats		dietary, 13 week	0, 50, 200, 1000 or 3000 ppm	-	no thyroid effect found		JMPR 1992
	Systemic toxicity	dogs		dietary, 13 week	0, 10, 100, 300 or 1000 ppm	NOAEL = 10 ppm = 0.29 mg/kg bw/day	cortisol response to ACTH was reduced	-	JMPR 1992
	Systemic toxicity	dogs		dietary, 1 year	0, 5, 30 or 180 ppm	NOAEL = 30 ppm = 0.82 mg/kg bw/day	liver changes and reduced cortisol response to ACTH	-	JMPR 1992
ADI: 0-0,002 mg/kg bw based on a NOAEL = 0.22 mg/kg bw/day in the long-term study in rats. Safety factor: 100									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlorobenzilate	carcinogenicity	rats					-		JMPR 1980
	carcinogenicity	mice					+		JMPR 1980
	mutagenicity	mice	male				no effect on the germinal epithelium and on spermatocytes		JMPR 1980
	mutagenicity	in vitro					-		JMPR 1980
	systemic toxicity	rats		dietary, 48/44 weeks	0, 40 or 800 ppm	NOAEL = 40 ppm in the diet = 2 mg/kg bw/day - ??? (se mode of action/effects)	retarded growth, red swollen eyelids, soft faeces. Organ to body weight rations for the 40 ppm group were significantly greater than for the controls - liver, kidney, testes. Non-specific changes in pancreas, adrenals, increased hemopoietic activity in the spleens	liver, kidney, testes, pancreas, adrenals, spleen	JMPR 1980/1968
	systemic toxicity	dogs		dietary, 2 year. Only 14-20 weeks for the high dose	0, 100, 500 or 5000/3000 ppm in the diet	NOAEL = 500 ppm in dry diet = 12.5 mg/kg bw/day	body weight depression, mild anaemia, increased liver and spleen to body-weight ratios. At 5000 ppm: + depressed food intake, incresed serum alkaline phosphatase. The albumen to globulin ratio was reversed. Extramedullary haematopoiesis was evident in liver and spleen + erythroid hyperplasia of the bone marrow	liver, spleen, blood	JMPR 1980/1968
	systemic toxicity	rats, mice		orally, suspension in gum arabid		LD50 = 3100-4850 mg/kg bw			JMPR 1968
	systemic toxicity	rats, mice		orally, technical material		LD50 = 702-729 mg/kg bw			JMPR 1968
ADI for humans: 0-0,02 mg/kg bw									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chloropropylat	repro-tox	rats		dietary, 3 generation	0, 25 or 50 ppm		No reproductive effects. Delay between weaning and starting the animals on the test-diet in the case of one litter.	-	JMPR 1968
	toxicity	rats		dietary, 12 weeks	0, 100, 1000, 5000 or 25000 ppm	-	reduced body weight		JMPR 1968
	toxicity	rats		50 % wettable formu-lation in the diet, 2 years	0, 40 or 125 ppm	NOAEL = 40 ppm = 2 mg/kg bw/day	decrease in prostate weight, increased incidence of chronic renal desease, fatty changes in liver	-	JMPR 1968
	toxicity	dogs		dietary, 3 months, experimental time: 2 years	0, 100, 500 or 3000 ppm	-	results reported after 1 year: reduced body weight gain at 500 ppm. At 3000 ppm: reduced body weight, gastric stress and trauma, elevated alkaline phosphatase and mortality.	-	JMPR 1968
ADI from 1968 withdrawn in 1972									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Bromopropylat	carcinogenicity (and toxicity)	rats		dietary, 119/131 weeks for ma-les/females	0, 100, 700 or 5000 ppm	NOAEL = 100 ppm = 3.7 mg/kg bw/day	increased water consumption, increased relative liver and thyroid weights, increased incidence of focal hepatocellular hypertrophy and fatty changes, pigmetation of hepatocytes	liver, thyroid	JMPR 1993
	repro-tox, 1	rats		dietary, 2-generation	0, 165, 750 or 2250 ppm	NOAEL = 165 ppm = 9 mg/kg bw/day	increased liver weight and hypertrophy of hepatocytes in F1 animals	liver	JMPR 1993
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 50, 300 or 700 mg/kg bw/day	NOAEL = 50 mg/kg bw/day	Maternal toxicity - reduced body-weight gain, increased incidence of skeletal variations of fully formed 14th ribs and rudimentary 14th ribs	-	JMPR 1993
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 50, 300 or 700 mg/kg bw/day	-	no embryo/fetotoxicity or teratogenic effects	-	JMPR 1993
	mutagenicity	in vitro/in vivo	-				not genotoxic		JMPR 1993
	systemic toxicity	mice		dietary, 24 months	0, 30, 150, 1000 or 3000 ppm	NOAEL = 150 ppm = 16 mg/kg bw/day	increased absolute and relative liver weights and hepatocellu-lar neoplastic lesions	liver	JMPR 1993
	systemic toxicity	dogs		dietary, 1 year	0, 100, 400 or 2000 ppm	NOAEL = 100 ppm = 2.7 mg/kg bw/day	reduced body-weight gain		JMPR 1993
ADI = 0-0.03 mg/kg bw based on NOAEL = 2.7 mg/kg bw/day in the one-year study in dogs. Safety factor: 100									

Metabolite of DDT

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPO-SURE	DOSE	NOAEL/LD50	MODE OF AC-TION/EFFECTS	TARGET ORGAN	REFERENCES
DDE, o, p'-	carcinogenicity	mice		dietary, 78 weeks, + 15 week observation period	0, 148 or 261 ppm	-	hepatocellular carcinomas. Increased weight loss and mortality in females	liver	IRIS website
	carcinogenicity	mice		dietary, lifetime (130 weeks)	0, 250 ppm		hepatomas	liver	IRIS website
	carcinogenicity	hamsters		dietary, 128 weeks	0, 500 or 1000 ppm	-	neoplastic nodules of the liver	liver	IRIS website
	carcinogenicity	rats		dietary, 78 weeks, + 35 week obser-vation period	437/242 or 839/462 ppm for ma-les/females	-	thyroid tumors in females		IRIS website
	carcinogenicity						evaluation: probable human carcinogen	-	IRIS website
	mutagenicity	in vitro (in mouse lymphoma (L5178Y) cells)	-				+		IRIS website
	systemic toxicity	rats				LD50 = 880 mg/kg			National Toxicology Program, website

APPENDIX 5

Benzene hexachloride and lindane

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
benzene hexachloride, BHC	carcinogenicity	mice		24 weeks	0, 100, 250, 500 ppm of each of the 4 isomers of BHC	-	hepatomas in animals treated with 500 ppm and 250 ppm alfa-isomer	-	JMPR 1973
	carcinogenicity	mice					hepatomas in animals treated with 200 ppm and 400 ppm alfa- og beta-isomer	-	JMPR 1973
No ADI because of lack of information									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Lindane, gamma-HCH, benzene hexachloride, BHC	carcinogenicity (and toxicity)	rats		oral, 2 year	0, 1, 10, 100 or 400 ppm	-	-		JMPR 1997
	(carcinogenicity and) toxicity	rats		oral, 2 year	0, 1, 10, 100 or 400 ppm	NOAEL = 10 ppm = 0.5 mg/kg bw/day	slight increase in mortality and effects on the liver	liver	JMPR 1997
	reprodutive toxicity	rats		oral, 2 generation	0, 1, 20 or 150 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day	reproductive and developmental tox.	-	JMPR 1997
	reprodutive toxicity	rats		oral, 2 generation	0, 1, 20 or 150 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day for parental toxicity	effects on bodyweight gain, increased kidney weights and hepatic effects	liver, kidney	JMPR 1997
	immunotoxicity	mice, rats, rabbits	-				functional effects and histological changes in the immune system		JMPR 1997
	toxicity	dog		oral		LD50 = 40 mg/kg bw			JMPR 1997
	eye test	rabbits					slightly irritating		JMPR 1997
Temporary ADI: 0-0,001 mg/kg bw based on NOAEL = 0.5 mg/kg bw/day in the 2-year study of toxicity and carcinogenicity in rats. Safety factor: 500									

APPENDIX 5

Cyclodiene and related compounds

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Heptachlor	carcinogenicity	rats		time-weighted dietary, 80 weeks	39 or 78 ppm (males) and 26 or 51 ppm (females)	NOAEL = 26 ppm = 1.3 mg/kg bw/day	decreased body-weight + increased mortality. Deficiencies in this study precluded proper evaluation of the carcinogenic portential	-	JMPR 1991
	carcinogenicity	mice		dietary, 90 weeks	initially: 10 or 20 ppm (males) and 20 or 40 ppm (females). Time-weighted conc.: 6 and 14 ppm (males) and 9 or 18 ppm (females)	-	hepatocellular carcinomas	liver	JMPR 1991
	systemic toxic-ity	mice		dietary, 30 days	0, 1, 5, 10, 25 or 50 ppm	NOAEL = 1 ppm = 0.15 mg/kg bw/day	enlargement of centrilobular and midzonal hepatocytes	liver	JMPR 1991
	systemic toxic-ity	dogs		dietary, 2 years	0, 1, 3, 5, 7 or 10 ppm	NOAEL = 1 ppm = 0.025 mg/kg bw/day	histopathological changes	liver	JMPR 1991
	repro-tox	dogs		dietary, 2-generation	0, 1, 3, 5, 7 or 10 ppm	NOAEL = 1 ppm = 0.025 mg/kg bw/day	increased mortality of F2 pups		JMPR 1991
	mutagenicity	in vitro/in vivo	-				not genotoxic		JMPR 1991
ADI: 0-0,0001 mg/kg bw based on the NOAELs derived from studies in dogs. Safety factor: 200									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Dieldrin	carcinogenicity	mice					+	liver	JMPR 1977
	carcinogenicity	rat, rabbit, rhesus monkey, chimpanzee	-				-		JMPR 1977
	repro-tox						no teratogenic effect		JMPR 1977
	mutagenicity	in vitro/in vivo	-				not genotoxic		JMPR 1977
	systemic toxicity	rats		dietary, 2 years	0, 0.5, 2, 10, 50, 100 or 150 ppm	NOAEL = 0.5 ppm = 0.025 mg/kg bw/day	liver changes		JMPR 1966
	systemic toxicity	dogs		dietary, 6 days/week	1, 3, 10, 25 or 50 ppm	NOAEL = 1 ppm = 0.025 mg/kg bw/day	liver changes. The highest doses were lethally	-	JMPR 1966
	systemic toxicity				high doses		effects on the central nervous system - primary site of action		JMPR 1966
ADI: 0-0,0001 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Endosulfan	carcinogenicity						-		JMPR 1998
	repro-tox	rats		dietary, 2-generation study	0, 3, 15 or 75 ppm	NOAEL = 75 ppm = 5 mg/kg bw/day for males and 6.2 mg/kg bw/day for females	no reproductive og developmental effects found	-	JMPR 1998
	repro-tox	rats		dietary, 2-generation study	0, 3, 15 or 75 ppm	NOAEL = 15 ppm = 1 mg/kg bw/day for males and 1.2 mg/kg bw/day for females	parental toxicity: increased liver and kidney weights	-	JMPR 1998
	repro-tox	rats		oral	0, 0.66, 2 or 6 mg/kg bw/day	NOAEL = 2 mg/kg bw/day	fetotoxicity		JMPR 1998
	repro-tox	rats		oral	0, 0.66, 2 or 6 mg/kg bw/day	NOAEL = 0.66 mg/kg bw/day	maternal toxicity		JMPR 1998
	repro-tox	rabbits		oral	0, 0.3, 0.7 or 1.8 mg/kg bw/day	NOAEL = 0.7 mg/kg bw/day	maternal toxicity - clinical signs		JMPR 1998
	repro-tox	rabbits		oral	0, 0.3, 0.7 or 1.8 mg/kg bw/day	NOAEL = 1.8 mg/kg bw/day - highest dose tested	developmental toxicity		JMPR 1998
	systemic toxic-ity	dog		dietary, 1 year		NOAEL = 10 ppm = 0.57 mg/kg bw/day			JMPR 1998
	systemic toxic-ity						increased renal weights and granular pigment formation after short-term administration. Chronic glomeru-lonephrosis or toxic nephropathy after long-term expo-sure.	kidney	JMPR 1998
	systemic toxic-ity	rats	female	oral		LD50 = 10 mg/kg bw			JMPR 1998
	eye test						not irritating		JMPR 1998
ADI: 0-0,006 mg/kg bw based on NOAEL = 0.6 mg/kg bw/day in the 2-year study of toxicity in rats. Safety factor: 100. ADI supported by other studies Acute RfD = 0-0.02 mg/kg based on NOAEL = 2 mg/kg bw/day in the study of neurotoxicity in rats. Safety factor: 100									

APPENDIX 5

PESTICIDES DERIVED FROM PLANTS AND OTHER ORGANISMS

Pyrethrum and related compounds

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Deltamethrin	carcinogenicity						? Data not evaluated		JMPR 1981/1982
	repro-tox	mice		orally, gavage, during gestation days 6 to 17	0, 0.1, 1 or 10 mg/kg bw/day	-	no teratogenic effects observed		JMPR 1981/1982
	repro-tox	rats		orally, during gestation days 6 to 18	0, 0.1, 1 and 10 mg/kg bw/day	-	no reproductive and teratogenic effects observed - but a slight delayed ossification at 10 mg/kg bw	-	JMPR 1981/1982
	repro-tox	rats		gastric intubation, during day 7-20 of gestation	0, 1.25, 2,5 or 5 mg/kg bw	-	no effect		JMPR 1981/1982
	systemic toxic-ity	rats		dietary, 2 year study	0, 2, 20 or 50 mg/kg	NOAEL = 2 mg/kg bw	slightly increased incidence of axonal degenera-tions in sciatic, tibial and/or plantar nerves	-	JMPR 1981/1982
	systemic toxic-ity	mice		dietary, 2 year study	0, 1, 25 or 100 mg/kg	NOAEL = 100 mg/kg bw (highest dose tested)	no effects found		JMPR 1981/1982
	systemic toxic-ity	dog		dietary, 2 year study	0, 1, 10 or 40 ppm	NOAEL = 40 ppm = 1 mg/kg bw (highest dose tested)	no effects found		JMPR 1981/1982
ADI: 0-0,01 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Fenvalerate	carcinogenicity	rats		dietary, 104 (males) or 119 (females) weeks	0, 50, 150, 500 or 1500 ppm	NOAEL = 150 ppm = 7.5 mg/kg bw	granulomata + giant cell infiltrates. Mechamism: a type of foreign body resonose due to the deposition of crystals of the cholesterol2-(4-chlorophenyl)-isovalerate ester in the tissues	-	JMPR 1981/1984
	carcinogenicity	mice		dietary, 91 (males) or 87 (females) weeks	0, 10, 30, 100 or 300 ppm	NOAEL = 30 ppm = 3.5 mg/kg bw	granulomata. Mechamism: a type of foreign body resonose due to the deposition of crystals of the cholesterol2-(4-chlorophenyl)-isovalerate ester in the tissues	-	JMPR 1981/1984
	carcinogenicity	dogs		dietary, 6 months	0, 250, 500 or 1000 ppm	NOAEL = 250 ppm	hepatic microgranulomas		JMPR 1984
	mutagenicity						no effect found - but limited studies		JMPR 1984
	systemic toxic-ity	rats		oral		LD50 = 3200 mg/kg			
ADI: 0-0,02 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Permethrin	carcinogenicity (and toxicity), 1	mice		dietary, two-year	0, 100, 500 or 2000 ppm	-	conflicting results, but conclusion: not carcinogenic		JMPR 1999
	(carcinogenicity and) toxicity, 1	mice		dietary, two-year	0, 100, 500 or 2000 ppm	NOAEL = 500 ppm = 75 mg/kg bw/day	changes in the weights of testis and liver at 2000 ppm	testis, liver	JMPR 1999
	carcinogenicity (and toxicity), 2	rats		dietary, two-year	0, 20, 100 or 500 ppm	-	-		JMPR 1999
	(carcinogenicity and) toxicity, 2	rats		dietary, two-year	0, 20, 100 or 500 ppm	NOAEL = 100 ppm = 5 mg/kg bw/day	clinical signs and changes in body and ovary weights and blood chemistry at 500 ppm	ovary	JMPR 1999
	repro-tox, 1	rats		dietary, three-generation study	0, 5, 30 or 180 mg/kg bw/day	NOAEL = 180 mg/kg bw/day, highest dose tested	reproductive toxicity - no effects found	-	JMPR 1999
	repro-tox, 2	rats		orally, days 6-16 of gestation	22.5, 71 or 225 mg/kg bw/day	NOAEL = 225 mg/kg bw/day, highest dose tested	maternal and developmental toxicity - no effects found	-	JMPR 1999
	repro-tox, 3	rabbits		orally, gavage, days 6-18 of gestation	400 mg/kg bw/day	NOAEL = 400 mg/kg bw/day, highest dose tested	maternal and developmental toxicity - no effects found	-	JMPR 1999
	repro-tox, 4	rabbits		orally, gavage, days 6-18 of gestation	600, 1200 or 1800 mg/kg bw/day	NOAEL = 1200 mg/kg bw/day	developmental toxicity		JMPR 1999
	neurotoxicity, 1	rats		single dose	150 mg/kg bw	NOAEL = 150 mg/kg bw	clinical signs of neurotoxicity		JMPR 1999
	neurotoxicity, 2	rats		13-week		NOAEL = 15 mg/kg bw/day	clinical signs of neurotoxicity		JMPR 1999
	systemic toxicity	dog		one-year		NOAEL = 5 mg/kg bw/day	reduced body weight at 100 mg/kg bw per day	-	JMPR 1999
ADI: 0–0.05 mg/kg bw (for technical-grade permethrin with cis:trans ratios of 25:75 to 40:60) based on the NOAEL of 100 ppm = 5 mg/kg bw/day, in the two-year study in rats, on the basis of clinical signs and changes in body and organ weights and blood chemistry at 500 ppm and the NOAEL in a one-year study in dogs of 5 mg/kg bw per day on the basis of reduced body weight at 100 mg/kg bw per day. Safety factor of 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Cypermethrin	carcinogenicity (and toxicity)	rats		dietary, 2 years	0, 1, 10, 100 or 1000 ppm		-		JMPR 1979
	(carcinogenicity and) toxicity	rats		dietary, 2 years	0, 1, 10, 100 or 1000 ppm	NOAEL = 100 ppm = 5 mg/kg bw/day	reduced body weight		JMPR 1979
	toxicity	dogs		dietary, 2 years	0, 3, 30, 300 or 1000 ppm	NOAEL = 300 ppm = 7.5 mg/kg bw/day	reduced body weight		JMPR 1981
	mutagenicity	in vitro/in vivo	-				-		JMPR 1979
	repro-tox	rats		orally, day 6-15 of gestation	0, 17.5, 35 or 70 mg/kg/day	-	no teratogenic or embryotoxic effects	-	JMPR 1979
	repro-tox	rabbits		orally, day 6-18 of gestation	0, 3, 10 or 30 mg/kg/day	-	no teratogenic effects		JMPR 1979
	neurotox	rats		orally, 14 days	0, 1250, 2500 or 5000 mg/kg/day	-	damage to sciatic nerves - may be reversible	-	JMPR 1979
ADI: 0–0.05 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Fenpropathrin/fenproponat	carcinogenicity (and toxicity)	rats		dietary, 2 years	0, 1, 5, 25, 125 or 500 ppm	-	-		JMPR 1993
	(carcinogenicity and) toxicity	rats		dietary, 2 years	0, 1, 5, 25, 125 or 500 ppm	NOAEL = 125 ppm = 5 mg/kg bw/day	reduced body-weight gain		JMPR 1993
	carcinogenicity (and toxicity)	rats		dietary, 2 years	0, 50, 150, 450 or 600 ppm	-	-		JMPR 1993
	(carcinogenicity and) toxicity	rats		dietary, 2 years	0, 50, 150, 450 or 600 ppm	NOAEL = 150 ppm = 7 mg/kg bw/day	clinical signs		JMPR 1993
	carcinogenicity (and toxicity)	mice		dietary, 104 weeks	0, 40, 150 or 600 ppm	-	-		JMPR 1993
	(carcinogenicity and) toxicity	mice		dietary, 104 weeks	0, 40, 150 or 600 ppm	NOAEL = 600 ppm = 56 mg/kg bw/day	-		JMPR 1993
	repro-tox, 1	rats		dietary, 3-generation	0, 5, 25 or 250 ppm	NOAEL = 25 ppm = 1.6 mg/kg bw/day	decreased pup weights in the F3A generation		JMPR 1993
	repro-tox, 2	rats		dietary, 3-generation	0, 40, 120 or 360 ppm	NOAEL = 40 ppm = 3 mg/kg bw/day	reduced body-weight gain, increased mortality in females, occurrence of tremors in pups	-	JMPR 1993
	repro-tox, 3	rats		intubation, days 6-15 of gestation	0, 0.4, 2 or 10 mg/kg bw/day	NOAEL = 2 mg/kg bw/day	maternal toxicity		JMPR 1993
	repro-tox, 3	rats		intubation, days 6-15 of gestation	0, 0.4, 2 or 10 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	embryotoxicity, teratogenicity		JMPR 1993
	repro-tox, 4	rats		orally, days 6-15 of gestation	0, 0.4, 1.5, 2, 3, 6 or 10 mg/kg bw/day	NOAEL = 3 mg/kg bw/day	maternal toxicity		JMPR 1993
	repro-tox, 4	rats		orally, days 6-15 of gestation	0, 0.4, 1.5, 2, 3, 6 or 10 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	embryotoxicity, teratogenicity		JMPR 1993
	repro-tox, 5	rabbits		orally, days 6-18 of gestation	0, 1.5, 3 or 6 mg/kg bw/day	NOAEL = 6 mg/kg bw/day	maternal toxicity, embryotoxicity, teratogenicity	-	JMPR 1993
	repro-tox, 6	rabbits		orally, days 7-19 of gestation	0, 4, 12 or 36 mg/kg bw/day	NOAEL = 4 mg/kg bw/day	maternal toxicity		JMPR 1993
	repro-tox, 6	rabbits		orally, days 7-19 of gestation	0, 4, 12 or 36 mg/kg bw/day	NOAEL = 36 mg/kg bw/day	fetotoxicity, teratogenicity		JMPR 1993

	neurotoxicity	hens, rats					no potential for delayed neurotoxicity	-	JMPR 1993
	mutagenicity	in vitro/in vivo	-				not genotoxic		JMPR 1993
	systemic toxicity	dogs		dietary, 1 year	0, 100, 250 or 750 ppm	NOAEL = 100 ppm = 3 mg/kg bw/day	reduced body-weight gain, clinical signs (tremors, emesis)	-	JMPR 1993
	systemic toxicity	rats		dietary, 13 weeks	0, 3, 30, 100, 300 or 600 ppm	NOAEL = 300 ppm = 17 mg/kg bw/day	reduced body-weight gain, clinical signs	-	JMPR 1993
ADI: 0–0.03 mg/kg bw based on NOAEL = 3 mg/kg bw/day in the multigeneration reproduction study in rats, the teratogenicity studies in rats and the 1 year feeding study in dogs. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Bifenthrin	Carcinogenicity	rats		dietary, 2-year	0, 12, 50, 100 or 200 ppm	-	not carcinogenic		JMPR 1992
	Carcinogenicity	rats		dietary, 2-year	0, 12, 50, 100 or 200 ppm	NOAEL = 100 ppm = 4 mg/kg bw/day for males and 7.5 mg/kg bw/day for females	tremors, reduction in body-weight gain	-	JMPR 1992
	repro-tox, 1	rats		gavage, gestation day 6-15	0, 0.5, 1 or 2 mg/kg bw/day	-	no evidence of teratogenicity		JMPR 1992
	repro-tox, 1	rats		gavage, gestation day 6-15	0, 0.5, 1 or 2 mg/kg bw/day	NOAEL = 1 mg/kg bw/day	tremors in the dams		JMPR 1992
	repro-tox, 2	rabbits		gavage	0, 2.7, 4 or 8 mg/kg bw/day	-	no teratogenic, foetotoxic or embryotoxic effects	-	JMPR 1992
	repro-tox, 2	rabbits		gavage	0, 2.7, 4 or 8 mg/kg bw/day	NOAEL = 2.7 mg/kg bw/day	tremors + twitching		JMPR 1992
	Mutagenicity						unlikely to present genotoxic hazard		JMPR 1992
	Systemic toxic-ity	rats		dietary, 90 days	0, 12, 50, 100 or 200 ppm	NOAEL = 100 ppm = 5 mg/kg bw/day	tremors		JMPR 1992
	Systemic toxic-ity	mice		dietary, lifetime (min. 20 months)	0, 50, 200, 500 or 600 ppm	NOAEL = 50 ppm = 7.6 mg/kg bw/dayin males and 200 ppm = 37 mg/kg bw/day in females	tumorigenic potential in mice can not be excluded	-	JMPR 1992
	Systemic toxic-ity	dogs		oral, capsules, 13 weeks	0, 2.5, 5.0, 10 or 20 mg/kg bw/day	NOAEL = 2.5 mg/kg bw/day	tremors		JMPR 1992
	Systemic toxic-ity	dogs		oral, capsules, 1 year	0, 0.75, 1.5, 3.0 or 5.0 mg/kg bw/day	NOAEL = 1.5 mg/kg bw/day	tremors		JMPR 1992
	Systemic toxic-ity	mice, rats	both	oral		LD50 = 43-56 mg/kg bw/day	-		JMPR 1992
ADI: 0-0,02 mg/kg bw based on NOAEL = 1.5 mg/kg bw/day in the 1-year study in dogs. Supported by the same NOAEL in the rat teratology study. Safety factor: 100.									

APPENDIX 5

HERBICIDES

Organic phosphorus herbicides

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Glyphosate	Carcinogenicity (and toxicity)	mice		Dietary, 24 months	0, 0.1, 0.5 pr 3.0 %	-	Not carcinogenic		JMPR 1986
	(carcinogenicity and) toxicity	mice		Dietary, 24 months	0, 0.1, 0.5 pr 3.0 %	NOAEL = 0.5 % = 814 mg/kg bw/day	Slight decreased body weight gain, mild hepatotoxicity at high doses	-	JMPR 1986
	Carcinogenicity (and toxicity)	rats		Dietary, 26 months	3, 10 or 31 mg/kg bw/day	-	-		JMPR 1986
	(carcinogenicity and) toxicity	rats		Dietary, 26 months	3, 10 or 31 mg/kg bw/day	NOAEL = 31 mg/kg bw/day	Reduced body weight in males		JMPR 1986
	Repro-tox, 1	rats		Dietary, 60 days, and 3 generation	0, 3, 10 or 30 mg/kg bw/day	NOAEL = 30 mg/kg bw/day	No effects found		JMPR 1986
	Repro-tox, 2	rats		Gavage, days 6-19 of gestation	0, 300, 1000 or 3500 mg/kg bw/day	-	Increased number of fetuses with malformations in high-dose group – number of litters with malformation was not increased – the effects are not supposed to be treatment related. No terato-genicicity observed.	-	JMPR 1986
	Repro-tox, 3	rabbits		Gavage, days 6-27 of gestation	0, 75, 175 or 350 mg/kg bw/day	-	mid- and high-dose levels: maternal toxicity – diarrhoea, soft tools, nasal discharge	-	JMPR 1986
	Repro-tox, 3	rabbits		Gavage, days 6-27 of gestation	0, 75, 175 or 350 mg/kg bw/day	-	No fetal anormalies – no teratogenicity	-	JMPR 1986
	mutagenicity	In vitro					-		JMPR 1986
	Systemic toxicity	dogs		Gelatin capsules, 1 year	0, 20, 100 or 500 mg/kg bw/day	NOAEL = 500 mg/kg bw/day	No effects found		JMPR 1986
	Systemic toxicity	mice		Dietary, 3 months	0, 0.5, 1.0 or 5.0 %	NOAEL = 0.5 %	Reduced body weight gain		JMPR 1986
	Eye test	Rabbits					Not irritating		JMPR 1986
ADI = 0-0.3 mg/kg bw (sum of glyphosate and AMPA) based on a 26-month study of toxicity in rats fed technical-grade glyphosate. Safety factor: 100.									

APPENDIX 5

Metabolite of glyphosate

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
AMPA	Repro-tox, 1	rats		Corn oil by gavage, day 6-15 of gestation	0, 150, 400 or 1000 mg/kg bw/day	NOAEL = 150 mg/kg bw/day	Maternal toxicity – soft stools		JMPR 1997
	Repro-tox, 1	rats		Corn oil by gavage, day 6-15 of gestation	0, 150, 400 or 1000 mg/kg bw/day	NOAEL = 400 mg/kg bw/day	Fetal toxicity - developmental toxicity	-	JMPR 1997
	mutagenicity	In vitro					-		JMPR 1997
	Systemic toxicity	rats		Dietary, 90 days	0, 400, 1200 or 4800 mg/kg bw/day	NOAEL = 400 mg/kg bw/day	Reduced body-weight gain, irritation of the mucosal and submucosal layers of the urinary tract, corresponding to hyperplasia of the urinary bladder, epithelial hyperplasia in the renal pelvis	-	JMPR 1997
	Systemic toxicity	dogs		Gelatine capsules, 90 days	0, 10, 30, 100 or 300 mg/kg bw/day	NOAEL = 300 mg/kg bw/day	No significant effects were observed		JMPR 1997
	Systemic toxicity	rats		Oral		LD50 = 8300 mg/kg bw			JMPR 1997
	Eye test	rabbits					Not irritating		JMPR 1997
ADI = 0-0.3 mg/kg bw (sum of glyphosate and AMPA) based on a 26-month study of toxicity in rats fed technical-grade glyphosate. Safety factor: 100.									

Carbamate herbicides

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlorpropham	toxicity	rat		oral		LD50 = 5000-8000 mg/kg bw			JMPR 1965
	toxicity, short term study	rat		oral, 90 days	310, 1250, 5000 or 20 000 ppm		increase in weight and food-intake. Higher mean liver weights at 1250 ppm and higher - no microscopic changes found		JMPR 1965
	toxicity	rabbit		oral		LD50 approx. 5000 mg/kg bw			JMPR 1965
No ADI and no-effect levels set because of lack of toxicological data									
Not evaluated since 1965. In 1965 no studies of cholinesterase inhibition									

FUNGICIDES AND RELATED COMPOUNDS

Chloroalkyl thio fungicides

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Captan	carcinogenicity	mice			> 6000 ppm		+ (induction was limited to the proximal part of the small intestine)	-	JMPR 1990
	repro-tox, 1	rats		dietary, one generation study. Animale mated after 102 days	0, 6, 12.5 or 25 mg/kg/day	NOAEL = 250 ppm = 12.5 mg/kg bw/day	Reproductive toxicity		JMPR 1995/1990
	repro-tox, 2	monkeys	pregnant females	oral, days 22-32 of gestation	6.35, 12.5 or 25 mg/kg bw/day	NOAEL = 12.5 mg/kg bw/day	Developmental toxicity		JMPR 1995
	mutagenicity	In vitro/In vivo					+/- (JMPR conclusion: not genotoxic – a effecient detoxification mechanism is present)		JMPR 1995
	Systemic toxic-ity	mice		56 days	0, 400, 800, 3000 or 6000 ppm	NOAEL = 400 ppm = 60 mg/kg bw/day	duodenal hyperplasia		JMPR 1995
	Systemic toxic-ity	dogs		dietary, 66 weeks	400, 4000 or 12000 ppm	NOAEL = 4000 ppm = 100 mg/kg bw/day	slightly enlarged kidneys and livers		JMPR 1995
ADI = 0-0.1mg/kg bw based on the NOAEL = 12.5 mg/kg bw/day in a study of reproductive toxicity in rats and monkeys. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STU-DY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Folpet	carcinogenicity	mice		Dietary, 2 year	0, 1000, 5000 or 10000ppm	NOAEL not found	Duedenal hyperplasia, adenomas + adenocarcinomas	-	JMPR 1995
	carcinogenicity	mice		Dietary, 2 year	0, 150, 450 or 1350 ppm	NOAEL = 150 ppm = 16 mg/kg bw/day	Tumours in the upper parts of the gastrointestinal tract (non-glandular stomach + duodenum)	-	JMPR 1995
	carcinogenicity	rats		Dietary, 104 weeks	0, 250, 1500 or 5000 ppm	NOAEL = 190 ppm = 10 mg/kg bw/day	Non-neoplastic lesions		JMPR 1995
	Mutagenicity	In vitro/In vivo	-				+/- (JMPR conclusion: not genotoxic – a effecient detoxifica-tion mechnism is present)	-	JMPR 1995
	Repro-tox (and toxicity), 1	rats		Dietary, 2 generation	0, 200, 800 or 3600 ppm		No reproductive toxicity		JMPR 1995
	(Repro-tox and) toxicity, 1	rats		Dietary, 2 generation	0, 200, 800 or 3600 ppm	NOAEL = 800 ppm = 40 mg/kg bw/day	Reduced body weights		JMPR 1995
	Repro-tox, 2	rats		Gavage on days 6-19 of gestation	0, 20, 80 320 or 640 mg/kg bw	NOAEL = 10 mg/kg bw/day	Maternal toxicity		JMPR 1995
	Repro-tox, 3	Rabbits		Oral intubation on days 6-28 gestation	0, 10, 20 or 60 mg/kg bw	NOAEL = 10 mg/kg bw/day	Maternal + fetotoxicity		JMPR 1995
	Repro-tox, 3	Rabbits		Oral intubation on days 6-28 gestation	0, 10, 20 or 60 mg/kg bw	NOAEL = 29 mg/kg bw/day	teratogenicity		JMPR 1995
	Systemic toxicity	dogs		Gelatine capsules, 1 year	0, 10, 60 or 140(120) mg/kg bw/day	NOAEL = 10 mg/kg bw/day	Decreased body weight + food consumption + serum biochemical changes	-	JMPR 1995
	Systemic toxicity	rats		Orally		LD50 > 5000 mg/kg bw			JMPR 1995
ADI = 0-0.1mg/kg bw based on the NOAEL = 10 mg/kg bw/day in the 2 year study of toxicity and carcinogenicity in rats, 1 year study of toxicity in dogs, studies of reproductive toxicity in rats and rabbits. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Dichlofluanid	carcinogenicity	mice		dietary, 24 months	0, 200, 1000 or 5000 ppm		not carcinogenic		JMPR 1983
	repro-tox	rabbits	female	orally, day 6-18 of gestation	0, 10, 30 or 100 mg/kg bw/day	NOAEL = 30 mg/kg bw/day	maternal, embryotoxic, faetotoxic effects. No teratogenic effects observed	-	JMPR 1983
	mutagenicity	in vivo (hamster)	-				-		JMPR 1983
	mutagenicity	in vitro					+		JMPR 1979
	systemic toxic-ity	rats		dietary, 9 weeks	0, 150, 500, 1500 or 4500 ppm	NOAEL = 500 ppm = 39.3 mg/kg bw/day	reduced body weight gain		JMPR 1983
	systemic toxic-ity	rats		dietary, 9 weeks	0, 150, 500, 1500 or 4500 ppm	NOAEL > 1500 ppm	thyroid effects		JMPR 1983
	systemic toxic-ity	dogs		dietary, 2 years	0, 100, 300, 1000 or 3000 ppm	NOAEL = 1000 ppm = 25 mg/kg bw/day	reduced body weight gain and food consumption, decreased interstitial tissue of the testis and vacuolation, degeneration of the adrenal cortex, increased mortality		JMPR 1974
	eye test	rabbits					moderate mucosal irritation		JMPR 1983
ADI = 0-0.3 mg/kg bw/day									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Tolylfluanid	carcinogenicty	mice		dietary, 104 weeks	0, 200, 1000 or 5000 ppm	-	-		JMPR 1988
	repro-tox, 1	rats		orally, days 6-15 of gestation	0, 100, 300 or 1000 mg/kg bw	NOAEL = 100 mg/kg bw/day	maternal and embryotoxicity. not teratogenic at any doses	-	JMPR 1988
	repro-tox, 2	rats		dietary, 2 generation, 2 litter/generation	0, 300, 1500 or 7500 ppm	NOAEL = 300 ppm = 15 mg/kg bw/day	no effects on reproduction. No malformation in the study.	-	JMPR 1988
	mutagenicity	in vitro/in vivo	-				+/-		JMPR 1988
	systemic toxicity	dogs		dietary, 12 months	-	NOAEL = 12.5 mg/kg bw/day	no adverse effects		JMPR 1988
	systemic toxicity	rats				LD50 > 5000 mg/kg bw			JMPR 1988
	systemic toxicity	guinea pigs, rabbits	-			LD50 = 250-500 mg/kg bw	-		JMPR 1988
ADI = 0-0.1 mg/kg bw/day									

Other aromatic hydrocarbons

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Diphenyl	systemic toxicity	rats				NOAEL = 500 ppm = 25 mg/kg bw/day	-		JMPR 1966
	systemic toxicity	dogs		orally, corn oil, 52 weeks	0, 2.5 or 25 mg/kg bw/day 5 times/week	NOAEL = 25 mg/kg bw/day	no pathological changes		JMPR 1966
	systemic toxicity	monkey				NOAEL = 50 mg/kg bw/day	-		JMPR 1966
	systemic toxicity	rats				LD50 = 3300-5000 mg/kg bw	-		JMPR 1966
	systemic toxicity	rabbits				LD50 = 2400 mg/kg bw			JMPR 1966
ADI = 0-0.125 mg/kg bw/day based on NOAEL = 25 mg/kg bw/day in the dogstudy. Safety factor: 200.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlorothalonil	carcinogenicity and toxicity, 1	rats		dietary, 2 year	0, 2, 4, 15 or 175 mg/kg bw/day	NOAEL = 1.5 mg/kg bw/day	non-neoplastic lesions in kidney and stomach - renal epithelial hypoplasia and forestomach hyperplasia/hyperkeratosis	-	JMPR 1992/1990
	carcinogenicity and toxicity, 1	rats		dietary, 2 year	0, 2, 4, 15 or 175 mg/kg bw/day	NOAEL = 3.3 mg/kg bw/day	neoplastic lesions - renal tumors, adenomas and carcinomas and non-glandular gastric papillomas and squamous cell carcinomas	-	JMPR 1992/1990
	carcinogenicity and toxicity, 2	mice		dietary, 2 year	0, 10/15, 40, 175 or 175 ppm	NOAEL = 15 ppm = 1.6 mg/kg bw/day	non-neoplastic gastric lesions - hyperplasia and hyperkeratosis of the forestomach	-	JMPR 1992/1987
	carcinogenicity and toxicity, 2	mice		dietary, 2 year	0, 10/15, 40, 175 or 175 ppm	NOAEL = 175 ppm = 21 mg/kg bw/day	neoplastic changes - papilloma formation	-	JMPR 1992/1987
	repro-tox	rats		dietary, 2 generation	0, 500, 1500 or 3000 ppm	NOAEL = 1500 ppm = 75 mg/kg bw/day	maternotoxicity without adverse effects on reproduction	-	JMPR 1992
	mutagenicity	in vivo/ in vitro	-				did not show genotoxic hazard for humans	-	JMPR 1992
	systemic toxicity	dogs	8/sex/group	dietary, 2 year	0, 60 or 120 ppm	NOAEL = 120 ppm = 3 mg/kg bw/day	renal tubule vacuolation		JMPR 1992/ JMPR 1990
ADI: 0-0,03 mg/kg bw based on NOAEL = 120 ppm = 3 mg/kg bw/day in the 2-year study in dogs. Safety factor: 100									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Hexachlorobenzene	Carcinogenicity	mice		Dietary, lifetime	0, 50, 100 or 200 ppm	-	+ (hepatomas at 100 and 200 ppm)		JMPR 1978
	Carcinogenicity	hamsters		Dietary, lifetime	0, 50, 100 or 200 ppm	-	+ (hepatomas, haemangioendotheliomas, thyroid adenomas at 50, 100 and 200 ppm)	-	JMPR 1978
	Repro-tox	rats		Dietary, 4 generations	0, 10, 20, 40, 80, 160, 320 or 640 ppm	-	Fertility was reduced at 320 ppm. Litter size decrease at 160 ppm. Reduced survival of pups at 160 ppm. Lactation affected at 80 ppm. Increased liver weights for pups from dams fed 40 ppm. No gross abnormalities in pups.	-	JMPR 1978
	Mutagenicity	In vitro					-		JMPR 1978
	Systemic toxic-ity	rats		Dietary, 15 weeks	0, 0.5, 2, 8 or 32 mg/kg bw	NOAEL = 0.5 mg/kg	Pathological and histopathological changes in liver and spleen	liver, spleen	JMPR 1978
	Systemic toxic-ity	Pigs		Dietary, 90 day	0, 0.05, 0.5, 5 or 50 mg/kg bw	NOAEL = 0.05 mg/kg	Increase in urinary excretion of coproporphyrin; induction of microsomal liver enzymes; increased liver, kidney and thyroid weights; histopahtological changes in liver and lymph nodes	kidney, liver, thyroid, lymph nodes	JMPR 1978
	immunotox	mice					Immuno-suppression at 167 mg/kg diet for 6 weeks	-	JMPR 1978
ADI from 1974 withdrawn in 1978									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Pentachlorobenzene	carcinogenicity						? (no studies available)		IRIS website
	mutagenicity	in vitro/in vivo	-				-		IRIS website
	repro-tox	rats		orally, day 6-15 of gestation	-		teratogen		National Toxicology Program, website
	systemic toxicity, 1	rats		dietary, 13 weeks	0, 2.2-164 mg/kg bw/day	NOEL = 24 mg/kg bw/day	histopathological lesions		TERA, website
	systemic toxicity, 2	mice	females	dietary, 13 weeks	0, 5.2-410 mg/kg bw/day	-	increased kidney weights at 330 ppm. Functional effects on thyroid from 33 ppm; increased liver weights from 100 ppm	thyroid	TERA, website
	systemic toxicity, 2	mice	females	dietary, 13 weeks	0, 5.2-410 mg/kg bw/day	NOEL = 22 mg/kg bw/day	histopathological lesions - centrilobular hepatocellular hypertrophy, minimal necrosis	liver	TERA, website
	systemic toxicity, 2	mice	males	dietary, 13 weeks	0, 5.2-410 mg/kg bw/day	LOEL = 5.2 mg/kg bw/day	histopathological lesions - centrilobular hepatocellular hypertrophy, minimal necrosis	liver	TERA, website
	systemic toxicity, 3	rats		dietary		LOAEL = 8.3 mg/kg bw/day	increased kidney weights, decreased heart weight, incresed hyaline droplets in proximal kidney tubules. At higher doses: hepatocellular enlargement	kidney, liver, heart	IRIS website and TERA, website
	systemic toxicity	rats		orally		LD50 = 1080 mg/kg			National Toxicology Program, website
	systemic toxicity	mice		orally		LD50 = 1175 mg/kg			National Toxicology Program, website
ADI = 0.0167 mg/kg reported in the 1980 Ambient Water Quality Criteria for Chlorinated Benzenes - based on a study of reproductive effects of short-term (10 days) feeding in rats. (IRIS website)									
TDI = 0.0001 mg/kg bw/day based on LOEL = 5.2 mg/kg/day in the 13 weeks study in mice. Safety factor: 5000 (TERA, website)									
Acute RfD = 0.0008 mg/kg bw/day based on LOAEL = 8.3 mg/kg bw/day in a toxicity study in rats (systemic toxicity, 3). Safety factor: 10000									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Pentachloroanisol	mutagenicity						?		National Toxicology Program, website
	systemic toxicity, 1	rats	5/sex/group	corn oil, gavage, 1/day, 5 days/week, 16 days	0, 100, 125, 150, 175 or 200 mg/kg bw	-	deaths at doses =/> 125 mg/kg bw after 2-3 days; dyspnea. Inactivity at all doses	-	National Toxicology Program, website
	systemic toxicity, 2	mice	5/sex/group	corn oil, gavage, 1/day, 5 days/week, 16 days	0, 100, 175, 250, 325 or 400 mg/kg bw	-	deaths at doses =/> 175 mg/kg bw after 2-3 days. Inactivity at all doses	-	National Toxicology Program, website
	systemic toxicity, 3	rats	10/sex/group	corn oil, gavage, 1/day, 5 days/week, 13 weeks	0, 40, 80, 120, 140 or 180 mg/kg bw		deaths at doses =/> 120 mg/kg bw during the first week. Decreased body weight gains. Increased liver and kidney weights at 40-80 mg/kg bw in males and 40-120 mg/kg bw in females	-	National Toxicology Program, website
	systemic toxicity, 4	mice	10/sex/group	corn oil, gavage, 1/day, 5 days/week, 13 weeks	0, 40, 80, 120, 140 or 180 mg/kg bw		deaths at doses =/> 120 mg/kg bw during the first week. Increased body weight gains of females. Inactivity for several hours after dosing. Increased liver weights at 80 mg/kg bw in males and 40-180 mg/kg bw in females. Increased relative kidney weights of females at 80-180 mg/kg.	-	National Toxicology Program, website
	systemic toxicity, 4	mice	10/sex/group	corn oil, gavage, 1/day, 5 days/week, 13 weeks	0, 40, 80, 120, 140 or 180 mg/kg bw	-	Lesions (males: =/> 40 mg/kg, females =/> 80 mg/kg): pulmonary congestion, edema, adrenal congestion, lymphoid depletion of lymph nodes and thymus, hepatocellular cytomegaly + karyomegaly, pigment accumulation	-	National Toxicology Program, website

	systemic toxicity, 5	rats	70/sex/group	corn oil, gavage, 5 days/week, 2 years	0, 10, 20 or 40 mg/kg bw for males and 0, 20 or 40 mg/kg bw for females	-	death in the high-dose group. Decreased body weight. Increased liver and kidney weights at 20 or 40 mg/kg. Increases in benign adrenal medulla pheochromocytomas. Adrenal medulla hyperplasia in females. Intracytoplasmic pigmentation. Congestion + hemorrhage of lungs, lymph nodes, thymus, adrenal cortex + meninges; hepatocellular centrilobular necrosis	-	National Toxicology Program, website
	systemic toxicity, 6	mice	70/sex/group	corn oil, gavage, 5 days/week, 2 years	0, 20 or 40 mg/kg bw	-	increased liver weights. Decreased body weights in males. Centrilobular hepatocyte cytomegaly and pigment accumulation in hepatocytes and Kupffer cells. Benign pheochromocytomas + hemangiosarcomas of the liver in males	liver	National Toxicology Program, website
	systemic toxicity, 7	mice		orally		LD50 = 318 mg/kg			National Toxicology Program, website
Can be evaluated as pentachlorophenol (Larsen)									

Anilino and nitrobenzenoid fungicides

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Dichloran	carcinogenicity (and toxicity), 1	mice		dietary, 18 months	0, 50, 175 or 600 ppm	-	- (but inadequate for complete evaluation of the carcinogenic potential)	-	JMPR 1998
	(carcinogenicity and) toxicity, 1	mice		dietary, 18 months	0, 50, 175 or 600 ppm	NOAEL = 175 ppm = 25 mg/kg bw/day	increased liver weights, centrilobular hepatocyte enlargement, centrilobular haemosiderosis, focal and singlecell liver necrosis, vacuolation of centrilobular hepatocytes	liver	JMPR 1998
	carcinogenicity (and toxicity), 2	rats		dietary, 2 year	0, 20, 100 or 3000 ppm or 0 or 1000 ppm	-	-		JMPR 1998
	(carcinogenicity and) toxicity, 2	rats		dietary, 2 year	0, 20, 100 or 3000 ppm or 0 or 1000 ppm	NOAEL = 1000 ppm = 59 mg/kg bw/day	changes in body-weight, food consumption, haematological parameters, spleen pigmentation, increased liver weights, centrilobular hepatocyte enlargement	liver	JMPR 1998
	repro-tox, 1	rats		dietary, 2 generation	0, 50, 250 or 1250 ppm	NOAEL = 250 = 21 mg/kg bw/day	reproductive toxicity - reduced weights of F1 and F2 pups.	-	JMPR 1998
	repro-tox, 1	rats		dietary, 2 generation	0, 50, 250 or 1250 ppm	NOAEL = 250 = 21 mg/kg bw/day	Systemic toxicity - reduced body-weight gains, increased liver weights	-	JMPR 1998
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 100, 200 or 400 mg/kg bw/day		NOAEL for maternal and developmental toxicity not established - reduced body-weight gains, resorbed litters in all treated groups. Not teratogenic	-	JMPR 1998
	repro-tox, 3	rabbits		gavage, days 6-18 of gestation	0, 8, 20 or 50 mg/kg bw/day	NOAEL = 8 mg/kg bw/day	maternal toxicity - reduced maternal body-weight gains	-	JMPR 1998
	repro-tox, 3	rabbits		gavage, days 6-18 of gestation	0, 8, 20 or 50 mg/kg bw/day	NOAEL = 20 mg/kg bw/day	developmental toxicity - slight increase in post-implantation losses, slight increase in incidence of minor anomalies of the gall-bladder, delays in ossification of all limb epiphyseal sites in fetuses	-	JMPR 1998
	mutagenicity	in vitro/in vivo	-				-/? meeting concluded: unlikely to be genotoxic	-	JMPR 1998

	systemic toxicity, 1	mice		dietary, 60 days	0, 300, 600 or 1200 ppm	NOAEL = 300 ppm = 15 mg/kg bw/day	hepatic lesions, splenic extramedullary haematopoiesis, increased methaemoglobin	liver, spleen	JMPR 1998
	systemic toxicity, 2	mice		gavage, 90 days	0, 15, 45, 135, 400 or 600 mg/kg bw/day	NOAEL = 15 mg/kg bw/day	polycythaemia in males, hypercholesterolaemia, increase in the incidence of splenic extramedullary haematopoiesis in females	liver, spleen	JMPR 1998
	systemic toxicity, 3	rats		dietary, 90 days	0, 1000, 3000 or 5000 ppm	-	reduced body-weight gain, effects on liver and thyroid in all treated groups	liver, thyroid	JMPR 1998
	systemic toxicity, 4	rats		dietary, 8 weeks	0, 500 or 750 ppm	NOAEL = 500 ppm = 44 mg/kg bw/day	reduced body-weight gain, decreased food consumption, increased liver weights, hepatic histopathological manifestations	liver	JMPR 1998
	systemic toxicity, 5	rats		dietary, 6 months	0, 30, 300 or 3000 ppm	NOAEL = 300 ppm = 22 mg/kg bw/day	reduced body-weight gain, increased liver and spleen weights	liver	JMPR 1998
	systemic toxicity, 6	rats		gavage, 4 weeks	0, 35, 140 or 350 mg/kg bw/day	NOAEL = 35 mg/kg bw/day	growth depression, hepatic hypertrophy and vacuolation	liver	JMPR 1998
	systemic toxicity, 7	dogs		dietary, 2 years	0, 20, 100 or 3000 ppm	NOAEL = 100 ppm = 1.7 mg/kg bw/day	changes in haematological and clinical chemical parameters, increases in liver, spleen, kidney weights, histological changes, irregular hepatic-cell size, moderate hepatic-cell hypertrophy, increased pigmentation of the liver and spleen	liver, spleen, kidney	JMPR 1998
	systemic toxicity, 8	humans	males	orally, 90 days	0 or 0.14 mg/kg bw/day	-	no effects		JMPR 1998
ADI = 0-0.01 mg/kg bw based on NOAEL = 1.7 mg/kg bw/Day for hepatic and haematological effects in the 2-year study in dogs. Safety factor: 200.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Quintozene	Carcinogenicity (and toxicity)	mice		Dietary, 103 week	0, 2500 or 5000 ppm	-	-		JMPR 1995
	(Carcinogenicity and) toxicity	mice		Dietary, 103 week	0, 2500 or 5000 ppm	NOAEL = 2500 ppm = 390 mg/kg bw/day	Reduced body-weight		JMPR 1995
	Carcinogenicity and toxicity	rats		Dietary, 2 year	0, 20, 3000 or 6000 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day	Follicular adenomas of the thyroid and hepatocellular adenomas	thyroid, liver	JMPR 1995
	Repro-tox, 1	rats		Dietary, multi-generation study	0, 20, 3000 or 6000 ppm	NOAEL = 20 ppm = 1 mg/kg bw/day	Reduced pup and adult body weights		JMPR 1995
	Repro-tox, 2	Rats		Dietary	0, 30, 600 and 1200 mg/kg bw/day	NOAEL > 1200 mg/kg bw/day	Maternal and developmental toxicity	-	JMPR 1995
	Repro-tox,3	Rabbits		dietary	0, 12, 120 or 250 mg/kg bw/day	NOAEL = 12 mg/kg bw/day	Maternal toxicity		JMPR 1995
	Repro-tox, 3	Rabbits		dietary	0, 12, 120 or 250 mg/kg bw/day	NOAEL = 120 mg/kg bw/day	Embryo- and fetotoxicity incl. reduced pup weight, increased resorption, increased incidence of stillbirths	-	JMPR 1995
	Systemic toxicity	Dogs		Dietary, 1 year	0, 15, 150 or 1500 ppm	NOAEL = 150 ppm = 4.2 mg/kg bw/day	Increased liver weight and hepatocellular hypertrophy, increased serum alkaline phos-phatase and cholesterol levels, decreased alanine aminotrans-ferase activity and creatinine levels	liver	JMPR 1995
	Systemic toxicity	Rats		Dietary, 13 weeks	0, 50, 3000 or 6000 ppm	NOAEL = 50 ppm = 3.1 mg/kg bw/day	Minimal changes in body and liver weight, decreased alanine aminotransferase activity	liver	JMPR 1995
	Systemic toxicity	Rats, dogs				LD50 = <1.5 g/kg bw			JMPR 1995
	Eye test	rabit					Mildly irritating		JMPR 1995
ADI = 0-0.01 mg/kg bw for quintozene containing less than 0.1% hexachlorobenzene. Based on NOAEL = 1 mg/kg bw/day in the 2 year study, study of thyroid toxicity, 2 generation study in rats. Safety factor: 100.									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Technazen	carcinogenicity	mice		dietary, 80 week	0, 750 or 1500 ppm	NOAEL = 1500 ppm = 155 mg/kg bw/day	-		JMPR 1994
	carcinogenicity	rats		dietary, 104 week	0, 750 or 1500 ppm	NOAEL = 1500 ppm = 56 mg/kg bw/day	-		JMPR 1994
	repro-tox, 1	rats		dietary, 2-generation	0, 300, 1000 or 5000/2000 ppm	NOAEL = 1000 ppm = 106 mg/kg bw/day	parental toxicity		JMPR 1994
	repro-tox, 1	rats		dietary, 2-generation	0, 300, 1000 or 5000/2000 ppm	NOAEL = 2000 ppm = 200 mg/kg bw/day	filial toxicity		JMPR 1994
	repro-tox, 2	rats		gavage	0, 15, 50 or 150 mg/kg bw/day	NOAEL = 50 mg/kg bw/day	maternal toxicity, embryo- and feto-toxicity on basis of minor skeletal defects. No teratogenic effects observed	-	JMPR 1994
	repro-tox, 3	rabbits		gavage	0, 15, 45 or 135 mg/kg bw/day	NOAEL = 45 mg/kg bw/day	maternal toxicity on basis of body weight loss and reduced food consumption	-	JMPR 1994
	repro-tox, 3	rabbits		gavage	0, 15, 45 or 135 mg/kg bw/day	NOAEL = 15 mg/kg bw/day	embryo- and fetotoxicity on basis of minor skeletal defects. No teratogenic effects observed	-	JMPR 1994
	systemic toxicity	rats		dietary, 90 days	0, 50, 500 or 5000 ppm	NOAEL = 500 ppm = 45 mg/kg bw/day	effects on body weight gain and on liver and kidneys	liver, kidney	JMPR 1994
	systemic toxicity	dogs		orally, 90 days	0, 2, 15 or 200 mg/kg bw/day	NOAEL = 15 mg/kg bw/day	effects on body and liver weights	liver	JMPR 1994
ADI: 0-0,02 mg/kg bw based on NOAEL = 15 mg/kg bw/day in the 90-day toxicity study in dogs and embryo- and fetotoxicity in rabbits. Safety factor: 1000.									

Benzimidazole

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Carbendazim	Carcinogenicity (and toxicity)	Mice (NMRKf – low spontaneous incidence of liver tumours)	-	Dietary, 96 weeks	0, 50, 150, 300 or 1000/2000/5000 ppm	-	-		JMPR 1995
	(Carcinogenicity and) toxicity	Mice (NMRKf)	-	Dietary, 96 weeks	0, 50, 150, 300 or 1000/2000/5000 ppm	NOAEL = 300 ppm = 34 mg/kg bw/day	Hepatotoxicity		JMPR 1995
	(Carcinogenicity and) toxicity	Mice (swiss and CD1)	-	Dietary, 80 weeks	Up to 5000 or 7500 ppm	< 150 or 500 ppm	Liver tumours		JMPR 1995
	Repro-tox	rats		Dietary, 3 generation study	0, 150, 300 or 2000 ppm	NOAEL = 2000 ppm = 120 mg/kg bw/day	Reproductive and developmental toxicity	-	JMPR 1995
	Repro-tox	rats		Dietary, days 6-15 of gestation	5-3000 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	Fetotoxicity and teratogenicity		JMPR 1995
	Repro-tox	rats		Dietary, days 6-15 of gestation	5-3000 mg/kg bw/day	NOAEL = 20 mg/kg bw/day	Maternal toxicity		JMPR 1995
	Repro-tox	rabbits		Gavage, days 7-19 of gestation	0, 10, 20 or 125 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	Fetotoxicity and teratogenicity (malformed vertebrae and ribs)	-	JMPR 1995
	Repro-tox	rabbits		Gavage, days 7-19 of gestation	0, 10, 20 or 125 mg/kg bw/day	NOAEL = 20 mg/kg bw/day	Maternal toxicity		JMPR 1995
	mutagenicity	In vitro/in vivo	-				Does not directly damage genetic material but causes chromosomal aberrations as a result of its interference with mitotic spindle proteins	-	JMPR 1995
	Systemic toxicity	dogs		Dietary, 2 year	Up to 4500 ppm	NOAEL = 100 ppm = 2.5 mg/kg/day	Hepatotoxicity		JMPR 1995
	Systemic toxicity	Rats				LD50 > 10000 mg/kg bw			JMPR 1995
ADI = 0-0.03 mg/kg bw based on NOAEL = 2.5 mg/kg bw/day in the 2 year study in dogs. Safety factor: 100									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Thiabendazole	systemic toxicity	humans	male	6 months	0 or 250 mg/day = 3-4 mg/kg bw	-	No effects found		JMPR 1977
	systemic toxicity	dogs		orally, 2 year	0, 20, 100 or 200 mg/kg bw/day	NOAEL = 20 mg/kg bw/day	haemosiderosis in spleen, liver, lymph noedes + bonemarrow	spleen, liver, lymph noedes, bonemarrow	JMPR 1970
	systemic toxicity	rats		2 year	0, 10, 40 or 160 mg/kg bw/day	-	increased weight of thyroid glands in males (not dose dependent) at 10 mg/kg bw/day	-	JMPR 1970
	repro-tox	mice		dietary, 5 generation	0, 200, 1000 or 5000 ppm	NOAEL = 200 ppm = 30 mg/kg bw/day	reduced body weight of weanlings + reduced number of mice born	-	JMPR 1970
	repro-tox	rats		dietary, 3 generation	0 or 500 ppm		no teratogenicity, decrased body weight		JMPR 1970
	systemic toxicity	sheep		gelatine capsules, 16 weeks	0, 10, 50, 100, 200, 400 or 800 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	loss of colloid in thyroid, reduced body weight	thyroid	JMPR 1970
	systemic toxicity	rats		suspended in 1 % methocel; 180 days	0, 12.5, 25, 50, 100, 200 or 400 mg/kg bw/day	NOAEL = 12.5 mg/kg bw/day	haemosiderosis of the thymus and colloid depletion in the thyroid, increased liver and kidney weight	thymus, thyroid, liver, kidney	JMPR 1970
	systemic toxicity	mice, rats, rabbits	-	orally		LD50 = 2400-3850 mg/kg bw	-		JMPR 1970
	eye test	rabbits					not irritating		JMPR 1970
ADI = 0-0.1 mg/kg bw (JECFA evaluation 1997)									

Phenylamide (acylalanine type)

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Metalaxyl	Carcinogenicity (and toxicity)	rats		Dietary, 104 weeks	0, 50, 250 or 1250 ppm	-	-		JMPR 1982
	(carcinogenicity and) toxicity	rats		Dietary, 104 weeks	0, 50, 250 or 1250 ppm	NOAEL = 50 ppm	Adverse effects		JMPR 1982
	carcinogenicity	mice		Dietary, 104 weeks	0, 50, 250 or 1250 ppm	NOAEL = 1250 ppm = 187.5 mg/kg bw	no carcinogenic effects found		JMPR 1982
	mutagenicity	In vitro					-		JMPR 1982
	Systemic toxicity	dogs		Dietary, 6 months	0, 50, 250 or 1000 ppm	NOAEL = 250 ppm = 7.4 mg/kg bw/day	Dose response for alkaline phosphatase, increased liver weight	liver	JMPR 1982
	Systemic toxicity	rats		Dietary, 90 days	0, 10, 50, 250 or 1250 ppm	NOAEL = 10 ppm	Decreased total leucocyte count, increased absolute and relative adrenal weights for males	blood, adrenal	JMPR 1982
	Systemic toxicity	rats		Gavage, 28 days	0, 10, 30 or 100 mg/kg/day for days 1-14; 0, 30, 100 or 3000 mg/kg/day for days 15-21; 0, 60, 200 or 600 mg/kg/day for days 21-28;	-	Absolute relative adrenal weights were significant increased for females in the high dose group. Increased absolute and relative liver weights	adrenal, liver	JMPR 1982
	Systemic toxicity	dogs		Dietary, 91 days	0, 50, 250 or 1250 ppm	NOAEL = 250 ppm	Significant increase in serum alkaline phosphatase levels	blood	JMPR 1982
	Systemic toxicity	Mice, rats, rabbits				LD50= 669-788 mg/kg bw	-		
	Eye test	Guinea pigs					Mildly irritating		JMPR 1982
ADI =0-0.03 mg/kg bw									

APPENDIX 5

Dicarboximide

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Iprodione	carcinogenicity (and mice toxicity)			dietary, 99 week	0, 160, 800 or 4000 ppm	NOAEL = 800 ppm = 115 mg/kg bw/day	tumourigenicity in the liver and ovary	liver, ovary	JMPR 1995
	(carcinogenicity and) mice toxicity			dietary, 99 week	0, 160, 800 or 4000 ppm	NOAEL = 160 ppm = 23 mg/kg bw/day	microscopic changes in liver and testes (non-neoplastic lesions - incl. hepatocellular enlargement and hypertrophy of interstitial cells in the testis)	liver, testes	JMPR 1995
	carcinogenicity (and rats toxicity)			dietary, 104 week	0, 150, 300 or 1600 ppm	NOAEL = 300 ppm = 12 mg/kg bw/day	interstitial cell tumours of the testes	testes	JMPR 1995
	(carcinogenicity and) rats toxicity			dietary, 104 week	0, 150, 300 or 1600 ppm	NOAEL = 150 ppm = 6 mg/kg bw/day	increased liver weight, histopathological findings in liver, kidneys, adrenals, testes and accessory glands	liver, kidney, adrenal, testes, accessory glands	JMPR 1995
	repro-tox, 1	rats		dietary	0, 300, 1000 or 3000/2000 ppm	-	reproductive performance was unaffected.	-	JMPR 1992
	repro-tox, 1	rats		dietary	0, 300, 1000 or 3000/2000 ppm	NOAEL = 300 ppm = 21 mg/kg bw/day	depressed body-weight		JMPR 1992
	repro-tox, 1	rats		dietary	0, 300, 1000 or 3000/2000 ppm		Off spring survival and growth were reduced at 3000/2000 ppm	-	JMPR 1992
	repro-tox, 2	rats		gavage	0, 40, 90 or 200 mg/kg bw/day	NOAEL = 200 mg/kg bw/day	maternal toxicity and tetratogenicity	-	JMPR 1992
	repro-tox, 2	rats		gavage	0, 40, 90 or 200 mg/kg bw/day	NOAEL = 90 mg/kg bw/day	embryofetal toxicity - slightly delayed fetal develop-ment	-	JMPR 1992
	repro-tox, 3	rabbits		gavage	0, 20, 60 or 200 mg/kg bw/day	NOAEL = 20 mg/kg bw/day	maternal toxicity - depressed weight gain	-	JMPR 1992
	repro-tox, 3	rabbits		gavage	0, 20, 60 or 200 mg/kg bw/day	NOAEL = 60 mg/kg bw/day	embryofetal toxicity - increased abortions and post-implantation loss	-	JMPR 1992
	repro-tox, 3	rabbits		gavage	0, 20, 60 or 200 mg/kg bw/day	NOAEL = 60 mg/kg bw/day	no teratogenic effects were found		JMPR 1992
	mutagenicity						not genotoxic		JMPR 1992
	systemic toxicity	mice, rats, dogs	-	orally		LD50 > 2000 mg/kg bw			JMPR 1995
	systemic toxicity	mice		dietary, 4 week	0, 600, 1900, 6000, 9500 or 15000 ppm	NOAEL = 600 ppm = 115 mg/kg bw/day	macroscopic hepatic changes	liver	JMPR 1992

	systemic toxicity	mice		dietary, 3 months	0, 1500, 3000, 6000 or 12000 ppm	-	increased liver and adrenal gland weights and hyper-throphy and/or vacuolation of hepatocytes and adrenal cortical cells	liver, adrenal gland	JMPR 1992
	systemic toxicity	rats		dietary, 3 months	0, 300, 1000 or 3000 ppm	NOAEL = 300 ppm = 21 mg/kg bw/day	swelling in the zona glomerulosa of the adrenal cortex	adrenal	JMPR 1992
	systemic toxicity	rats		dietary, 3 months	0, 1000, 2000, 3000 or 5000 ppm	NOAEL = 100 ppm = 78 mg/kg bw/day	reduced body weight gain and histopathological changes in the adrenal glands, ovaries and uterus	adrenal glands, ovaries, uterus	JMPR 1992
	eye test	rabbits					irritating		JMPR 1995
ADI = 0-0.06 mg/kg bw based on NOAEL = 6 mg/kg bw/day in the 2-year study of carcinogenicity and toxicity in rats. Safety factor: 100. (JMPR 1995)									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Procymidone	carcinogenicity	mice		Dietary, 104 weeks	0, 30, 100, 300 or 1000 ppm	NOAEL = 100 ppm = 15 mg/kg bw/day	Liver tumours	Liver	JMPR 1989
	Carcinogenicity (and toxicity)	rats		Dietary, 104 weeks	0, 100, 300, 1000 or 2000 ppm	NOAEL = 300 ppm = 14 mg/kg bw/day	Testicular interstitial cell hyperplasia, interstitial cell tumours	testes	JMPR 1989
	(Carcinogenicity and) toxicity	rats		Dietary, 104 weeks	0, 100, 300, 1000 or 2000 ppm	NOAEL = 100 ppm = 5 mg/kg bw/day	Reduced body weight gain		JMPR 1989
	Repro-tox, 1	rats		Dietary, 2-generation	0, 50, 250 or 750 ppm	NOAEL = 250 ppm = 12.5 mg/kg bw/day	Infertility, abnormalities of the male sexual organs (hyperspadias) in adults and pups. Reduction of ano-genital distance	-	JMPR 1989
	Repro-tox, 2	rats		Orally, corn oil, days 6-15 of gesta-tion	0, 30, 100 or 300 mg/kg bw/day	NOAEL = 300 mg/kg bw/day	No embryotoxic and teratogenic effects	-	JMPR 1989
	Repro-tox, 3	rabbits		Orally, corn oil, days 7-19 of gesta-tion	0, 30, 150, 750 or 1000 mg/kg bw/day	NOAEL = 750 mg/kg bw/day	No embryotoxic and teratogenic effects	-	JMPR 1989
	Special study on serum hormone levels	rats		Dietary, 3 months	0, 100, 300, 700 or 2000 ppm	No hormonal effect level = 300 ppm	Increased testosterone levels		JMPR 1989
	mutagenicity	In vitro/in vivo	-				Not genotoxic		JMPR 1989
	Systemic toxicity	dogs		Capsule, 6 months	0, 20, 100 or 500 mg/kg bw/day	NOAEL = 100 mg/kg bw/day	Increased liver weight, hepatocellular hyperplasia	liver	JMPR 1989
ADI = 0-0.2 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Vinclozolin	Carcinogenicity and toxicity, 1	Mice (C57B1)	-	Dietary, 18 months	0, 15, 150, 3000 or 8000 ppm	NOAEL = 150 ppm = 24 mg/kg bw/day	Hepatotoxicity. Hepatocellular carcinomas were seen at 8000 ppm	liver	JMPR 1995
	Carcinogenicity (and toxicity), 2	Mice (NMRI)	-	Dietary, 112 weeks	0, 16, 490, 1460 or 4370 ppm	-	-		JMPR 1995
	(Carcinogenicity and) toxicity, 2	Mice (NMRI)	-	Dietary, 112 weeks	0, 16, 490, 1460 or 4370 ppm	NOAEL = 490 ppm = 63 mg/kg bw/day	Increased liver weight – but no histological change.	-	JMPR 1995
	Carcinogenicity (and rats toxicity), 3			Dietary, 2 year	25-4500 ppm	NOAEL = 25 ppm = 1.4 mg/kg bw/day	Antiandrogenicity. Adrenal tumours were seen at 3000 ppm. Hepatocellular carcinomas were seen in males at 4500 ppm. Hepatoxicity were 150 ppm	adrenal, liver	JMPR 1995
	Repro-tox, 1	rats		Dietary, 70 days prematng	0, 20 or 40 ppm	NOAEL = 40 ppm = 4 mg/kg bw/day	No effects seen		JMPR 1995
	Repro-tox, 2	rats		Orally, days 6-19 of gestation	0, 15, 50 or150 mg/kg bw/day	NOAEL = 15 mg/kg bw/day	Teratogenicity – changes in anogenital distance	-	JMPR 1995
	Repro-tox, 3	Rats		Orally, days 6-19 of gestation	0, 50, 100 or 200 mg/kg bw/day	NOAEL = 100 mg/kg bw/day	Fetotoxicity – developmental delay		JMPR 1995
	Repro-tox, 4	Rats		Orally, days 6-19 of gestation	0, 200 or 400 mg/kg bw/day	NOAEL = 400 mg/kg bw/day	Maternal toxicity - clinical signs of toxicity	-	JMPR 1995
	Repro-tox, 5	Rabbits		Orally, days 6-18 of gestation	0, 50, 200 or 800 mg/kg bw/day	NOAEL = 50 mg/kg bw/day	Maternal toxicity		JMPR 1995
	Repro-tox, 5	Rabbits		Orally, days 6-18 of gestation	0, 50, 200 or 800 mg/kg bw/day	NOAEL = 200 mg/kg bw/day	Fetotoxicity, no signs of teratogenicity	-	JMPR 1995
	Mutagenicity	In vitro/in vivo	-				JMPR conclusion: not genotoxic		JMPR 1995
	Systemic toxicity, 1	dogs		Dietary, 12 months	0, 35, 75, 150 or 1500 ppm	NOAEL = 75 ppm = 2.4 mg/kg bw/day	Pathological changes in the liver, spleen, prostate, testis and adrenals. Antiandrogenicity	liver, spleen, prostate, testis, adrenals	JMPR 1995
	Systemic toxicity, 2	Rats, 2 studies	-	Dietary, 3 months	0, 300, 1000 or 3000 ppm or 0 or 50 ppm	NOAEL = 50 ppm = 4 mg/kg bw/day	Effects on the adrenal glands (incl. lipidosis)	adrenal glands	JMPR 1995
	Systemic toxicity, 3	mice		Dietary, 3 months	100-5000 ppm	NOAEL = 100 ppm = 20 mg/kg bw/day	-		JMPR 1995
	Systemic toxicity, 4	Rats				LD50 > 15000 mg/kg bw			JMPR 1995
	Eye test	rabbits					Not irritating		JMPR 1995
ADI = 0-0.01 mg/kg bw based on the NOAEL = 1.4 mg/kg bw/day in the 2 year study of carcinogenicity of rats. Safety factor: 100									

APPENDIX 5

Azole fungicides

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Imazalil	carcinogenicity (and toxicity)	rats		dietary, 30 months	0, 25, 100 or 400 ppm	-	-		JMPR 1985
	(carcinogenicity and) toxicity	rats		dietary, 30 months	0, 25, 100 or 400 ppm	NOAEL = 100 ppm = 5 mg/kg w/day	non-oncogenic effects - reduced body and brain weight in males.	brain	JMPR 1985
	Repro-tox, 1	mice		Orally, Day 6-16 of gestation	0, 2.5, 10 or 40 mg/kg bw/day of imazalil sulfate	-	No significant effects observed		JMPR 1986
	Repro-tox, 2	rabbits		Gavage, day 6-18 of gestation	0, 0.63 or 2.5 mg/kg bw/day of imazalil nitrate	-	Embryotoxic and maternally toxic effects in both dose groups		JMPR 1986 (Mars-boom, 1974)
	Repro-tox, 3	Rabbits		Gavage, day 6-18 of gestation	0, 0.16 or 0.63 mg/kg bw/day of imazalil nitrate	-	No effects found		JMPR 1986 (Marsboom and Dirkx, 1981)
	Repro-tox, 4	rabbits		Gavage, day 6-18 of gestation	0, 1.25, 2.5 or 5 mg/kg bw/day of imazalil nitrate	NOAEL = 2.5 mg/kg bw/day	Embryotoxic and maternally toxic effects – decreased body weight in dams and pups. No teratogenic effect		JMPR 1986 (Marsboom and Dirkx, 1985)
	Systemic toxicity	dogs		Capsule, 12 months	0, 1.25, 2.5 or 20 mg/kg bw/day	NOAEL = 2.5 mg/kg bw/day	Clinical signs, decreased body-weight gain, increased liver weight	liver	JMPR 1991
ADI = 0-0.03 mg/kg bw based on NOAEL = 2.5 mg/kg bw/day in the dog study. Safety factor: 100									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Triadimefon	carcinogenicity and) toxicity)	mice		dietary, 24 months	0, 50, 300 or 1800 ppm	-	-		JMPR 1981
	(carcinogenicity and) toxicity	mice		dietary, 24 months	0, 50, 300 or 1800 ppm	NOAEL = 300 ppm = 40 mg/kg bw/day	growth was affected, increased erythrocyte counts, necropsies, increased liver weights, increased AP, GOT and GPT activities…	liver, blood	JMPR 1981
	carcinogenicity (and toxicity)	rats		dietary, 24 months	0, 50, 500 or 5000 ppm	-	-		JMPR 1981
	(carcinogenicity and) toxicity	rats		dietary, 24 months	0, 50, 500 or 5000 ppm	NOAEL = 50 ppm = 2.5 mg/kg bw/day	increased liver weight, reduced erythocyte counts, enzyme reduction…	liver, blood	JMPR 1981
	repro-tox	rats		dietary, 3 generation	0, 50, 300 or 1800 ppm	NOAEL = 50 ppm	reduced body weight of pups. Reproductive toxicity at 1800 ppm - a later study confirmed that.	-	JMPR 1981/1985
	special study on induction of liver enzymes	rats		dietary, 7 days			Induced hepatic mono-oxygenase activity at 30 mg/kg in males and 10 mg/kg in females	liver	JMPR 1984
	special study on induction of liver enzymes	mice		dietary			induced aldrin epoxidation at 50 mg/kg	-	JMPR 1984
	systemic toxicity	dogs		dietary, 104 weeks	0, 100, 330, 1000 or 2000 ppm	NOAEL = 330 ppm = 8.25 mg/kg bw/day	microsomal enzyme induction		JMPR 1981
	mutagenicity	in vitro					-		JMPR 1981
ADI = 0-0.03 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Triadimenol	Carcinogenicity (and toxicity)	Rats		Dietary, 24 months	0, 125, 500 or 2000 ppm	-	-		JMPR 1989
	(carcinogenicity and) toxicity	rats		Dietary, 24 months	0, 125, 500 or 2000 ppm	NOAEL = 125 ppm = 7 mg/kg bw/day	Hepatotoxicity	liver	JMPR 1989
	Repro-tox, 1	rats		Dietary, 100 days before mating, 2 generation	0, 20, 100 or 500 ppm	NOAEL = 125 ppm = 5 mg/kg bw/day	Reproductive toxicity - decreased parental pup body weights.	-	JMPR 1989
	Repro-tox, 2	rats		Gavage, days 6-15 of gestation	0, 30, 60 or 120 mg/kg bw/day	NOAEL = 60 mg/kg bw/day	Embryo-fetotoxicity		JMPR 1989
	Repro-tox, 2	rats		Gavage, days 6-15 of gestation	0, 30, 60 or 120 mg/kg bw/day	NOAEL = 30 mg/kg bw/day	Maternal toxicity – reduced food consumption + body weight gain	-	JMPR 1989
	Repro-tox, 3	Rabbits		Gavage, days 6-18 of gestation	0, 8, 40 or 200 mg/kg bw/day	NOAEL = 40 mg/kg bw/day	Embryo-fetotoxicity – increased resorption	-	JMPR 1989
	Repro-tox, 3	Rabbits		Gavage, days 6-18 of gestation	0, 8, 40 or 200 mg/kg bw/day	NOAEL = 8 mg/kg bw/day	Maternal toxicity – slightly decreased body weight gain	-	JMPR 1989
	mutagenicity	In vitro/in vivo	-				-		JMPR 1989
	Systemic toxicity	Rats		Dietary, 90 days	0, 120, 600 or 3000 ppm	NOAEL = 120 ppm = 8 mg/kg bw/day	Increased liver weights and liver hypertrophy	liver	JMPR 1989
	Systemic toxicity	Dogs, 3 studies	-	Dietary	0-240 ppm	NOAEL = 100 ppm = 7.5 mg/kg bw/day	Hepatotoxicity	liver	JMPR 1989
ADI = 0-0.05 mg/kg bw									

Fungicides no otherwise classified

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Diphenylamine	carcinogenicity (and toxicity)	mice		dietary, 78 weeks	0, 520, 2625 or 5200 ppm	-	-		JMPR 1998
	(carcinogenicity and) toxicity	mice		dietary, 78 weeks	0, 520, 2625 or 5200 ppm	NOAEL = 520 ppm = 73 mg/kg bw/day	haematological effects, reduced body-weight gain, reduced survival	blood	JMPR 1998
	carcinogenicity (and toxicity)	rats		dietary, up to 2 year	0, 200, 750, 3750 or 7500 ppm (males) and 0, 150, 500, 2500 or 5000 ppm (females)	-	-		JMPR 1998
	(carcinogenicity and) toxicity	rats		dietary, up to 2 year	0, 200, 750, 3750 or 7500 ppm (males) and 0, 150, 500, 2500 or 5000 ppm (females)	NOAEL = 150-200 ppm = 7.5 mg/kg bw/day	haematological and histological effects	blood	JMPR 1998
	repro-tox, 1	rats		dietary, 2 generation	0, 500, 1500 or 5000 ppm	NOAEL not observed. LOAEL = 500 ppm = 40 mg/kg bw/day	enlarged spleens in F1 females, increased spleen congestion and haemosiderosis. Hepatocyte hypertrophy in F0 females	spleen, liver	JMPR 1998
	repro-tox, 1	rats		dietary, 2 generation	0, 500, 1500 or 5000 ppm	NOAEL = 500 ppm = 46 mg/kg bw/day	developmental toxicity - reduced body weights in F2 pups	-	JMPR 1998
	repro-tox, 1	rats		dietary, 2 generation	0, 500, 1500 or 5000 ppm	NOAEL = 1500 ppm = 120 mg/kg bw/day	reproductivite toxicity		JMPR 1998
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 10, 50 or 100 mg/kg bw/day	NOAEL = 50 mg/kg bw/day	maternal toxicity - enlarged, blackish, heavier spleens	spleen	JMPR 1998
	repro-tox, 2	rats		gavage, days 6-15 of gestation	0, 10, 50 or 100 mg/kg bw/day	NOAEL = 100 mg/kg bw/day - highest dose tested	developmental toxicity		JMPR 1998
	repro-tox, 3	rabbits		gavage, days 7-19 of gestation	0, 33, 100 or 300 mg/kg bw/day	NOAEL = 300 mg/kg bw/day - highest dose tested	developmental toxicity		JMPR 1998
	mutagenicity	in vivo/in vitro		-			-/+ the meeting concluded: it is unlikely to present a human genotoxic hazard.	-	JMPR 1998
	systemic toxicity	dogs		gelatine capsules, 1 year	0, 10, 25 or 100 mg/kg bw/day	NOAEL = 10 mg/kg bw/day	haematological and clinical chemical changes	blood	JMPR 1998
	systemic toxicity	dogs		gelatine capsules, 90 days	0, 10, 25 or 50 mg/kg bw/day	NOAEL = 50 mg/kg bw/day	no treatment-related effects		JMPR 1998
	systemic toxicity	rats		dietary, 90 days	0, 150, 1500, 7500 or 15000 ppm	NOAEL = 150 ppm = 12 mg/kg bw/day	changes in clinical chemical parameters, increased organ weighs, gross and histological changes in females	-	JMPR 1998
	systemic toxicity	mice		dietary, 90 days	0, 10, 520, 2600 or 5200 ppm	NOAEL = 10 ppm = 1.7 mg/kg bw/day	changes in haematological parameters, findings of necropsy	blood	JMPR 1998
	systemic toxicity	rats				LD50 = 3000 mg/kg bw			JMPR 1998
ADI = 0-0.08 mg/kg bw based on NOAEL = 150 ppm = 7.5 mg/kg bw/day in the 2-year study of toxicity and carcinogenicity in rats. Safety factor: 100.									

APPENDIX 5

PLANT GROWTH REGULATORS
Quaternary nitrogen compounds

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Mepiquat	carcinogenicity						not undergone a complete evaluation and determination	-	IRIS website
	carcinogenicity	rats		dietary, 2 years		NOEL = 100 ppm = 50 mg/kg/day	decreased food intake and body weight gain	-	IRIS website
	(carcinogenicity and) toxicity	mice				NOEL = 1000 ppm = 150 mg/kg/day	systemic toxicity - increase incidence of leucocytes in males and serum free cholesterol in females	blood	IRIS website
	repro-tox	rats		3-generation		NOEL = 3191 ppm = 340 mg/kg/day	maternal and developmental toxicity	-	IRIS website
	systemic toxicity	dogs	4/sex/group	dietary, 90 days	0, 100, 300, 1000 and 3000 ppm	NOEL = 1000 ppm = 25 mg/kg/day	sedation and tonoclonic spasms, decreased food intake and body weights, decreased hemoglobin and RBC, increased hematocrit and reticulocytes	blood	IRIS website
	systemic toxicity	rats		dietary, 90 days		NOEL = 3000 ppm = 150 mg/kg/day	decreased body weight and food consumption, decreased absolute weights of heart, kidneys, liver, lungs and spleen in males	heart, kidney, liver, lungs, spleen	IRIS website
	systemic toxicity	rats		orally		LD50 = 464 mg/kg bw			The e-Pesticide Manual, 11. ed., 1999
	eye test	rabbits					not irritating		The e-Pesticide Manual, 11. ed., 1999
Tentative ADI = 0.15-0.3 mg/kg bw/day (Otto Meyer) ADI = 1.5 mg/kg bw (The e-Pesticide Manual, 11. Ed., 1999)									
RfD = 0.03 mg/kg/day based on NOEL = 1000 ppm = 25 mg/kg/day in the 90 days dog study. Safety factor: 1000. (IRIS website)									

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlormequat	carcinogenicity (and toxicity)	mice		dietay, 2 year	0, 150, 600 or 2400 ppm	-	-		JMPR 1997
	(carcinogenicity and) toxicity	mice		dietay, 2 year	0, 150, 600 or 2400 ppm	NOAEL = 150 ppm = 23 mg/kg bw/day	tubular down-growth in the ovaries, incidence of endometrial hyperplasia	ovaries	JMPR 1997
	carcinogenicity (and toxicity)	rats		dietary, 2 year	0, 280, 940 or 2800 ppm	-	-		JMPR 1997
	(carcinogenicity and) toxicity	rats		dietary, 2 year	0, 280, 940 or 2800 ppm	NOAEL = 940 ppm = 42 mg/kg bw/day	reduced body weight		JMPR 1997
	Repro-tox, 1	rabbits		Gavage, day 6-18 after insemination	0, 1.5, 3, 6 or 12 mg/kg bw/day	NOAEL = 6 mg/kg bw/day	Maternal toxicity		JMPR 1999
	Repro-tox, 1	rabbits		Gavage, day 6-18 after insemination	0, 1.5, 3, 6 or 12 mg/kg bw/day	NOAEL = 12 mg/kg bw/day	Developmental toxicity		JMPR 1999
	Repro-tox, 2	rats		Dietary, 2-generation	0, 300, 900 or 2700 ppm	NOAEL = 900 ppm = 69 mg/kg bw/day	Reproductive toxicity - reduced male fertility and number of delivered pups	-	JMPR 1999
	Repro-tox, 2	rats		Dietary, 2-generation	0, 300, 900 or 2700 ppm	NOAEL = 300 ppm = 23 mg/kg bw/day	Systemic toxicity; clinical signs such as tremors	-	JMPR 1999/1997
	Systemic toxicity	dogs		Dietary, 1 year	0, 150, 300 or 1000 ppm	NOAEL = 150 ppm = 4.7 mg/kg bw/day	Diarrhoea, salivation		JMPR 1999
	Systemic toxicity	rodents				LD50 = 200-1000 mg/kg bw	-		JMPR 1999
	Systemic toxicity	Monkeys				LD50 > 800 mg/kg bw			JMPR 1999
	Systemic toxicity	Dogs, cats				LD50 approx. 50 mg/kg bw	-		JMPR 1999
ADI = 0-0.05 mg/kg bw based on NOAEL = 4.7 mg/kg bw/day in the 1 year study of dogs. Safety factor: 100.									
Acute RfD = 0.05 mg/kg bw based on NOAEL = 4.7 mg/kg bw/day in the 1 year study in dogs									

APPENDIX 5

MICELLANEOUS PESTICIDES
Syntetic acaricides

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Chlorfenson	Systemic toxicity	rats		Dietary, 2 years	0, 0.63, 1.25, 2.5, 5 ,15 or 50 mg/kg bw/day	NOAEL = 1.25 mg/kg bw/day	Histological changes in the liver and kidneys + increase of the weights of the organs	Liver, kidney	JMPR 1965
	Systemic toxicity	rats		Dietary, long-term study	0, 100, 300 or 3000 ppm	-	Histological changes in the liver and kidneys	Liver, kidney	JMPR 1965
	Systemic toxicity	Rats	female	Dietary, 130 days	0, 300, 1000, 3000 or 10000 ppm	NOAEL = 300 ppm	Increased liver and kidney weights, slight histological degenerative changes in these organs	Liver, kidney	JMPR 1965
	Systemic toxicity	dogs		Dietary, 6 months	0, 5, 15 or 50 mg/kg bw/day	NOAEL = 5 mg/kg bw/day?	Maybe a slight increase in the liver weight – but no histological chages in the organ	liver	JMPR 1965
	Systemic toxicity	rats				LD50 = 2000 mg/kg bw			JMPR 1965
ADI = 0-0.01 mg/kg bw									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Tetrasul	systemic toxicity	rats		dietary, 2 years		NEL = 10 mg/kg diet = 0.5 mg/kg bw	-		The Pesticide Manual, 8 ed., 1987
	systemic toxicity	rats		orally		LD50 = 6810-8250 mg/kg	-		The Pesticide Manual, 8 ed., 1987
	systemic toxicity	mice		orally		LD50 = 5010 mg/kg			The Pesticide Manual, 8 ed., 1987
Tentative ADI = 0-0.03 mg/kg bw based on the toxicitystudy in rats (Otto Meyer)									

APPENDIX 5

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Tetradifon	repro-tox	rats		2-generation		NOEL = 200 mg/kg diet			The e-Pesticide Manual, 11. ed., 1999
	repro-tox	rats, rabbits	-				no teratogenic effects		The e-Pesticide Manual, 11. ed., 1999
	mutagenicity						not mutagenic		The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	various		long term studies		NOAEL = 2.5-15 mg/kg bw/day	-		Otto Meyer
	systemic toxicity	rats		dietary, 2 years		NOAEL = 300 mg/kg diet			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	rats				LD50 > 2500 mg/kg			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity	mice				LD50 > 500 mg/kg			The e-Pesticide Manual, 11. ed., 1999
	systemic toxicity			long-term or repeated exposure	-			kidneys and liver	International Labour Organization, website
	eye test	rabbits					slight irritating		The e-Pesticide Manual, 11. ed., 1999
Tentative ADI = 0.03 mg/kg bw based on NOAEL = 2.5-15mg/kg bw/day in the long term studies. Safety factor: 100. (Otto Meyer)									

APPENDIX 5

PHENOLES = METABOLITES

CHEMICAL	TYPE OF STUDY	SPECIES	SEX	EXPOSURE	DOSE	NOAEL/LD50	MODE OF ACTION/EFFECTS	TARGET ORGAN	REFERENCES
Ortho-phenylphenol	carcinogenicity	mice		2 years		NOAEL < 250 mg/kg bw/day	carcinogenic in male mice (adenomas)	liver	JMPR 1999
	carcinogenicity (and toxicity)	rats		2 years		NOAEL = 800 ppm = 39 mg/kg bw/day	carcinogenic in male rats		JMPR 1999
	carcinogenicity						meeting concluded: not carcinogenic to humans	-	JMPR 1999
	mutagenicity	in vivo/in vitro	-				-/?		JMPR 1999
	repro-tox	mice				NOAEL < 1500 mg/kg bw/day	lowest dose tested. Maternal and fetotoxicity		JMPR 1999
	repro-tox	mice				NOAEL = 2100 mg/kg/day	highest dose tested. Not teratogenic		JMPR 1999
	repro-tox	rats		2-generation		NOAEL = 460 mg/kg/day	no reproductive toxicity		JMPR 1999
	repro-tox and carcinogenicity	rats		2-generation		NOAEL = 92 mg/kg/day	no reproductive toxicity. Urinary bladder tumours in males	urinary bladder	JMPR 1999
	repro-tox	rats				NOAEL = 150 mg/kg/day	developmental toxicity, maternal toxicity, not teratogenic	-	JMPR 1999
	repro-tox	rats				NOAEL = 300 mg/kg/day	developmental toxicity		JMPR 1999
	repro-tox	rats				NOAEL = 700 mg/kg/day	teratogenicity		JMPR 1999
	repro-tox	rabbits				NOAEL = 100 mg/kg/day	maternal toxicity		JMPR 1999
	repro-tox	rabbits				NOAEL = 500 mg/kg/day	fetotoxicity		JMPR 1999
	repro-tox	rabbits				NOAEL = 750 mg/kg/day	teratogenicity		JMPR 1999
	neurotoxicity	rats, mice, dogs	-				no developmental neurotoxicity		JMPR 1999
	systemic toxicity	dogs		1 year		NOAEL = 750 mg/kg/day	highest dose tested		JMPR 1999
	systemic toxicity	rats		1 year		NOAEL = 39 mg/kg bw/day	-		JMPR 1999
	systemic toxicity	mice, rats		orally		LD50 = 600-3500 mg/kg bw	-		JMPR 1999
	eye test	rabbits					irritating		JMPR 1999
ADI = 0-0.4 mg/kg bw based on NOAEL = 39 mg/kg bw/day in the 2-year study of toxicity and carcinogenicity of the urinary bladder in male rats. Safety factor: 100									

Appendix 6: Toxicology of single compounds - overview

CHEMICAL	NOAEL (mg/kg bw/day)	EFFECTS UPON WHICH NOAEL ARE BASED	ADI (mg/kg bw)	MODE OF ACTION/EFFECTS	TARGET ORGAN	ABSORPTION, DISTRIBUTION; EXCRETION	METABOLISM	MAJOR METABOLITES	CHEMICAL GROUP	REFERENCES
AMPA	31	Glyphosate: reduced body weight in males	0.3	Hyperplasia of the urinary bladder. Fetal toxicity - developmental toxicity	Urinary bladder	Absorbed and excreted unmetabolized - urine	Not metabolised	No metabolites	Metabolite of glyphosate	JMPR 1997
Azinphos-methyl	0.48	Repro-tox	0.005	Inhibit AChE (plasma, erythrocyte, brain). Mutagenic *in vitro*. Repro-tox	Nervous system	Absorbed from the digestive tract. Organs of elimination: liver and kidney, relatively high concentrations found in blood. Mostly excreted in the urine	Oxidation, GSH-transferases, hydrolysis, methylation, oxidation.	Azinphos-methyl oxygen analog, mercaptomethylbenzazimide, glutathionyl methyl benzazimide, desmethyl isoazinphos-methyl	Dimethoxy compounds of category IV	JMPR 1991
Benzene hexachloride, BHC	-	-	- No ADI because of lack of information	Hepatomas	Liver	-	-	-	Benzene hexachloride and lindane	JMPR 1973
Bifenthrin	1	Tremors in the dams	0,02	Tremors + twitching. Tumorigenic potential in mice can not be excluded	-	Absorbed. Eliminated mainly via faeces - 20-30 % in bile; 5-10 % in urine	Hydrolysis, hydroxylation	From hydroxylated parent compound: hydroxymethyl bifenthrin, 4'-OH bifenthrin, 3' or 4'-OH-hydroxymethyl bifenthrin. Hydrolytic products: 4'-OH-2-methyl-3-phenylbenzoic acid. 4'-OH-2-methyl-3-phenylbenzyl alcohol. Dimethoxy-2-methyl-3-phenylbenzoic acid. Dimethoxy-2-methyl-3-phenylbenzyl alcohol	Pyrethrum and related compounds	JMPR 1992
Bromopropylate	2.7	Reduced body-weight gain	0.03	Effects on the liver. Increased	Liver, thyroid gland	Excreted mainly in the faeces	Preferentially by cleavage of the	Benzilic acid + amino acid conjugates of this	DDT and its analoges	JMPR 1993

				thyroid weights. Increased incidence of skeletal variations			isopropyl ester			
Captan	12.5	Repro-tox	0.1	Carcinogenic. Duodenal hyperplasia. Slightly enlarged kidneys and livers. Reproductive- and developmental toxicity. Mutagenic *in vitro*	Duodenal	Degradation in the gastrointestinal tract. Excretion mainly via urine.	Oxidation, hydrolysis	Thiophosgene -> thiazolidine-2-thione-4-carboxylic acid. 4,5-cyclohexene-1,2-dicarboximide	Chloroalkyl thio fungicides	JMPR 1995
Carbaryl	14.7	Non-neoplastic lesions	0,003	Carcinogenic. Inhibit AChE (brain, erythrocyte). Tumours in thyroid gland, liver, urinary bladder, vascular. Effects on thyroid gland, reproductive organs, kidney	Thyroid gland, liver, urinary bladder, kidney, vascular, reproductive organs	Rapidly absorbed and excreted, mainly in the urine	Ring hydroxylation, hydrolysis, conjugation	1-naphthol	Anticholinesterase carbamates and procarbamates	JMPR 1996
Carbendazim	2.5	Hepatotoxicity	0.03	Liver tumours. Hepatotoxicity. Reproductive-, developmental and fetotoxicity, teratogenicity. Not mutagenic but causes chromosomal aberrations	Liver	Readily absorbed, rapidly metabolized. Excreted in faeces and urine	-	2-[(methoxycarbonyl)amino]-1H-benzimidazol-5-yl hydrogen sulfate	Benzimidazole 13)	JMPR 1995
Chlorfenson	-	-	0.01	Histological changes in the liver and kidneys	Liver, kidney	absorbed from the alimentary tract. Accumulation in depot fat of dogs at high doses	No data	No data	Syntetic acaricides	JMPR 1965/1963
Chlormequat	4.7	Diarrhoea, salivation	0.05	Tubular downgrowth in the ovaries, incidence of endometrial hyperplasia. Diarrhoea, salivation. Developmental-, reproductive	Ovaries	Rapidly absorbed, complete elimination - via urine - unchanged	-	Salts of chlorcholine. A polar but unidentified metabolite was found in faeces	Quaternary nitrogen compounds	JMPR 1997

				toxicity						
Chlorobenzi-late	-	-	0,02	Carcinogenic in mice. Mild anae-mia, increased organ to body-weight ratios for liver, spleen, kidney, testes, et cetera. Non-specific changes in pancreas, adrenals	Liver, spleen, kidney, testes, blood, pancreas, adrenals	Excreted in the urine and faeces. No accumulation	Hydrolysis	4,4'-dichlorobenzilic acid	DDT and its analoges	JMPR 1968
Chloropropy-late	-	-	-	Decrease in prostate weight, increased inci-dence of chronic renal desease, fatty changes in liver. Gastric stress and trauma, elevated alkaline phosphatase	Liver, kidney, prostate, stomach	Excreted in the urine and faeces. No accumulation	-	Dichlorobenzilic acid	DDT and its analoges	JMPR 1968
Chlorothalonil	3	Renal tubule vacuolation	0,03	Neoplastic and non-neoplastic lesions in kidney and stomach	Kidney, stomach	Excreted mainly in faeces; 2-4 % in urine. Metabolized in the gastrointestinal tract	-	-	Other aro-matic hydro-cabons	JMPR 1990
Chlorpropham	-	-	- (lack of data)	Increased liver weight	Liver	No data	No data	No data	Carbamate herbicides	JMPR 1963/1965
Chlorpyrifos	1	Inhibit AChE (brain, erythro-cyte). Neonatal, fetal and perinatal toxicity	0,01	Inhibit AChE (brain, erythro-cyte). Neonatal, fetal and perinatal toxicity. Mild delayed neuropa-thy	Nervous system	Rapidly and exten-sively absorbed, eliminated in the urine and faeces	Oxidative desul-furation (to chlor-pyrifos oxon), hydrolysis: chlor-pyrifos oxon -> diethylphosphate and 3,5,6-trichloropyridinol	chlorpyrifos oxon -> diethylphosphate and 3,5,6-trichloropyridinol. Metabolites found in urine from rats: glucuronide and sulfate conjugates. From humans diethylphosphorus metabolites	Diethoxy compounds of category IV	JMPR 1999
Chlorpyrifos-methyl	0.1 (human) and 1 (rat)	Humans: No effects. Rats: histological alteration in the adrenals	0.01	Inhibit AChE. Mutagenic *in vitro*. Effects on the liver and adrenal gland	Nervous system. Liver. Adrenal gland	Rapidly absorbed and excreted - mostly in urine and faeces	-	3,5,6-trichloro-2-pyridinol	Dimethoxy compounds of category IV	JMPR 1975

		adrenals		adrenal gland						
Cypermethrin	-	-	0.05	Damage to sciatic nerves - may be reversible	Nervous system	Well absorbed from the gastrointestinal tract, metabolize, excreted - urine. Little bioaccumulation	Ester cleavage, oxidation and conjugation	3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid. 3-(4-hydroxyphenoxy) benzoic acid. N-(3-phenoxybenzoyl) glycine	Pyrethrum and related compounds	JMPR 1981
DDE, o, p'-	-	-	-	Hepatocellular carcinomas. Thyroid tumors in females. Probable human carcinogen. Mutagenic *in vitro*	Liver, thyroid gland	-	-	-	Metabolite of DDT	IRIS, website; National Toxicology Program. Chemical Health and Safety Data, website
DDT	-	-	0,02	Hepatomas	Liver	Absorbed, mostly excreted unchanged in the faeces. DDT and its metabolites accumulate in human body fat	-	-	DDT and its analoges	JMPR 1965
Deltamethrin	-	-	0,01	Slightly increased incidence of axonal degenerations in sciatic, tibial and/or plantar nerves	Nervous system	-	Ester cleavage, oxidation at the 4'-position of the alcohol moiety	esters monohydroxylated at the 2', 4' and 5 positions of the alkohol moity; 2,2-dimethyl.3-(2,2-dibromovinyl) cyclopropane carboxylic acid and its glucuronide and glycine conjugates + and others	Pyrethrum and related compounds	JMPR 1981
Diazinon	0.025	Humans: no effect	0.002	Inhibit AChE (erythrocyte, brain). Repro-tox. Fetotoxicity	Nervous system.	Almost completely absorbed and eliminated - mainly in the urine	Oxidase/hydrolase-mediated clevage of the ester bond	2-isopropyl-6-methyl-4(1H)-pyrimidinone	Diethoxy compounds of category IV	JMPR 1993
Dichlofluanid	-	-	0.3	Thyroid effects. Maternal, embryotoxic, faetotoxic effects. Decreased interstitial tissue of the testis and vacuolation, degeneration of the adrenal cortex. Mutagenic	Thyroid gland	Metabolite excreted in urine	-	Thiazolidine-2-thiocarbonic acid	Chloroalkyl thio fungicides	JMPR 1979

				in vitro						
Dichloran	1.7	Hepatic and haematological effects	0.01	Effects on the liver, spleen, thyroid and kidney. Changes in haematological and clinical chemical parameters. Reproductive and developmental toxicity	Liver, spleen, thyroid gland, kidney, blood	Rapidly absorbed, metabolized, eliminated in urine (> 70%) and faeces	-	Unine: sulfate and glucuronide conjugates of 2,6-dichloro-4-hydroxyaniline. Faeces: derivatives of glutathione conjugates	Anilino and nitrobenzenoid fungicides	JMPR 1998
Dicofol	0.22	Histopathological changes in liver, vacuolation in adrenal cortical cells	0,002	Liver adenomas/carcinomas. Ovarian stromal cell hypertrophy. Effects on liver. Thyroid follicular epithelial hypertrophy. Repro-tox. Embryofoetal toxicity.	Liver, thyroid gland, orary	Absorbed from the gastrointestinal tract. Accumulated in adipose tissue. Dicofol is more polar than DDT and therefore less persistent in the body. In some extent excreted unchanged in the faeces.	-	In human urine: dichlorobenzilic acid. In rodent brain, fat, liver: dichlorobenzophenone, dichlorobenzhydrol	DDT and its analoges	JMPR 1992
Dieldrin	-	-	0,0001	Carcinogenic - liver in mice. Effects on the central nervous system	Liver, central nervous system	Excretion in the bile - both changed and unchanged. Accumulation unchanged in body fat - slowly lost	-	6,7-trans-dihydroxy-dihydroaldrin	Cyclodiene and related compounds	JMPR 1977
Dimethoate	1.2	Reproductive toxicity	0.002	Inhibit AChE (brain, erythrocyte). Repro-tox., Mutagenic *in vitro*	Nervous system	Rapidly and extensively absorbed from gut; rapidly excreted - urine	Thiono oxidation, hydrolysis	Omethoate	Dimethoxy compounds of category IV	JMPR 1996
Dioxathion	-	-	0.0015	Inhibit AChE	Nervous system	Absorbed, excreted - mainly in the urine	Hydrolysis, oxidation	Diethyl pyhosphoric acid, O,O-diethyl phosphorothionic and O,O-diethyl phosphorodithionic acid	Diethoxy compounds of category IV	JMPR 1968
Diphenyl	25	Pathological changes	0.125	-	-	Excreted both as changed and unchanged	Hydroxylation	4-hydroxydiphenyl	Other aromatic hydrocabons	JMPR 1966

Diphenyl-amine	7.5	Haematological and histological effects	0.08	Haematological and histological effects. Effects on spleen and liver Developmental and reproductivite toxicity. Mutagenic *in vitro*	Blood, spleen, liver	Extensively ab-sorbed, rapidly excreted - in urine	Ring hydroxyla-tion, formation of glucuronide and sulfate conjugates	4,4'-dihydroxydiphenylamine, 4-hydroxydiphenylamine, indophenol, 3-hydroxydiphenylamine, 2-hydroxyphenylamine	Fungicides not other-wise classi-fied	JMPR 1998
Dithiocar-bamates (analysed as a sum of 6 dithiocar-bamates)	-	-	0.003 (low-est ADI)	Effects on thyroid gland. Develop-mental toxicity, mainly terato-genicity (some of the dithiocar-bamates)	Thyroid gland	Accumulate in tissues. Rapid excre-tion in urine	-	Carbon disulfide, carbonyl sulfide, hydrogen sulfide	Anticholi-nesterase carbamates and procar-bamates	Tordoir et al., 1994
Endosulfan	0.6	In a 2-year study of toxicity in rats	0,006	Increased liver and kidney weights. Chronic glomerulonephro-sis or toxic neph-ropathy after long-term exposure. Developmental-and fetotoxicity	Liver, kidney	Absorbed, mainly distributed in kidney and liver. No accu-mulation	Oxidation, hy-drolysis	Polar substances not yet identified. Endosulfan sulfate, diol, hydroxy-ether, ether, lactone	Cyclodiene and related compounds	JMPR 1998
Ethion	0.2	Reproductive toxicity	0.002	Inhibit AChE (plasma, brain). Embryotoxic-ity/fetotoxicity	Nervous system	Slowly absorbed. Complete metabo-lism. Renal excretion	-	-	Diethoxy compounds of category IV	JMPR 1986
Etrimfos	-	-	0,003	Inhibit AChE	Nervous system	Mainly excreted in the urine	-	6-ethoxy-2-ethyl-4-hydroxypyrimidine; desmethyl etrimfos	Dimethoxy compounds of category IV	JMPR 1980
Fenitrothion	-	-	0,005	Histological changes of the liver and spleen. Inhibit AChE (plasma, erythro-cyte, brain)	Liver, spleen, nervous system	Easily absorbed from the gastro-intestinal tract and distributed into various tissues, mainly excreted in the urine	Fenitrothion -> 3-methyl-4-nitrophenol and fenitrothion -> 3-methyl-dimethyl para-oxon -> 3-methyl-4-nitrophenol	3-methyl-4-nitrophenol, O-desmethyl-fenitrothion. Most important with respect to toxicity: 3-Methyl-dimethyl para-oxon	Dimethoxy compounds of category IV	JMPR 1969

Fenpro-pathrin/fenpro ponat	3	Reduced body-weight gain, mortality in females, occur-rence of tremors in pups	0.03	Embryotoxicity, teratogenicity	-	Absorbed. Rapid excretion - via urine and faeces. Effective metabolism to polar products	Oxidation at the methyl groups of the acid moiety and at the 2'- and 4'-positions of the alcohol moiety, ester cleavage, conjugation with glucuronic acid, sulfuric acid and glycine	Urine: 3-phenoxybenzoic acid + as glycine conju-gate. 4'-OH-phenoxybenzoic acid-sulfate. 2'-OH-phenoxybenzoic acid-sulfate. Faeces: CH2OH (trans)-fenpropathrin	Pyrethrum and related compounds	JMPR 1993
Fenson	-	-	-	-	-	-	-	-	Syntetic acaricides	-
Fenthion	0.07	Acethylcholi-nesterase inhibi-tion (erythrocyte)	0,007	Inhibit AChE (brain, erythro-cyte). Repro-tox., weakly genotoxic	Nervous system	Readily absorbed and rapidly excreted in the urine.	-	Fenthion phenol [4-methylthio-meta-cresol] and its sulfoxide and sulfone; the phenols glucuronide and sulfate conjugates	Dimethoxy compounds of category IV	JMPR 1995
Fenvalerate	-	-	0,02	Hepatic micro-granulomas	Liver	The acid or alcohol moity were excreted. The cyano-fragment was rather persistent. Minimal potential for bioaccumulation of fenvalerate	-	Cows: 4-chloro-alpha-(1-methylethyl)-benzene acetic acid	Pyrethrum and related compounds	JMPR 1984
Folpet	10	Non-neoplastic lesions, repro-tox., decreased body weight + food consumption + serum biochemi-cal changes	0.1	Duedenal hyper-plasia, adenomas + adenocarcino-mas. Tumours in the upper parts of the gastrointesti-nal tract. Non-neoplastic lesions. Fetotoxicity, teratogenicity. Mutagenic *in vitro*	Duodenal, gastro-intestinal tract	Excretion mostly in the urine and expired air	Folpet (hydroly-sis) -> phthalim-ide -> phthalic acid + chloride ions + organic sulfurs. Thiophos-gene -> carbon dioxide.	Rats: disulfonic acid. Mice: thiazolidine (Pro-posed metabolic pathways see p 183 in JMPR 1995)	Chloroalkyl thio fungi-cides	JMPR 1995
Glyphosate	31	Reduced body weight in males	0.3	Mild hepatotoxic-ity	Liver	Incompletely ab-sorbed from the gastrointestinal tract. Excreted unchanged in urine + perhaps in bile + enterohepatic circulation	Not metabolised	No metabolites	Organic phosphorus herbicides	JMPR 1986

Heptachlor	0.025	Histopathological changes. Increased mortality of F2 pups in repro-tox study	0,0001	Hepatocellular carcinomas	Liver	Well absorbed in the gastrointestinal tract, metabolized to heptachlor epoxide, which accumulate in adipose tissue, cross the placenta. Major exretion via faeces	-	Heptachlor epoxide	Cyclodiene and related compounds	JMPR 1991
Hexachlorobenzene	-	-	-	Hepatomas, thyroid adenomas. Pathological and histopathological changes in liver and spleen. Increased liver, kidney and thyroid weights. Repro-tox. Immuno-suppression	Kidney, thyroid gland, liver, spleen	Excreted mainly in faeces - unchanged and changed	-	In rats: pentachlorophenol, pentachlorothiophenol, tetrachlorohydroquinone, tetrachlorothiophenol, 2,4,5-trichlorophenol. In monkeys: pentachlorophenol, pentachlorobenzene	Other aromatic hydrocabons	JMPR 1978
Imazalil	2.5	Clinical signs, changed body and liver weight	0.03	Changed brain and liver weight. Embryotoxic and maternally toxic	Brain, liver	Rapidly absorbed and excreted - mainly in the urine	A) oxidative decarboxylation or b) oxidative dealkylation, oxidation, decarboxylation	-	Azole fungicides 7), 13)	JMPR 1980
Iprodione	6	Effects on liver, kidneys, adrenals, testes and accessory glands	0.06	Tumourigenicity in the liver and ovary. Non-neoplastic lesions in liver and testes. Interstitial cell tumours of the testes. Histopathological findings in liver, kidneys, adrenals, testes and accessory glands and uterus. Changes in organweight. Maternal- and embryofetal toxicity and tetratogenicity	Liver, ovary, testes, adrenal gland, uterus, kidney	Extensively absorbed from the gastrointestinal tract, metabolized, rapidly excreted - urine. At high doses excretion in faeces of parent compound	Dealkylation on the isopropyl carbamoyl chain, hydroxylation of the aromatic ring, rearrangement and opening of the hydantoin ring	The desisopropylated derivative. N-(3,5-dichloro-4-hydroxyphenylbiuret)	Dicarboximide 13)	JMPR 1992

Lindane, gamma-HCH, benzene hexachloride, BHC	0.5	Increased mortality, effects on liver	0,001	Effects on the liver. Reproductive and developmental tox. Functional effects and histological changes in the immune system. Increased kidney weight	Liver, kidney, immune system	Excreted in the urine. Accumulated in fat, kidney, muscle, brain.	Pentachlorocyclohexene -> isomeric trichlorobenzene -> isomeric trichlorophenols + sulphate + tetrachlorophenols	Pentachlorocyclohexene -> isomeric trichlorobenzene -> isomeric trichlorophenols + sulphate + tetrachlorophenols	Benzene hexachloride and lindane	JMPR 1989/1977
Malathion	29	Decreased survival, changes in haematological parameters, inhibit AChE (brain), changes in body and organ weights	0.3	Inhibit AChE (brain, erythrocyte, plasma). Liver adenomas. Increased liver, kidney and thyroid/parathyroid weights. Reprotox., developmental toxicity	Nervous system. Liver, kidney, thyroid/parathyroid	Rapidly absorbed, biotranformed, excreted mainly in the urine but also in the faeces	Hydrolysis, oxidation	Alfa and beta monocarboxylic acids and dicarboxylic acid of malathion	Dimethoxy compounds of category IV	JMPR 1997
Mecarbam	-	-	0,002	Inhibit AChE	Nervous system	Rapidly absorbed, metabolized and excreted in the urine	In rats: hydroxylation, oxidative desulfuration, degradation of the carbamoyl moiety	Phosphorothioate and a phosphorodithioate	Anticholinesterase carbamates and procarbamates	JMPR 1986
Mepiquat	-	-	0.15-0.3 (Tentative IFT-ADI). ADI = 1.5 (The e-Pesticide Manual, 11. Ed., 1999	Changes in blood chemistry. Decreased weights of heart, kidneys, liver, lungs and spleen in male rats. Maternal and developmental toxicity	Blood, heart, kidney, liver, lungs, spleen	Excreted in urine (48 %) and faeces (38%) - 90 % unchanged	-	-	Quaternairy nitrogen compounds	The e-Pesticide Manual, 1999
Metalaxyl	-	-	0.03	Decreased total leucocyte count. Significant increase in serum alkaline phosphatase levels. Increased adrenal and liver weight.	Adrenal, liver, blood	Rapidly metabolized and excreted in urine and faeces - females mainly via urine, males mainly via faeces	Methyl ester hydrolysis, N-dealkylation, methyl ether cleavage, benzylic methyl oxidation	Glucuronic acid conjugates	Phenylamide (Acylalanine type) 13)	JMPR 1982
Methidathion	0.16	Liver effects	0.001	Inhibit AChE (brain, erythrocyte). Hepatocellular tumours in	Nervous system. Liver	Extensively absorbed. Excretion via the urine and expired CO2	O-demethylation with desmonomethyl derivative	Sulfide, sulfoxide, sulfone and desmonomethyl derivative	Dimethoxy compounds of category IV	JMPR 1992

				males. Cholestasis, increased liver enzymatic activity in serum						
Methoxychlor	3500 ppm	Not carcinogenic	0,1	-	-	Absorbed, metabolised - detoxicated in the liver - metabolited excreted into the intestine an excreted in the faeces. Low accumulation.	Hydrolysis of the methyl ether leading to a polar phenol which is rapidly excreted	2-(p-hydroxyphenyl)-2-(p-methoxyphenyl-1,1,1-trichloroethane. 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane	DDT and its analoges	JMPR 1965/1977
Monocrotophos	0.006	Human: inhibit AChE (erythrocyte)	0.0006	Inhibit AChE (plasma, erythrocyte, brain). Embryo/fetotoxicity. Mutagenic	Nervous system	Readily absorbed and rapidly excreted in the urine - unchanged or as metabolites. Significant accumulation in the body	Hydrolysis, keto group reduction	3-hydroxy-N-methyl butyramide	Dimethoxy compounds of category IV	JMPR 1993
Orthophenylphenol	39	Carcinogenicity	0.4	Urinary bladder tumours in male rats. Carcinogenic in male mice (liver adenomas) and rats. JMPR concluded: not carcinogenic to humans. Maternal-, developmental- and fetotoxicity, teratogenic	Liver, urinary bladder	Rapidly and extensively absorbed (95%) and distributed. Excretion is also rapid - mainly in urine (90%) and in faeces (5%)	Conjugation of 2-phenylphenol or hydroxylation at the 5-position of the phenol ring, followed by conjugation with glucuronide or sulfate	Glucuronide conjugates of ortho-phenyl-phenol and 2,5-dihydroxybiphenyl	Phenoles = metabolites	JMPR 1999/1985
Parathion	0.4	Inhibition of acetyl cholinesterase (brain), retinal atrophy	0.004	Inhibit AChE (plasma, erythrocyte, brain). Retinal atrophy. Developmental, reproductive and perinatal toxicity	Nervous system.	Readily absorbed, excreted primarily in the urine	-	Paraoxon; diethyl phosphorothioic acid. Paraoxon -> diethyl phosphate, diethyl phosphorothioate, de-ethyl paraoxon, para-nitrophenol	Diethoxy compounds of category IV	JMPR 1995
Parathion-methyl	-	-	0.003	Inhibit AChE (plasma, erythrocyte, brain). Effects on the sciatic nerves Repro-tox. Mutagenic *in vitro*	Nervous system	Rapidly absorbed -> bloodstream. "Difference between its oral and intravenous toxicity are believed to be associated with first-pass effects in the liver". Rapidly	Conjugation with glutathione. Hydrolysis or O-demethylation (to para-nitrophenol)	Paraoxon-methyl (by liver microsomal oxidases), para-nitrophenol, dimethyl phosphate	Dimethoxy compounds of category IV	JMPR 1995

						excreted - urine				
Penta-chloroanisol	-	-	- not evalua-ted	Effects on the liver. Increased liver and kidney weights. Conges-tion + hemorrhage of the lungs, lymph nodes, thymus, adrenal cortex + meninges	Liver, kidney, lungs, thymus, lymph nodes, adrenal cortex, meninges	No data	No data	No data	Other aro-matic hydro-cabons	National Toxi-cology Pro-gram, website. Chemical Health and Safety Data, website
Pentachloro-benzene	-	Reproductive effects	0.0167	Histopathological lesions. Changes in kidney, liver and heart weights. Increased hyaline droplets in proxi-mal kidney tu-bules. Functional effects on thyroid. Teratogen	Liver, kidney, thyroid gland, heart	-	-	Pentachlorophenol, 2,3,4,5-tetrachlorophenol	Other aro-matic hydro-cabons	IRIS, website
Permethrin	5	Clinical signs, changes in body and ovary weights, blood chemistry	0.05	Clinical signs of neurotoxicity. Changes in ovary, testis and liver weight. Develop-mental toxicity	Nervous system, ovary, testis, liver	Rapidly absorbed, distributed and excreted. Metabo-lism was extensive	Ester cleavage, hydroxylation, oxidation and ultimately conju-gation	4-hydroxypermethrin, dichlorovinyl acid and phenoxybenzyl alcohol	Pyrethrum and related compounds	JMPR 1999
Phenthoate	-	-	0.003	Inhibition of cholinesterase activity (erythro-cyte)	Nervous system	Rapidly absorbed, distributed, and metabolites excreted (primarily urine)	Oxidation, hy-drolysis	Phenothoate acid, des-methyl phenthoate acid	Dimethoxy compounds of category IV	JMPR 1980
Phorate	0.05	-	0.0005	Inhibition of cholinesterase activity (erythro-cyte, brain), decreased body weight, clinical signs consistent with cholinergic toxicity	Nervous system	Rapidly absorbed and excreated (urine)	No accumulation of a toxic metabo-lite	Non-phosphorylated metabolites ((ethylsul-fonyl)methyl sulfoxide; ethyl(methylsulfonyl)meth yl sulfoxide; (ethylsul-fonyl)(methylsulfonyl)-metane; (ethyl-sulfinyl)(methylsulfinyl)-methane)	Diethoxy compounds of category IV	JMPR 1996

Phosalone	1.8	Inhibit AChE	0.02	Inhibit AChE (erythrocyte, plasma, brain) - after conversion to phosalone oxon. Maternal and fetotoxicity	Nervous system	Moderately well absorbed - excreted in the urine	Hydrolysis	Phosphorothioates, phosphorodithioates, 3-methyltrhiomethyl 6-chloro-benzoxalone, 3-methylsulfonylmethyl 6-chlorobenzoxazolone	Diethoxy compounds of category IV	JMPR 1993
Phosmet	1.3	Maternal toxicity	0,01	Cholinesterase inhibition (brain), effects on liver and kidney, reprotox., developmental- and fetotoxicity (skeletal anomalies). Mutagenic *in vitro*	Nervous system. Liver, kidney	Rapidly absorbed, distributed and excreted - mostly in the urine (as metabolites)	Hydrolysis	N-(methylsulfinyl-methyl)phthalamic acid; N-(methylsulfonylmethyl)-pthalamic acid	Dimethoxy compounds of category IV	JMPR 1994
Pirimicarb	-	-	0,02	Inhibit AChE (plasma, erythrocyte)	Nervous system	-	?	?	Anticholinesterase carbamates and procarbamates	JMPR 1982, 1981 and 1978
Pirimiphos-methyl	0.25	Human: inhibit AChE (erythrocyte)	0.03	Inhibit AChE (plasma, erythrocyte, brain). Feto-, embryotoxicity and teratogenicity. Mutagenic *in vitro*	Nervous system	Excreted in the urine and faeces	Cleaving of the P-O-C bond of pirimiphos-methyl, N-de-ethylaion and/or conjugation	2-ethylamino-4-hydroxy-6-methylpyrimidine	Dimethoxy compounds of category IV	JMPR 1992
Procymidone	-	-	0.2	Liver tumours. Testicular interstitial cell hyperplasia, interstitial cell tumours. Reprotox	Liver, testes	Rapidly excreted primary via the urine	Oxidized at the methyl group, hydrolyzed at the imide and amide linkages.	N-(3,5-dichlorophenyl)-1-carboxy-2-methyl cyclopropane-1,2-dicarboxyimide and the products yelded from hydrolysis of this at the imide	Dicarboximide 13)	JMPR 1989

Prothiofos	-	-	0.0001	Inhibit AChE. Non-systemic insecticide with contact and stomach action	Nervous system	Rapidly absorbed, metabolised and excreted	Oxidation, hydrolysis, conjugation+cleavage of the propyl group of prothiofos and prothiofos-exon: prothiofos-oxon -> 2,4-dichlorophenol -> 2,4-dichlorophenylethyl hydrogen phosphorothioate + 2,4-dichlorophenylethyl hydrogen phosphate	Prothiofos-oxon -> 2,4-dichlorophenol -> 2,4-dichlorophenylethyl hydrogen phosphorothioate + 2,4-dichlorophenylethyl hydrogen phosphate	Mixed substitute Compounds of category IV	The e-Pesticide Manual, 1999
Pyrazophos	0.4	Increased body and thymus weight, inhibit AChE (erythrocyte), effects on kidney and blood	0.004	Inhibit AChE (erythrocyte, brain). Histopathological abnormalities in the kidneys. Increased thymus weight, increased lymphocyte counts. Hemangiomas in mesenteric lymph nodes in males. Maternal and embryofetal toxicity	Nervous system. Kidney, thymus, blood	Absorbed, excreted - urine (71-78 %) (as its hydrolysis products), faeces (16-24 %) (mainly the parent compound)	Hydrolysis	2-hydroxy-5-methyl-pyrazolo(1,5-a)pyrimidine-6-carboxylic acid	Diethoxy compounds of category IV	JMPR 1992
Quinalphos	NOEL = 0.15-0.3	Inhibit AChE	0.0015 (IFT-ADI)	Inhibit AChE (plasma). Fetotoxic, maternal toxicity. Oncogenicity	Nervous system	Rapidly absorbed, excreted - urine (83 %) and bile (17%)	-	2-hydroxyquinoxaline and its conjugates	Diethoxy compounds of category IV	The e-Pesticide Manual, 1999
Quintozene	1	Adenomas. Reduced body and adult body weight	0.01	Follicular adenomas of the thyroid and hepatocellular adenomas. Embryo-, feto- and developmental toxicity	Thyroid gland, liver	Slowly absorbed, excreted in faeces and urine. Bile levels in ruminants were high, enterohepatic circulation is probable	A) reduction of the nitrogroup to an amino group. Or b) replacement of the nitro group with af sulfur-containing group	Pentachloroaniline. Methylpentachlorophenyl sulfide. N-acetyl-S-(pentachlorophenyl)cysteine	Anilino and nitrobenzenoid fungicides	JMPR 1995

Technazen	15	Repro. Tox. Effects on body and liver weights	0,02	Effects on liver and kidney. Maternal toxicity, embryo- and fetotoxicity on basis of minor skeletal defects.	Liver, kidney	Excreted in the urine and faeces	Via the tetra-chlorobenzene glutathione conju-gate pathway	Tetrachloro-phenyl-mercapturate conjugate, tetrachloroaniline, tetra-chlorothioanisole	Anilino and nitrobenze-noid fungi-cides	JMPR 1994
Tetradifon	2.5-15	-	0.03 (Tenta-tive IFT-ADI)	-	Kidneys, liver	70 % was excreted via the bile in the faeces	No data	No data	Syntetic acaricides	The e-Pesticide Manual, 1999
Tetrasul	-	-	0.03 (Tenta-tive IFT-ADI)	-	-	No data	No data	No data	Syntetic acaricides	The e-Pesticide Manual, 1999
Thiabendazole	-	-	0.1	Haemosiderosis of the thymus and colloid depletion in the thyroid. Haemosiderosis in spleen, liver, lymph noedes + bonemarrow. Increased liver and kidney weight	Thymus, thyroid gland, spleen, liver, lymph noedes, bonemar-row, kidney	Rapidly absorbed from the gastrointes-tinal tract.	Hydroxylation	5-hydroxythiabendazole, - free and conjugated as a glucuronide and sulfite ester	Benzimida-zole 13)	JMPR 1970
Thiometon	-	-	0.003	Inhibit AChE. Marginal repro-tox effect. Mutagenic *in vitro**	Nervous system	Rapidly absorbed, distributed and excreted - mostly in the urine (un-changed)	O-demethylation to O,O-dimethylphos-phoric acid - excretion in expired air. No unchanged thiometon found in the urine.	O,O-dimethylphosphoric acid	Dimethoxy compounds of category IV	JMPR 1979. *Moriya et al., 1983
Tolclofos-methyl	6.5	Inhibit AChE (brain), increased kidney weight	0,07	Inhibit AChE (brain). Effects on kidney and liver	Nervous system. Liver, kidney	Rapidly excreted - mostly in the urine. <1% retained in tissues	Oxidation of P=S to P=O, oxidation of the 4-methyl group, cleavage of the P-O-aryl and P-O-methyl linkages	2,6-dichloro-4-methylphenol; O,O-dimethyl-O-(2,6-dichloro-4-carboxylphe-nol)phosphate; O-methyl-O-hydrogen-O-(2,6-dichloro-4-carboxyphenyl)phosphate; 3,5-dichloro-4-hydroxyboenzoic acid; 3,5-O-dichloro-4-hydroxybenzyl glycine	Dimethoxy compounds of category IV	JMPR 1994

Tolylfluanid	-	-	0.1	Maternal and embryotoxicity. Mutagenic *in vitro*	-	Rapidly absorbed, metabolized, elimi-nated in urine and faeces. Accumula-tion in thyroid gland	-	Thiazolidine-2-thione-4-carboxylic acid. 4-dimethylaminosulphonyl-aminobenzoic acid	Chloroalkyl thio fungi-cides	JMPR 1988
Triadimefon	-	-	0.03	Effects on liver and blood. Repro-tox.	Liver, blood	Rapidly and readily absorbed from the gastrointestinal tract. Excreted in urine and faeces	Hydroxylation, oxidation	Triadimenol acid	Azole fungicides 7), 13)	JMPR 1981
Triadimenol	-	-	0.05	Hepatotoxicity, increased liver weight. Reproduc-tive-, embryo-fetotoxicity	Liver	Rapidly absorbed and excreted - in the urine	Hydroxylation of the tert-butyl moiety, oxidation to carboxylic acid	-	Azole fungicides 7), 13)	JMPR 1989
Vinclozolin	1.4	Antiandrogenic-ity. Hepatoxicity	0.01	Hepatotoxicity. Hepatocellular carcinomas. Antiandrogenic-ity. Adrenal tumours. Pathological changes in the liver, spleen, prostate, testis and adrenals. Terato-genicity – changes in anogenital distance. Fetotox-icity	Liver, spleen, prostate, testis, adrenals	Well absorbed, extensively metabo-lized. Excreted in urine and faeces	Cleavage of the 2,3-bond in the oxazolidine ring, dihydorxylation of the vinyl group	N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutanoic acid amide	Dicarbox-imide 13)	JMPR 1995