Pesticides in food: assessing the risk to children

Gezondheidsraad
Health Council of the Netherlands

To the Minister of Health, Welfare and Sport

Subject : Report on the vulnerability of children to pesticides
Your reference : GZB/VVB-993063
Our reference : -2235/HvD/ts/638-D
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Dear Minister,

At the request of your ministerial predecessor and the former State Secretary of Agriculture, Nature Management and Fisheries, as expressed in letter reference GZB/VVB-993063, I am pleased to enclose a report on the vulnerability of children to pesticides. The report has been prepared by a specially convened committee of the Health Council and reviewed by both the Standing Committee on Health and Environment and the Standing Committee on Food. As requested, I am also sending a copy of the report to the Minister of Agriculture, Nature and Food Quality.

In its report, the Committee states that there is not presently any evidence that children's health is being adversely affected by the presence of pesticide traces in their food. Nevertheless, the Committee does believe that the pesticide approval procedures in international use leave something to be desired. Through various channels, numerous initiatives are being taken with a view to improving the situation, and the Committee would like to see the Netherlands give its active support to these moves. I endorse the Committee's recommendations and share the view of the Standing Committee on Food that there is no reason to modify existing government advice on the consumption of fruit and vegetables by children.

Yours sincerely,

(signed)

Professor JGAJ Hautvast
Vice-President

P.O. Box 16052
NL-2500 BB The Hague
Telephone +31 (70) 340 7451
Telefax +31 (70) 340 75 23
E-mail: hfg.van.dijk@gr.nl

Visiting Address
Parnassusplein 5
NL-2511 VX The Hague
The Netherlands
www.gr.nl

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to:

the Minister of Health, Welfare and Sport

the Minister of Agriculture, Nature and Food Quality

No. 2004/11E, The Hague, June 7, 2004

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature & Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Executive summary

Background to this advisory report

Pesticides are widely-used chemical preparations: they protect agricultural crops against disease and infestation, they remove weeds from pavements, and they combat vermin in and around homes. These products may also be harmful to other organisms, including humans. That is why they are only allowed onto the market after extensive safety assessment. In the Netherlands, the Board for the Authorisation of Pesticides (CTB) is responsible for making that assessment. This agency assesses issues such as whether sprayed agricultural produce is safe for consumers. To do so, it uses a scientific, internationally accepted method. In 1996, the United States revised several aspects of the usual approach, with a view to providing better protection for children. In Europe, the authorities have also started to reappraise their approval procedures for pesticides. As a precautionary step, standards for pesticides in baby food and baby formula have been tightened-up. In the Netherlands, and indeed throughout the world, the issue has given rise to an ongoing debate about whether the usual approval procedure would actually cause children to be harmed by sprayed food.

Against this background, the Minister of Health, Welfare and Sport, also on behalf of the former State Secretary of Agriculture, Nature Management and Fisheries submitted the following questions to the Health Council:

- Are children more vulnerable to xenobiotic compounds in general and to pesticides in particular?

- What does this mean for the derivation of safe levels for chronic and short (generally a single) exposure to these substances?
- How can the risk assessment take into account the fact that levels of child exposure to pesticides are different from average exposure levels because children have different patterns of food consumption?

The Committee that drew up the advisory report has taken into consideration all the phases of childhood, from embryo to adolescent. It first provides a description of how exposure and response to pesticides varies between children and adults. It then discusses the current risk assessment procedure for these substances. Finally, it deals with the question of whether that procedure is sufficiently well tailored to children. The advisory report concludes with a series of recommendations.

Exposure of children to pesticides

The food consumption patterns of children are different from those of adults. The younger the child, the greater the difference. After birth, children are breast-fed or bottle-fed. After four to six months, weaning starts and, from about ten months onwards, children gradually start eating the same as the rest of the family. Per kilogram of body weight, children aged from one to six consume greater amounts of certain foods than adults. The foods in question are milk and dairy products, fruit juices and cordials, fruit and vegetables, cereal products, sweets, and sweet sandwich fillings. Therefore, per kilogram of body weight, they are more exposed than adults to the pesticides in these products.

Young children crawl a lot. They also often put their hands and all sorts of objects in their mouths. This behaviour can similarly result in higher exposure through the oral pathway. Per kilogram of body weight, children also have more body surface area and breathe in more air than adults. This results, relatively speaking, in a higher exposure to substances via the skin and the respiratory system.

Processing of substances by the body of a child (toxicokinetics)

Children are physiologically different from adults. The younger the child, the greater the difference. This affects the uptake of substances in the body, the distribution through tissue, metabolisation by enzymes and excretion from the body.

Before birth, children are protected from the outside world by their mother's bodies. However, many xenobiotic compounds can pass through the barriers between the mother and the child. After birth, the gastrointestinal tract constitutes an important uptake route. However, at birth the gastrointestinal tract has not yet reached full matu-

rity. As a result, neonates absorb some substances better than adults, and actually absorb others less well.

The way substances spread through the body also depends on age. In particular, substances which dissolve well in water spread to a relatively larger volume in babies' bodies than in adults. From the circulatory system, they also penetrate more easily into tissues and organs, including the brain.

In foetuses and in neonates, the ability to metabolise chemical substances with enzymes is still limited. As a result, the detoxification and excretion of poisonous substances take place slowly. On the other hand, the lower level of enzyme activity provides protection against substances that are metabolised by enzymes into more harmful substances. In addition, the kidneys and the liver do not yet function at adult levels in neonates, so excretion is slower.

All the toxicokinetic differences between children and adults referred to here largely disappear during the first year of life. Older children may even surpass adults in terms of their ability to metabolise substances with enzymes and then excrete them. This is because children have a higher metabolic rate.

Effect of substances on the body of a child (toxicodynamics)

While chemical substances may produce the same types of effects in adults and children, these can occur at different exposure levels. In addition, substances can result in effects that are unique to children, and that are associated with adverse effects on the development of organs or organ systems. During development, there are periods in which organs can be particularly vulnerable to the influence of chemical substances. These periods are known as windows of vulnerability. In recent years, there has been a growing awareness that disturbances of the maturation of organs may ultimately lead to impaired functioning, even if the organs are apparently healthy. These functional shortcomings sometimes only emerge in adulthood.

Animal trials have shown that some pesticides can affect the development of organs and organ systems. Young laboratory animals can suffer damage to the brain, reproductive organs and immune systems after exposure to doses of pesticides that do not induce any perceptible harm in adult animals. In adult animals, low doses usually only produce effects after long-term exposure. However, in young animals, short or even a single exposure can be enough to produce an effect. In so far as the same effects have been studied and found in both adult and young animals, they are often more severe and longer-lasting in the latter.

There have been no studies to determine whether low levels of exposure to pesticides through food can have similar effects on human development. More is known about the effect of certain pollutants (such as lead, dioxins, and PCBs), certain medi-

cines (DES, thalidomide) and natural stimulants (alcohol). These can adversely affect the development of children, particularly unborn children, at levels of exposure that are not harmful to adults.

The current appraisal procedure for food safety

The Board for the Authorisation of Pesticides (CTB) assesses the risks of long-term exposure to pesticides through food. To do so, it determines the amount of the pesticide in question that consumers can ingest each day, throughout their lifetime, without running an appreciable risk. This Acceptable Daily Intake (ADI) is expressed as milligrams per kilogram of body weight per day. The assessment is based on data from animal trials, which the manufacturer is required to provide. The CTB uses this data to derive the highest exposure level at which no harmful effects are found in laboratory animals. The ADI is then calculated by applying uncertainty factors that compensate for the differences between animals and humans, and for differences between people. Current thinking is that, in the case of substances that can damage genetic material and thereby cause cancer, it is impossible to determine an exposure level at which no effects occur. Substances of this kind do not qualify for approval.

The CTB also makes an initial, rough estimate of the amount of pesticide that consumers might ingest with their food. For safety's sake, the worst-case scenario is assumed here. In other words, high levels of consumption and high levels of pesticides. Consumption by children aged from one to six is dealt with separately.

The CTB then compares the estimated level of exposure to the level that is considered safe. If exposure, both in the general population and among children aged between one and six, remains below the ADI, the CTB will consider the pesticide to be acceptable. If the calculated exposure is higher, the CTB will conduct a more refined calculation based more on the actual circumstances, in which it takes into account, for example, the consequences for exposure of how food is prepared (washing, peeling, cooking). If the ADI is still exceeded, approval will only be possible after changes to the pesticide's instructions for use, leading to a reduction of exposure to below the permissible standard.

For a number of years now, it has been clear that the level of pesticides in individual products – for example, a single apple – is sometimes higher than was assumed on the basis of analyses of pooled samples. This is particularly important in the case of pesticides with a high acute toxicity. In order to protect consumers against peak exposures, the Acute Reference Dose (ARfD) is determined for these substances as well as the ADI. This is the amount of a particular pesticide that consumers can ingest during a single meal or a single day without appreciable risk. Here also, the CTB makes a separate estimate of the exposure for children aged between one and six, in addition to intake by

the general population. If this initial worst-case calculation indicates that the ARfD may be exceeded, a more refined, more practice-oriented estimate will follow. If the ARfD is still being exceeded, adjustments will be required to the instructions for using the pesticide.

Conclusions about tailoring the current safety assessment procedure to children

The Committee of the Health Council that drew up this advisory report concludes that children can be both more and less vulnerable to substances than adults. Vulnerability can vary according to the substance and depends on the stage of development of the child in question. Current toxicological research therefore includes all developmental stages of organisms. However, this research is neither sufficiently profound nor broad enough in terms of design to identify every single effect on the main organs and organ systems in developing organisms. In particular, effects on the development of the nervous system and the immune system may remain unnoticed. This may also apply to effects resulting from endocrine disruption. Changes which are most likely to remain unnoticed are those resulting from exposure during development that only emerge in later life.

Information about the influence of pesticides on the development of the nervous system, the immune system and endocrine-regulated processes is important for the derivation of safe levels for both short and chronic exposure to pesticides. It is unclear to what extent the lack of this relevant data about development toxicity is offset by the traditional uncertainty factor for variations in vulnerability between individuals. This means that is also unclear whether the current calculations of safe intake levels always provide adequate protection for children, including unborn children, in periods of heightened vulnerability during the course of their development. In some cases, protection of the foetus may require stricter standards for acute exposure (ARfDs) than are required for the protection of other age groups. The question of whether one wishes to derive separate, less stringent standards for the latter is a policy issue. The Committee finds that the current risk assessment procedure correctly takes into account the specific consumption patterns of children aged between one and six.

At present, there are no concrete indications of genuine adverse effects on children's development caused by the presence of pesticide traces in food. However, this matter requires further research. Furthermore, effects involving aspects such as behaviour, learning ability, motor skills, immunity or fertility are difficult to identify. Research has shown that some substances can have an adverse effect on the development of children. Examples are PCBs, dioxins and lead.

Recommendations of the Committee

The number of toxicological studies that manufacturers are required to conduct as standard need not be increased. Nevertheless, improvements are required to existing research protocols. In particular, studies of reproduction toxicity involving several generations of laboratory animals should be designed on broader lines, to allow for the identification of any effects on the development of the nervous system, immune system and endocrine-regulated processes of development. If this standard research yields an indication that there may be an adverse effect on the development of organisms, there should be follow-up research into the specific problem. In effect, this is already a requirement, but there are still no validated research methods available for a range of follow-up studies. There are international initiatives in progress with a view to drawing up research protocols. The committee recommends Dutch support for these activities.

As long as the current research procedures do not achieve full success in determining possible harm to development, data about the toxicity of pesticides is, in effect, incomplete. The Committee believes that the best way to tackle this problem is to have experts assess each pesticide individually. If, on the basis of all available toxicological data and in the absence of adequate research or follow-up research, there is reasonable cause for supposing that developing organisms are more vulnerable than adult organisms, the Committee believes that an additional uncertainty factor, in addition to the factors traditionally used, is appropriate when calculating the ARfD and the ADI. In many cases, it will not be feasible to supply scientific justification for the size of any additional uncertainty factor. Where this is the case, a default value of 10 could be chosen. This value is traditionally used to account for the absence of key data. Alternatively, values 3 or 10 can be chosen. These are used in the United States. As soon as the missing data becomes available, it will be necessary to determine whether it justifies a change to the ADI or ARfD. International collaboration is indispensable here, in order to prevent different countries setting different ADIs and ARfDs.

The United States is currently reassessing all pesticides in terms of their safety for children. In the case of many pesticides, the additional uncertainty factor assigned beforehand to all pesticides for the protection of children was removed after completion of this reassessment. However, the additional factor was retained for a few pesticides. In the Netherlands and in Europe as a whole, priority should be given to a reassessment of these substances to determine their harmfulness to developing organisms. The same applies to pesticides where there is a small margin between the calculated or measured exposure and the level of exposure considered safe.

Human food consumption patterns change over the course of time. This is also true of children. The Committee therefore considers it to be very important for future food

consumption surveys to obtain information about children. It recommends including consumption by children aged between six months and one year in the surveys and, where necessary, including them in the risk assessment.

The possibility of simultaneous exposure to several pesticides with a common mechanism of toxicity and of simultaneous exposure to the same compounds from various sources (food, water, domestic uses) merits systematic attention in the risk assessment of individual pesticides. However, appropriate methods are still being developed. The Committee advocates the use of Dutch expertise as support for the international activities in this field.

Pesticides policy, including the way in which the risks associated with the substances are assessed, is increasingly determined by the European Union. The Committee therefore urges the Dutch government to put forward its recommendations for discussion within the European Union (including the European Food Safety Authority), where decisions can be taken at the central level.

Chapter 1

Introduction

1.1 Background

Pesticides are widely available chemical preparations used to protect agricultural crops against disease and infestation, to remove weeds from pavements and to combat vermin in and around the home, in storage areas and in places where animals are kept. These products can be harmful not only to their 'target' organisms, but also to other organisms, including humans. Consequently, the marketing and use of pesticides is subject to legal controls in most countries. In the Netherlands, regulation is provided through the Pesticides Act (BMW62). Under this act, a pesticide is allowed onto the market only if, when used in accordance with the manufacturer's instructions, it has its intended effect and poses no hazard to human health or the environment. The government agency that assesses pesticides to determine whether these conditions are met is the Board for the Authorisation of Pesticides (CTB). The Board bases its assessment on a comprehensive scientific dossier that the pesticide manufacturer has to submit when applying for product approval.

When considering whether a pesticide is a hazard to human health, one of the matters that the CTB looks at is the potential risk to consumers arising out of the presence in harvested agricultural produce of small amounts of pesticide left over from treatment of the growing crop. The Board accordingly makes an estimate of the health risk associated with the presence of such pesticide residues, and authorises the product only if the risk is judged to be negligible. To this end, the CTB follows internationally accepted procedures.

In the 1980s, reservations were expressed in the United States regarding the effectiveness of the established methodology as a means of protecting infants and children against trace pesticides. In 1993, a report was published entitled *Pesticides in the diets of infants and children* (NRC93). In this report, a committee of the US National Research Council (NRC) recommended revision of the safety assessment procedures in certain respects. It was argued, for example, that use should be made of toxicological data relevant specifically to infants and children and that the exposure of these population groups should be more accurately estimated. Furthermore, it was recommended that the calculation of safe levels of exposure should allow for the fact that, during development, unborn babies, infants and children are particularly vulnerable to such substances. In 1996, the US government implemented these recommendations in the Food Quality Protection Act (see, for example, Lan04, Suh00a), and the Environmental Protection Agency (EPA), the body responsible for pesticide approval in the USA, began revising its procedures accordingly. Since then, interest in the influence of pesticides on children's health has grown rapidly (Kim01a); much has been written on the subject, both in academic journals and in the lay press. It has become clear that major differences of opinion exist regarding the adequacy of the original assessment procedure and the need for modification (compare, for example, Sch00c and Jub03).

In Europe, the authorities have also been prompted to re-examine their pesticide approval procedures. Within the EU, this has led to strict detection-threshold-based limits on pesticide concentrations in food products intended specifically for infants (EU98, EU99). However, no extra safety margin is currently applied to allow for the vulnerability of children (JMPR00a). The issue has also been debated in the Netherlands. The Consumers' Association and the Netherlands Society for Nature and Environment have expressed fears that thousands of Dutch children might be suffering impaired health – particularly damage to the nervous system – as a result of consuming produce that has been sprayed with pesticide (Luij00). However, other bodies take the view that the risks to children are negligible (Han01, Voe01) and fear that consumers may be unnecessarily deterred from eating sufficient fresh fruit and vegetables.

1.2 Request for advice

In view of the developments in the United States and the debate taking place in the Netherlands, the Minister of Health, Welfare and Sport – acting also on behalf of the former State Secretary of Agriculture, Nature Management and Fisheries – asked the Health Council to prepare an advisory report. The ministers wished to hear the Council's views regarding the possible heightened sensitivity of children to xenobiotic substances in general and pesticides in particular. They additionally inquired about the significance that any heightened sensitivity might have for the calculation of safe levels of long-term

and short-term (typically single-incident) consumption of such substances. The Council was also asked to suggest how the risk assessment procedure might make allowance for deviating patterns of exposure, such as those associated with the specific eating habits of children. The full text of the Minister's letter is reproduced in Annex A.

1.3 The Committee and its working methods

On 19 June 2002, the Vice-President of the Health Council formed a committee of experts to address the Minister's questions. The membership of the Committee is given in Annex B.

The use of pesticides needs to be safe for all consumers, irrespective of age. Indeed, unborn children and even individual reproductive cells can be exposed to pesticides. Any changes resulting from such exposure can have permanent health implications. Hence, the Committee has interpreted the term 'child' as embracing the antenatal phases of development (fertilised ovum, embryo and foetus), as well as the postnatal phases (infant from birth to one year old; toddler one to four years old; small child four to six years old; older child six to twelve years old; adolescent twelve to eighteen years old). Where the Committee uses the word 'children' without qualification, it is intended to refer to individuals in all antenatal and postnatal development phases.

As requested by the minister, the Committee has focused primarily on pesticides. However, pesticides constitute a very diverse group of substances. What they have in common is that they are all used to control unwanted organisms and that they are all covered by the legal framework of the Pesticides Act. They nevertheless differ from one another greatly in terms of their chemical structure and mechanism of action. Hence, the Committee believes that its conclusions and recommendations are applicable not only to pesticides, but in principle also to chemicals in general. Conversely, the Committee has felt justified in making use of information concerning other types of substance, such as medicines, heavy metals, dioxins and PCBs, where appropriate data could not be found regarding the fate of pesticides in the body and or regarding their effects on the body during the various phases of development. The Committee believes that conclusions based upon such 'alternative' information are in principle valid in relation to pesticides as well.

Finally, the Committee has considered whether the present pesticide approval procedure takes sufficient account of the observed differences between children and adults and therefore affords adequate protection to children.

1.4 Structure of this report

In chapters 2, 3 and 4, the Committee sets out the reported differences between children and adults, both in terms of exposure to pesticides (chapter 2) and in terms of sensitivity to such substances. Differences in sensitivity arise out of differences in the way substances are dealt with by the body (chapter 3) and the way substances affect organs and organ systems (chapter 4). Chapter 5 describes the present procedure for assessing the risks to consumers posed by pesticides in food. In the subsequent chapters, the Committee assesses the adequacy of the present procedure as a means of protecting children against pesticides. Hence, consideration is given to toxicological research (chapter 6), the use of uncertainty factors (chapter 7) and the estimation of exposure (chapter 8). Finally, a series of conclusions and recommendations are presented in chapter 9.

Chapter 2

Differences between children and adults: exposure patterns

The treatment of growing crops to prevent disease and infestation can result in traces of pesticide remaining in the harvested produce. These traces are generally referred to as residues. The gastrointestinal tract is by far the most important route by which people can be exposed to such residues; the levels of exposure via the skin and airways are barely significant. The latter two routes can, however, play a more important role in the context of exposure to pesticides used for non-agricultural purposes, such as domestic pest and weed control. Furthermore, the same pesticides are often used for both agricultural and domestic purposes. The following discussion of how exposure patterns in children differ from those seen in adults accordingly takes all exposure routes into consideration.

2.1 Exposure via the oral pathway

The extent to which consumers are exposed to pesticide residues depends on what and how much they eat and drink, and on the residue concentrations in their food and drink. Individual food consumption patterns differ widely, partly as a result of age-related differences in needs and preferences. In the first months of life, infants are given only human milk or infant formula. From four to six months old, milk intake is supplemented with small amounts of solids, with a gradual transition to ‘family’ food from about ten months. The Dutch National Food Consumption Surveys carried out in 1987/1988, 1992 and 1997/1998 provide a picture of eating patterns in the Dutch population above the age of one, but exclude infants less than twelve months old (Voe98). However, the eat-

ing habits of children aged between nine and eighteen months have recently been studied (Voe02). In absolute terms, small children were found to consume less food and drink of almost all types than adults do. This was not the case, however, with fruit or with milk and milk products, the largest quantities of which were consumed by children of nine months. Of the total amount of fruit consumed by children of nine months, 41 per cent was found to be in the form of potted baby food and 56 per cent in the form of fresh fruit. By the age of eighteen months, fresh fruit accounted for 84 per cent of the total (Voe02). Follow-on formula represented 80 per cent of the milk and milk products consumed by nine-month-olds, but it represented just 7 per cent of such foodstuffs consumed by eighteen-month-olds. The opposite trend was seen in the consumption of full-cream and particularly semi-skimmed milk (Voe02).

When food intake was expressed in terms of consumption per kilogram bodyweight, a very different picture emerged. The study found that, in relative terms, small children in particular ingest more of almost all kinds of food and drink than adults (see Table 1). The discrepancy was most apparent where fruit and vegetables, milk and milk products, fruit juices and cordials, cereal products and confectionery and sweet spreads were concerned. Similar patterns have been observed by researchers in other countries (Lar98, Law98, NRC93, Øst98, Plu92, Sch98).

Table 1 Average consumption of foodstuffs of various types (in grams per day per kilogram bodyweight) by individuals of different ages. The data on individuals aged nine, twelve and eighteen months is taken from the VIO[a] and relates to both boys and girls (Voe02); the data on individuals of other ages is taken from the most recent Dutch National Food Consumption Survey and relates to males only (Voe98). The figures on female food consumption follow a similar pattern.

age	9 months	12 months	18 months	1-4 yrs	4-7 yrs	7-10 yrs	10-13 yrs	13-16 yrs	22-50 yrs
bodyweight (kg)	9.3	10.3	11.9	14.1	20.1	29.3	39.7	55.8	81.8
type of food									
potatoes	2	2	3	4	4	3	3	3	2
bread	3	4	4	5	5	4	4	3	2
non-alc. drinks	15	21	33	31	23	21	18	18	17
fruit	18	16	13	7	4	3	2	2	1
cake, biscuits	1	1	2	2	2	1	1	1	0
cereal products	2	2	2	3	1	1	1	1	1
vegetables	3	3	3	4	3	3	2	2	2
milk, milk products	69	61	47	38	27	17	13	8	4
ready meals	11	9	5	2	0	0	0	0	1
sugar, confectionery, sweet spreads	0	1	1	2	2	2	1	1	1
meat, meat products, etc.	1	2	3	4	3	3	2	2	2

[a] The Nutrients Intake Study (VIO), carried out in 2002 by TNO Nutrition and Food Research for Nutricia BV Nederland (see Voe02).

The most recent Dutch National Food Consumption Survey also provided a little insight into the eating habits of pregnant women. It was found that food consumption during pregnancy did not differ significantly from the norm for women aged twenty-two to fifty, except in that pregnant women consumed a lot less alcohol (Voe98). However, only fifty pregnant women took part in the survey, so it would not be appropriate to draw any general conclusions from the findings.

Because children's diets differ from adults', corresponding differences exist in their exposure to pesticide residues present in food. It has been known for several decades that breastfed infants can be exposed to environmental pollutants through their mothers' milk. The research that has been done in this field has focused mainly on organochlorine compounds, such as dioxins, PCBs and old, chlorine-based insecticides, such as DDT (LaK01). Much less data is available on the presence of modern pesticides in human milk. A British study that addressed this question found no organophosphates in human milk (WPPR98), but a subsequent study in India did detect such substances (San03). In France, atrazine has recently been found in human milk (Bal03). Only minimal traces of pesticide are now permitted in infant formula, or in potted baby food and the like sold in the European Union. As a precautionary measure, the concentration of each pesticide is limited to 0.01 mg/kg, which is more or less the lowest detectible concentration (EU98, EU99). Children experience higher levels of exposure to pesticide residues in other types of food and drink, because of their greater consumption per kilogram bodyweight. Finally, small children in particular are apt to put their dirty fingers and any objects they may come across in their mouths (Fre01, Ste01). This tendency, and the ingestion of soil, can also result in children experiencing disproportionate levels of exposure to toxic substances (Gur98). Consistent with this heightened exposure, American researchers recently found elevated concentrations of dialkyl phosphates – metabolites of organophosphate insecticides – in the urine of children (Bar04).

2.2 Exposure via the skin and the respiratory system

For a given level of effort, children consume more energy per kilogram bodyweight than adults. As a result, children breathe in more air per kilo bodyweight per minute. Since at the age of ten or so children are much more active than infants, the former have a greater total intake of air per kilo bodyweight per day than the latter (Plu92). Consequently, children are also exposed to greater quantities of airborne toxins per kilogram bodyweight.

Children have a greater skin area per kilogram bodyweight than adults. In a newborn infant, the ratio averages roughly 0.061 m^2/kg. As the child develops, the figure gradually falls to an average of 0.027 m^2/kg on reaching adulthood (Ren98a, see also EPA97). This means that, in a situation where the entire body is exposed to a substance

(e.g. due to swimming in polluted water), a child will experience a higher level of exposure relative to its bodyweight (Plu92). In addition, children's behaviour patterns (crawling, going barefoot, etc) are liable to result in greater exposure via the skin (Gur98, Fre04).

2.3 Conclusions

Relative to their bodyweight, children eat and drink more than adults, breathe in more air and have a larger skin area. These characteristics and certain behaviour patterns associated with childhood, such as crawling and putting fingers and foreign objects in the mouth, mean that children are often exposed to larger amounts of pesticide per kilogram bodyweight than adults.

Chapter 3

Physiological differences between children and adults and their influence on the toxicokinetics of substances

The sensitivity of an organism to a toxic substance is determined by the toxicokinetics and the toxicodynamics of the substance in question. The term 'toxicokinetics' covers all the processes that dictate the fate of a substance in the body: the substance's absorption by the body, its transportation and distribution within the body, its binding to other substances, its conversion by enzymes and its excretion. These processes determine how much of the dose to which the organism is exposed is ultimately capable of causing harm. 'Toxicodynamics' is a term used to describe the process of interaction between a substance and structures and processes in the body, and the biochemical and physiological consequences of such interaction. In this chapter, the Committee describes the physiological differences between children and adults and how such differences influence the toxicokinetics of substances. In doing so, the Committee draws mainly on a number of review articles that have appeared in the academic press (Plu92, Ren98a, Sch02, Sno92) and a comprehensive recent report published by RIVM (Zwa02). The most important findings, which are based primarily on pharmaceutical research, are summarised in Table 2.

3.1 The absorption of substances by the body

An unborn child obtains all its nutrients through its mother's body and can be exposed to toxic substances via the same route. In the early stages of an embryo's development, the yolk sac acts as a barrier between mother and child, a role that is later taken over by the placenta. However, these barriers are effective only against very large or highly charged

molecules (Cre00, Øst98, Pet98). Most substances present in a pregnant woman's blood consequently reach a similar concentration in the blood of her unborn child (Cre00, Sch02, Why03). Evidence for the ability of pesticides to reach the foetus is provided by the presence of these substances or their metabolites in the amniotic fluid during pregnancy (Bra03), as well as in umbilical blood (Why03) and the meconium (Ost02, Why01) immediately after birth.

Following birth, major changes occur in the transport of substances. The gastrointestinal tract, the respiratory tract and the skin become the media through which substances can enter the body. Where chemical compounds in food are concerned, the oral route is of particular importance. The pH of a newborn baby's gastric juices is high compared with that of an adult. This has implications for substances_f ionisation levels. Digestive enzymes, such as pepsin, trypsin and α-amylase are relatively inactive in neonates. In the first months of life, gastric emptying is irregular and the intestines' peristaltic contractions are slow and irregular. The immature intestinal wall is more permeable to large molecules, including proteins, and to metals. Following birth, the previously sterile gastrointestinal tract is quickly colonised by bacteria, resulting in gradual shifts in the intestinal flora. The way these factors influence absorption through the gastrointestinal tract differs depending on the compound concerned; one substance will more readily be absorbed by a neonate than an adult, but the reverse will be the case with another substance. From the age of about twelve months, however, a child's gastrointestinal tract works in much the same way as an adult's.

The skin and the airways are not very important routes for the absorption of pesticides present in food. They are nevertheless relevant in the context of overall exposure. The epidermis forms an effective barrier against fluid loss, infections by invasive microorganisms and, in most cases, against the effects of toxic substances. Being provided by the outermost layer of the epidermis, the stratum corneum, whose development does not start until about the twenty-fourth week of pregnancy and takes roughly ten weeks, such protection is not available to an unborn foetus (Car00, Eva86). A neonate born at the end of a full-term pregnancy has a fully functional epidermis that affords adequate protection (Ath86).

Little is known about any differences that may exist between children and adults in terms of the speed at which substances are absorbed by the body via the lungs. However, fat-insoluble substances appear to be absorbed via the airways more quickly in young rats between three and twelve days old than in adult rats (Hem78a,b).

Table 2 Toxicokinetically significant physiological differences between children and adults (based on Ren98a, Sch02, Zwa02).

Absorption	
Skin area/Weight	Ratio between skin area and bodyweight is roughly 2.3 greater in a neonate than in an adult
Stomach pH	6-8 at birth, <4 within 2-4 days, then falls gradually to adult value, i.e. 1.5
Digestive enzymes	Activity limited at birth, then quickly increases in first year of life
Gastric emptying	Variable and irregular for 6-8 months after birth
Intestinal peristalsis	Slow and irregular in infants
Intestinal flora	Rapid colonisation following birth, changes gradually
Skin	Thinner and more permeable in seriously premature infants due to incomplete development of stratum corneum, which is formed in the 2 to 3 weeks after birth; very little difference between full-term neonate and adult
Lungs	Relative to bodyweight, children have a larger area of alveoli than adults; ventilation speed in children greater; number of alveoli continues to increase in first eight years of life, after which alveoli merely increase in size; lungs continue to grow until early adulthood
Distribution	
Total body water	90% of bodyweight in 2nd trimester, ~75% at birth, falls to 60% (adult level) by 3 months
Extracellular water	60% of bodyweight in 2nd trimester, 40-45% at birth, falls to ~30% by 1 year
Intracellular water	Rises during early pregnancy to ~35% in 2nd trimester, remains around 40% during infancy
Total body fat	Rises from 1-3% of bodyweight in midgestation to ~16% at birth; peaks at ~25% in infants aged 6-9 months, then falls over a period of 6-7 years to adult level
Total plasma-protein	Lower in neonates and infants than in older children and adults; has less bonding affinity
Brain	Relatively speaking, neonates have larger brains with lower myelin levels, higher blood flows, and immature blood-brain barriers
Detoxification by enzymes	
Phase I and II	Low activity levels at birth, particularly in terms of P450 oxidation and glucuron conjugation; different enzyme systems mature at different speeds, generally reaching adult levels within 6-12 months
Excretion	
Via bile	Limited capacity at birth
Glomerular, kidney filtration	Low in neonates, quickly rises in first year
Tubular, kidneysecretion	Matures later than glomerular filtration, reaches adult level within 1 year
Kidney function and excretion	Greater in older infants and small children than in older children and adults

3.2 Distribution within the body

The dispersal and distribution within the body of a toxic substance following intake is influenced by the body's physical composition. In proportional terms, the body of a child contains more water than that of an adult. Most of the extra water is found outside the cells (Sch02). The fat percentage is subject to age-dependent variations and is rela-

tively low in neonates and adolescents. The degree of bonding with plasma proteins is also important in this regard, since it determines the extent to which a substance remains in circulation, rather than finding its way into organs and tissues, where it is liable to cause damage (Plu92, Sch02). Relatively little plasma protein bonding occurs in neonates and infants. This fact, together with the relatively high water content of the body, means that there is a tendency for water-soluble substances in particular to become distributed through a comparatively large volume of a baby's body.

The brain accounts for a larger proportion of the body in an infant or child than in an adult. Furthermore, the blood supply to the brain, expressed per kilogram brain tissue, is greater in children (Sno92). In combination with the high lipid concentration of the brain, this has implications for the distribution of fat-soluble substances through the body. The blood-brain barrier, which affords the brain protection against many toxic substances, takes time to mature. In rats, this barrier is fully developed between one week and several weeks after the birth. It is not known exactly when the barrier becomes fully functional in humans (NRC93, Sch02). However, it is clear that, until the barrier is working properly, substances can reach the brains of foetuses and neonates in relatively large quantities.

3.3 Metabolism

Once absorbed into the body, a substance can be converted by enzymes. This biotransformation takes place primarily in the liver, but to a lesser extent in other organs such as the lungs and intestines as well. A wide variety of enzymes are involved, each of which can occur in several forms. A functional distinction is made between phase I and phase II enzymes (Ber95, Cre98, Cre00). The former transform substances by processes such as oxidation, while the latter attach the resulting products to water-soluble substances such as glucuronic acid, sulphate, glutathione or acetylate, thus creating so-called 'conjugates'. The latter process has two effects. First, it enhances solubility in water, making it easier to excrete the substance in the urine. Second, it can diminish the toxicity of the original substance. However, this process can in fact make some substances more toxic, in which case it is referred to as bioactivation.

The enzyme systems involved in these processes develop only in the course of the foetal stage. As development progresses, changes take place, both in the nature of the enzymes and in their activity. Foetuses and neonates have relatively little biotransformation capacity (Cre00), particularly for oxidation reactions and glucuronic acid conjugation. The low levels of enzyme activity can result in retarded detoxification and therefore prolonged internal exposure. However, the threat from substances that are bioactivated is diminished. In general, a child's biotransformation capacity reaches an adult level within the first year of life (Plu92, Zwa02). Indeed, enzyme activity can be greater

in childhood than in adulthood, possibly because children have a higher metabolic rate. In this regard, it is instructive to note that ‘half-life dip’ is a familiar concept in paediatric medicine; there is a period in childhood when medicines need to be administered in relatively high doses, because they are broken down more quickly than in adults (Øst98).

3.4 Excretion

Before birth, the foetus disposes of (endogenous) excess and harmful substances primarily through the umbilical cord and the placenta and thus via the mother’s body (Gin04). After birth, chemicals leave the body in modified or unmodified form via the kidneys (in the urine), the liver/gall bladder (via the bile in the faeces), the lungs (in exhaled air) or the skin (in perspiration). For most substances, the kidneys are the primary excretion organs. In the immediate postnatal period, however, the blood supply to the kidneys is relatively small, not reaching a level comparable to that in an adult until the age of about five months (Sch02, Zwa02). Excretion via the kidneys depends on the activity of roughly a million nephrons, each of which is made up of a filtering unit (glomerulus) and a tubule (tubulus). Some substances are removed from the blood by the passive filtering action of the glomeruli. Others are actively expelled via the tubules. Both functions operate at a relatively low level in a neonate. Glomerular filtering matures first, within five to six months. Tubular secretion does not reach full capacity until the child is between eight months and a year old (Plu92, Sch02, Zwa02). On the other hand, reduced plasma protein bonding in the first months after birth can facilitate excretion. Research with newborn laboratory animals has found that excretion in bile, by the liver, also takes some time to reach a functionally mature level. It is not yet known whether the same is true in humans (Plu92).

3.5 Conclusions

As long as mother and child are connected via the placenta and umbilical cord, there will tend to be little difference between the two in the concentrations of most blood-borne substances. This situation changes when the baby is born. In infancy, and particularly the first few months of life, the body does not deal with substances in the same way that it does later. The overall significance of such toxicokinetic differences can vary from substance to substance. In general, however, enzymatic detoxification and excretion operate more slowly in neonates, and especially in premature neonates, than in adults. As a result, for a given external dose of a toxic substance, a very small child typically experiences more prolonged internal exposure than an adult. Such differences largely disappear within the first year of life. Children aged twelve months and above are gener-

ally no less able – and often better able – to expel chemicals from the body than adults. In consequence, a given daily intake level will generally be associated with a lower burden per kilogram bodyweight. Where bioactivated substances are concerned, the opposite is the situation: neonates are at less risk than adults, whereas older children are at more risk than adults.

Chapter 4

Physiological differences between children and adults and their influence on the toxicodynamics of substances

The mechanism of action of a toxic substance – the way in which it causes harm – is generally the same in related species of organism. In many cases, the mechanism of action also remains the same at different stages in the development of a given species (NRC93). Consequently, a chemical will often affect the organs of a child in the same way as it affects those of an adult. If a substance causes, say, liver necrosis in adults, it is likely to do so in children too (Nie01), although factors such as the toxicokinetic differences outlined above can bring about differences in the severity of an effect. Nevertheless, chemicals can also have effects that are specific to developing individuals, typically involving impaired development of organs and organ systems. In this chapter, the Committee explores the pertinent issues. The findings presented draw mainly on animal research. Where data on the influence of chemicals on the development of organs and organ systems in humans is available, it comes principally from pharmaceutical research and studies of environmental pollutants.

4.1 Critical windows of exposure

Every organ in the body develops according to a precise 'timetable', with each change taking place at a particular point. During development, there may be periods – some long, some short – during which an organ is particularly sensitive to the adverse effects of chemicals. These periods are referred to as critical windows of exposure (Sel00). In some cases, effects can occur at levels of exposure that would have no significant impact

on an adult organism. Furthermore, the repercussions of impaired organ development can be prolonged or even permanent.

Disorders can be induced as early as the first week after fertilisation, or even earlier, but under such circumstances they rarely result in abnormalities (Den98, Nie01). Serious damage is liable to destroy the embryo. If only a small number of cells are damaged, they can in the early stages of development probably be replaced by others. In a human embryo, all the organs are formed in a period between twenty-one days and fifty-six days after fertilisation. Hence, it is in this period that the embryo is most sensitive to the induction of (structural) abnormalities by chemical compounds (Den98). Perhaps the best-known example of this is the effect on the development of the arms and legs induced by the sedative, thalidomide (Softenon). Serious structural abnormalities cannot be caused after the eighth week of pregnancy. However, the organs are by no means fully mature after week eight; some organs and organ systems continue to develop well beyond birth. In recent years, scientists have increasingly come to believe that, while it may have no morphologically visible impact, interference during this phase of development can cause permanent functional abnormalities, which may not fully manifest themselves until adulthood (Den98, Nie01, Sel00, Uyl98). Studies following the offspring of 'DES daughters' (the daughters of women who were prescribed the synthetic oestrogen Diethylstilbestrol) have suggested that effects may continue into the third generation (Kli02). Although the development of any organ can in principle be compromised, the greatest concerns surround organ systems whose development is complex and prolonged, such as the central nervous system, the immune system and the reproductive organs. Each of these organ systems is accordingly considered in turn below.

Finally, the Committee would draw attention to the recent publication by the EPA of a draft report on children and cancer (EPA03, Sch03). On the basis of animal research findings, the report's authors conclude that exposure to carcinogenic substances in early life is more likely to trigger cancer than a similar level of exposure for a similar length of time during adulthood. This finding applies to carcinogenic substances that are also mutagenic. Where substances that cause cancer by other mechanisms of action are concerned, the situation currently remains less clear. However, since the use of mutagenic substances in pesticides is prohibited, no further consideration is given to this matter here.

4.2 The central nervous system

4.2.1 Development history

The development of the nervous system is a precisely regulated process, in terms of both timing and siting (Ric00a, Rod95). From the first structure, the neural tube, the nervous

system grows into an organ system with a complex structure and a wide variety of functions. Development entails numerous processes. Cells divide and multiply, migrate to the different sectors of the brain and differentiate into various cell types. Connections between cells are continually formed and broken again when no longer needed; insulating material is deposited by special glial cells and biochemical changes take place. The various centres of the brain develop in different periods before and after birth. Different species of mammal do not differ so much in terms of the order in which the various developmental processes take place, but in terms of the timing of the developments relative to the moment of birth. The brains of many mammals go through a period of rapid development, the so-called 'brain growth spurt'. In humans, this spurt begins in the third trimester of pregnancy and continues into the early postnatal years. By comparison, the brain growth spurt in most commonly used laboratory animals, such as rats and mice, occurs three to four weeks after birth. The morphological development of the nervous system is accompanied by the formation of numerous functional systems (for instance for locomotion, cognition, memory, sensory perception), which are not localised in particular parts of the nervous system, but have components spread throughout the nervous system. A wide variety of neurotransmitters, synapses and receptors enable the effective transmission of signals between nerve cells, thus providing for the integrated and coordinated operation of brain systems.

4.2.2 The influence of toxic substances

The developing nervous system provides much more potential targets for the effects of neurotoxic substances than the mature nervous system (Rod95). Furthermore, the so-called 'blood-brain barrier' and detoxification mechanisms offer relatively little protection during the early phases of development (see chapter 3). Consequently, there are various periods of heightened vulnerability during the brain's development between the embryonic stage and adolescence. Animal research has shown that exposure to neurotoxic substances can interfere with development, resulting in permanent changes in the structure and/or function of (parts of) the nervous system (EPA00a, IEH96). Such changes are liable to involve not only the structures and processes of the nerve cells themselves, but also cells of other types, such as glial cells. Depending on their nature and seriousness, these changes may manifest themselves immediately or in later life. The great plasticity of the developing nervous system can temporarily mask or reverse changes that occur.

Recent animal studies have shown that exposure to pesticides can have numerous effects on the developing nervous system that do not relate directly to the best-known mechanism of action for the (category of) substance in question, for example, acetylcholinesterase inhibition by organophosphates (see Table 3). In many cases, the effects con-

Table 3 Examples of pesticides that can influence nervous system development in laboratory animals.

Substance	Animal species studied	Effects	References
Chlorpyrifos	Rat, mouse	Acetylcholinesterase inhibition, modified brain morphometry, inhibition of DNA and protein synthesis, reduced cognitive performance, cell loss, increased adenylyl cyclase activity, deficiencies in cholinergic synaptic activity, increased motor activity, modified social behaviour	Hob98, Whi95, Qia02, Lev02, Cam97, Mey03, Slo01, Ric03
Carbaryl	Rat	Modified morphometry in brain	Rob97
Bioallethrin	Mouse	Modified cholinergic receptor density in brain, increased motor activity	Ahl94, Eri90, Eri91
Deltamethrin	Rat, mouse	Increased motor activity, increased activity in dopaminergic system, reduced response to acoustic stimuli, retarded cytogenesis and morphogenesis, damage to blood vessels in brain	Eri90, Eri91, Laz01, She00, Pat97

cerned involve functional, morphological or neurochemical endpoints that are not investigated in the context of ordinary toxicological testing (see chapter 5). Where a number of pesticides are concerned, the effects are induced at dosages which produce no effects in standard tests (Eri91, Laz01, Lev02, Pat97, Qia02, Qia03, Slo01, Slo02, Son97). Often, researchers have been unable to establish safe exposure levels for the effects in question, since these are evident even at the lowest doses administered. Some of the effects manifest themselves in adult animals, for example through increased motor activity (Ah94), reduced cognitive capacity (Lev02), modified social behaviour (Ric03) or heightened vulnerability to substances in adulthood (Eri97; Eri00). A fuller summary of the reported effects is given in Annex C.

It is not easy to say whether the developing nervous system is more susceptible to the effects of exposure to pesticides than the mature nervous system. Of particular significance in this context is whether differences exist in vulnerability at the low levels of exposure that (unborn) children typically experience. Studies involving a number of pesticides in various categories (organophosphates, pyrethroids) have shown that, at high dosages, young animals can be more vulnerable than adults to certain of the pesticides' effects (She00). At low dosages, however, the picture is less clear. Some studies appear to suggest that young animals are less vulnerable (She94), while others have produced evidence that the opposite is the case (Liu99, Pop97). Such dose-dependent variations in vulnerability may be attributable to differences in the vulnerability of processes or structures in the nervous system or to kinetic differences (see chapter 3). The functional, morphological and neurochemical outcomes referred to above as not being addressed by standard toxicity studies have in most cases been studied only in animals exposed early in life. It is not therefore possible to say with confidence whether exposure in adulthood would induce an equivalent response.

In most cases, it is not possible to investigate the effects on humans using comparative experimental methods similar to those used in animal studies, since it is necessary to employ invasive techniques to determine what effect exposure has had. Academic literature includes a single reference to a case involving the almost complete absence of skeletal muscle in a newborn baby whose mother had suffered dermal exposure to malathion, a organophosphate insecticide, during the eleventh or twelfth week of pregnancy (Lin87). Since the embryo would have been going through a period of neuromuscular development at the time of the mother's exposure, a causal relationship is plausible. Nevertheless, a single anecdotal account carries little scientific weight.

Around the world, one now sees epidemiological studies being set up to look into the possible consequences of exposure to neurotoxic pesticides before birth or during the early years of life, but little meaningful data is yet available (see also Hei01, IEH02, EU04). The studies that have so far examined the relationship between antenatal exposure to organophosphates and foetal growth or pregnancy duration have produced contradictory results. This may be due to differences in the way researchers have characterised exposure (analysis of the mother's or child's blood or of the mother's urine; measurement of the pesticide itself, or of metabolites that may or may not be pesticide specific or of cholinesterase activity; timing and frequency of sampling).

In a study in New York, Perera *et al.* found an inverse correlation between umbilical blood concentrations of chlorpyrifos and diazinon and the weight and length of the neonate. No correlation was found with cranial circumference in infancy (Per03, Why04). The association between pesticide concentration and birth length and weight was statistically significant in children born before 2000-2001. After 2001, rules were introduced by the EPA to prevent domestic use of the relevant pesticides. Children born after the rules took effect experienced much less exposure to the pesticides, and there ceased to be any statistically significant correlation with the growth of the foetus.

Berkowitz *et al.*, who also studied New York residents, failed to find any correlation between the presence of a metabolite of chlorpyrifos in the mother's urine and neonatal bodyweight, length or cranial circumference (Ber04). A significant, albeit minor, reduction in infant cranial circumference was nevertheless detected in cases where the mother exhibited low PON1 activity. PON1 is an enzyme, which plays a role in the detoxification of chlorpyrifos.

A comparable study in a Californian farming community was unable to detect a negative correlation between exposure to organophosphates and foetal growth (Esk04). The researchers did, however, find that reduced pregnancy duration was associated with two of the indicators of exposure used: cholinesterase activity in umbilical blood and the presence of non-specific metabolites of organophosphates in the mother's urine. Reduced pregnancy duration was most clearly linked to greater exposure during the latter part of the pregnancy. The researchers suggested that this could have been due to the

organophosphates inhibiting the breakdown of acetylcholine, a substance that stimulates uterine contractions.

In view of the neurotoxic characteristics of organophosphates and the previous observation that cranial circumference is a useful predictor of IQ, various research teams are planning to monitor the neurocognitive development of the children involved in the studies (Ber04, Why04). Research in farming communities in Mexico has found that children of four to five years old growing up in a community where intensive use was made of pesticides exhibited less endurance, poorer hand-eye coordination, poorer memory and less well-developed drawing skills than children of a similar age growing up in a community where very little use was made of pesticides (Gui98).

A technical working group set up to support the European Commission's SCALE* initiative recently looked at a number of substances and substance groups and classified the evidence for a correlation between exposure and neurological developmental abnormalities in children (Table 4) (EU04). Where pyrethroids and organophosphates were concerned, the evidence for a link was classed as level 2 and level 2*, respectively. Classification as level 2 means that animal tests have revealed a (reasonable) correlation, but sometimes at unrealistically high exposure levels. Assignment to level 2* implies that animal studies or *in vitro* studies have discovered effects that involve mechanisms known to be relevant for humans or that occur at exposure levels or via exposure routes that are relevant for humans.

More information is available on the influence of certain environmental pollutants and medicines on the development of the human nervous system (EU04). Antenatal exposure to lead and mercury, for example, can lead to learning difficulties and mental retardation, respectively (Uyl98). Antenatal exposure to background levels of PCBs and dioxins is associated with adverse effects on birth weight, thyroid function and psychomotor development (Koo94, Koo96, Pat98). If used by a mother during pregnancy, some medicines are known to be capable of causing health problems in the child. Use of the antihypertensive clonidine, for example, has been associated with sleeping problems in the child, use of the anti-abortive DES with childhood anxiety and depression, and use of the contraction inhibitor ritodrine with motor, social and emotional problems in the child (Uyl98). It has also been speculated that ante- and perinatal exposure to neurotoxic substances could be involved in the development of autism (EU04, Lon00), ADHD (EU04, Ric00b, Win03) and neurodegenerative diseases such as Alzheimer's and Parkinson's (Eri00, Wei00a). Evidence for any such link has yet to be found, however.

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* SCALE: Scientific evidence, focused on Children, meant to raise Awareness, improve the situation by use or Legal instruments and ensure a continual Evaluation of the progress made (EU04)

Table 4 Evidence for a correlation between neurological developmental abnormalities and exposure to a number of substances and other environmental factors (EU04).

Substance (substance group)	Level 1	Level 2* and Level 2	Level 3
PCBs en dioxins	X		
PBDEs		X*	
Organophosphates		X*	
Organochlorine pesticides	X (DDT)	X (others)	
Pyrethroids		X	
Dithiocarbamates			X
Solvents		X	
Lead	X		
Iodine	X		
Mercury	X		
Manganese		X	
Aluminium		X	
Cadmium		X	
Arsenic			X
Monosodium glutamate		X	
Stress	X		
Noise	X		
Anticonvulsants	X		
Steroids	X		
DES	X		
Radiation	X		
Alcohol and smoking	X		

Level 1: Based on reliable human studies showing a correlation between one or more neurological developmental abnormalities and exposure to a substance or group of substances.
Level 2*: Based on animal studies or on *in vitro* studies showing effects on the development of the nervous system involving mechanisms of action that are relevant for humans or that occur at exposure levels or via exposure routes that are relevant for humans.
Level 2: Based on animal studies showing a (reasonable) correlation, but sometimes at unrealistically high exposure levels.
Level 3: Based on assumptions, hypotheses and incidental case descriptions.

4.3 The immune system

4.3.1 Development history

Development of the immune system in vertebrates has been researched most thoroughly in relation to humans and mice (Hol00). The process begins with the formation of what are known as pluripotent stem cells on the yolk sac. These non-specialised cells have the

ability to develop into all the different types of cell involved in the provision of immunity. Once the circulatory system has become closed, the stem cells migrate to the primary lymphoid organs (liver, bone marrow, thymus) and secondary lymphoid organs (including the spleen and the lymph glands). Under the influence of contact with the cells of those organs and the specific circumstances in the relevant locations, the stem cells differentiate into a wide variety of cell types, which gradually mature and develop their particular functional properties. As part of these processes, the cells learn to distinguish between self and non-self. This enables the cells to provide effective resistance against xenobiotic agents, such as micro-organisms, without giving rise to auto-immunity (which involves the immune system responding to components of the body itself, ultimately triggering pathological processes). The most important cell types include: B-lymphocytes, which produce antibodies and thus provide humoral resistance; various types of T-lymphocytes, which provide so-called cellular resistance; macrophages, which have the ability to engulf substances or particles (phagocytosis) before destroying them or presenting them to other immune cells; granulocytes, which release active substances. By means of complex interaction, these various types of cell give the body resistance against infection and disease.

The immune system develops in much the same way in humans and mice (and other vertebrate laboratory animals). Nevertheless, there is an important temporal difference: while the human immune system is already fully formed at birth, a mouse's or rat's immune system continues to develop for quite some time after birth. As a result, a newborn human baby has greater resistance than a newborn mouse. Nevertheless, important developments do take place in the human immune system after birth; it is some while before all immune functions are fully active. Furthermore, shifts occur in the balance between the activities of the various cell types. Finally, interaction with the environment leads to the acquisition of an immunological 'memory', enabling the immune system to respond more rapidly and more effectively if it re-encounters a harmful micro-organism or toxic substance.

4.3.2 Sensitivity to toxic substances

Although quite a lot is known about the influence of pesticides, and chemicals in general, on the function of the mature immune system (see, for example, Lue02, Rod96, Voc99, WHO96a), we still know relatively little about the way chemicals can interfere with development of the immune system. It is nevertheless likely that such interference can have serious and prolonged or even permanent consequences for health. The reason being that destruction of pluripotent stem cells, which are particularly numerous in the perinatal period, may be expected to have much more far-reaching implications than the destruction of a few specialist cells in an adult. Furthermore, damage to the thymus in

the perinatal period has more serious consequences than damage in adulthood. It is mainly around the time of birth that T-cells in the thymus learn to distinguish between self and non-self, and that selection of T-cells with the appropriate antigen specificity takes place. This process is largely complete soon after birth, and the thymus gradually withers thereafter.

On the basis of what is known about the successive processes involved in development of the immune system, some researchers distinguish five periods, or critical windows of exposure, in which there may be increased vulnerability to toxic substances (Die00, Lus03). However, toxicological research has not to date focused to any significant extent on individual critical exposure windows. In the animal studies, exposure generally continues for a large part of gestation and often after birth during lactation and even after weaning. Studies are designed in this way in order to cover all possible periods of increased vulnerability, but in consequence it is not possible to say when exposure is most harmful or to attribute particular effects on elements of the immune system to particular exposure periods. The data does nevertheless allow general comparison of the consequences of antenatal/perinatal exposure with the consequences of exposure in adulthood, even though studies are rarely designed with this aim in mind.

Table 5 Examples of substances known to be toxic to the developing immune system.

Substance	Animal species studied	Main effects	References
Atrazine	Rat	Reduced production of antibodies; reduced function of certain T-lymphocytes	Roo03
TCDDs (and PCBs)	Rat, human	Thymus atrophy; impaired T-lymphocyte maturation and function (rats); increased susceptibility to infection (humans)	Vos89, Fin89, Lus79, Vos74, Fai77, Geh99, Bar96, Wei00b
Hexachlorobenzene	Rat, mouse, human	Stimulation of antibody production (rats); reduced T-lymphocyte function (mice); increased prevalence of middle-ear infection (humans)	Vos79a,b, Vos83, Bar87, Vos86, Dew00
Chlordane	Mouse	Reduction in certain T-lymphocyte functions due to influence on macrophages; reduced stem cell numbers	Spy82, Bar85a,b, Bar90a,b
Benzo[*a*]pyrene	Mouse	Thymus atrophy, reduced stem cell numbers, reduced T and B-lymphocyte function; increased prevalence of tumours	Urs80, Urs82, Urs84, Hol94b
Lead	Rat, chicken	Reduction in certain T-lymphocyte functions; shift in Th1-Th2 balance (rats and chickens)	Lus78, Fai79, Che99, Mil98, Bun00, Bun01a,b, Lee01a, Lee02, Die02
Acyclovir	Rat	Thymus agenesis; thymus atrophy; increased susceptibility to infection	Sta91, Sta92
Dexamethasone	Rat	Permanently increased vulnerability to induction of autoimmune conditions	Bak00

Few substances – and hardly any modern pesticides – have been studied with a view to determining their toxicity to the developing immune system (Bar96, Die00, Hol94a, Hol00, Lov03, Lus03). A number of examples are given in Table 5. Their effects are considered in more detail in Annex C. From the available data, it seems that the toxicity of a substance to the immune system depends partly on the developmental stage that the system has reached at the time of exposure. Not only the nature of the effects, but also their seriousness and duration exhibit such dependency. Although in many cases the sparse data suggests that the developing immune system is more vulnerable than the mature system, it does not follow that this is necessarily the case with all immunotoxic substances. The consequences of antenatal or perinatal exposure do nevertheless generally appear to be more serious or prolonged. It is not always clear just what significance changes in separate immune parameters have for the health of the organism. However, there is reason to believe that such changes can lead to increased susceptibility to infection, to increased tumour prevalence or to allergic or auto-immune conditions.

4.4 Hormone-dependent sexual development

4.4.1 Development history

Hormones are signalling substances. They enable molecules, cells, tissues and organs within an organism to function in harmony with one another and in interaction with the environment. Numerous different hormones are produced by a variety of organs and tissues around the body. They are then transported to all parts of the body in the bloodstream, sometimes bound to transport proteins. Cells that carry substances called receptor proteins are able to bond with particular hormones. The formation of a hormone-receptor complex sets a chain of reactions in progress, leading to, for example, the activation of certain genes and thus the production of special proteins by the cells concerned.

In vertebrates, including humans, hormones play an important role in gender differentiation. The gender of an individual is genetically determined upon fertilisation. However, male and female reproductive organs (gonads) remain morphologically identical until day 13.5 in the gestation of a mouse and until the end of week 6 in a human pregnancy. Thereafter, differences begin to develop. In the male sex organ, the testicle, genetic activity triggers production of the male sex hormones (androgens), MIS (Müllerian Inhibiting Substance) and testosterone. These hormones cause the embryo to develop as a male. MIS inhibits the development of female characteristics, while testosterone induces formation of the epididymis, seminal duct and seminiferous tubules. A metabolite called dihydrotestosterone triggers the development of the prostate and the external sex organs (penis, scrotum), as well as secondary gender characteristics such as

beard growth. In females, these male hormones are not formed, so development automatically follows the female path. The ovaries begin producing female sex hormones (oestrogens), of which oestradiol is the most important. Sex hormones play an essential role in the further sexual development of the body. For example, they control developments during puberty, the maturation of the ova, the formation of spermatozoa and, in mammals, gestation, birth and the production of milk.

Sex hormones are produced not only in the testicles and ovaries, but also in the adrenal glands, which produce predominantly androgens. This is the case in both men and women. Furthermore, testosterone can be enzymatically converted into oestradiol. Hence, both male and female sex hormones are active in the bodies of both sexes, although not immediately.

It is not only the reproductive organs that need to develop male or female characteristics. Other organs, such as the liver, the skeletal muscles, the immune system (Big99) and the brain also have to be 'gender programmed'. Enzymes are active in the brain, which can convert testosterone into dihydrotestosterone and into oestradiol. The various hormones also play a major role in the development of male and female behaviour patterns. In this context, the relative significance of each hormone differs, depending on the animal species and type of behaviour concerned.

The formation of sex hormones and reproductive cells is closely controlled by the brain and the pituitary. Control is effected by means of hormones and, for example, brings about a degree of cyclicity, with feedback mechanisms playing an important role. In this context, reference is often made to a hypothalamic-pituitary-gonadal axis. The involvement of the nervous system enables the organism to respond to external stimuli and means that, for example, visual stimuli can influence the body's hormonal balance. Finally, it should be pointed out that the thyroid also produces hormones, which are very important for the proper development of various organs, including the brain (Por00) and the gonads (Lem00). In turn, the production of thyroid hormones is regulated by the brain and the pituitary.

Clearly, the (sex) hormone balance is the consequence of extremely complex interaction involving numerous hormones and receptors. The correct development of an organism requires that the various substances are present in the right concentrations, the right proportions, at the right place and at the right time. Fuller descriptions of this interaction can be found in an earlier Health Council advisory report (GR99) and in the academic literature (Pry00).

4.4.2 The influence of toxic substances

During the 1980s and 1990s, there was growing concern that exposure to naturally occurring or anthropogenic chemicals might disrupt the hormone balance (Col93,

NRC93). In recent years, a great deal of research has been conducted into the endocrine-disrupting capacity of chemicals, into the associated mechanisms of action and into the possible implications both for natural fauna and for humans. Indeed, the Health Council has itself published two advisory reports dealing with such matters (GR97, GR99). In these reports, the Council makes the point that, while in many cases it is demonstrable or probable that endocrine-disrupting chemicals do affect wildlife populations, no evidence of an acute threat to public health has yet been produced. The Teratology Society (Bar99) and the Scientific Committee for Toxicity, Ecotoxicity and the Environment of the European Union (EU00) have made similar statements. Nevertheless, it was felt that the possibility of endocrine-disrupting chemicals affecting human health should be taken seriously, since the population at large is exposed to such substances and effects on reproduction and development – albeit perhaps subtle effects – are biologically plausible (Bar99, GR97).

Because the system by which the hormonal balance is maintained is so complex, opportunities for influence by toxic substances abound. Such substances can disrupt hormonal action in various ways (GR99; Big99):

By attaching themselves to hormone receptors and thus behaving like hormones, resulting in the creation of an active receptor-'hormone' complex (hormone mimicry)

By attaching themselves to hormone receptors without activation occurring, but in a way that prevents bonding of the real hormone (hormone antagonism)

By disrupting the production, conversion, breakdown, transportation or excretion of hormones

A growing number of substances are now known to be capable of disrupting the sex hormone balance, or suspected of being able to do so (EU01). These substances include various pesticides, some of which are listed in Table 6. This topic is covered in greater detail in Annex C.

Table 6 Examples of endocrine-disrupting pesticides.

Substance	Animal species studied	Main effects	References
Vinclozolin	Rat	Anti-androgenic, affecting both physical make-up and behaviour	Gra94, Kel94, Gra99a, Mon99, Hel000, Wol00, Gra01, Hot02
Procymidone	Rat	Anti-androgenic, causing feminisation of male offspring	Ost99, Gra01, Nel03
Linuron	Rat	Anti-androgenic, causing abnormalities of the male sex organs, Leydig cell tumours	McI00, Coo93
Atrazine	Rat	Disruption of hypothalamic-pituitary-gonadal axis; spontaneous abortion; delayed parturition; delayed puberty; inhibited testosterone production; prostatitis	EPA02c, Nar01, Law00, Sto00, Fri02, Sto99

Research has shown that the nature and seriousness of the effects of endocrine disruption are not merely dose-related; the moment of exposure is equally significant (Pry00). Effects such as malformations and infertility cannot therefore be predicted on the basis of research involving the exposure of adult animals only. Furthermore, exposure to endocrine-disrupting substances during critical phases of development can often have permanent effects, in contrast to the reversible changes that typically occur in adult animals in response to temporary exposure (Big99, Gra01). Functional changes frequently manifest themselves only after puberty. There is evidence that, where endocrine disruption is concerned, the relationship between dose and effect is less straightforward than with other effects and that U-shaped and inverted-U-shaped dose-effect curves can occur. In other words, it appears that a medium dose may induce less pronounced effects (or, as the case may be, *more* pronounced effects) than either a very low dose or a very high dose. Furthermore, the nature of the effect can be dose-dependent. Hence, it can be difficult to deduce from tests involving high dosages what the effects of low dosages might be (Big99, Gra01). This is particularly inconvenient, since, where actual human exposure is concerned, it is low doses that are most relevant.

4.5 Conclusions

From animal research it appears that some substances, including pesticides, can influence the development of organs and organ systems. Young laboratory animals are liable to suffer damage to their brains, sex organs and immune systems following exposure to dosages of pesticide that have no apparent adverse effect on adult animals. At low dosages, adult animals are often affected only after prolonged exposure. In young animals, by contrast, brief periods of exposure or isolated exposure incidents can have health implications. Where the same effects have been looked for and detected in adult animals as in young animals, they frequently prove to be more serious and prolonged in the latter group.

Whether low levels of exposure to food-borne pesticides can similarly affect human development has not been scientifically investigated. However, a considerable amount of research has been done into the influence of certain environmental pollutants, medicines and alcohol and maternal smoking. This research has shown that such substances can affect the development of a child or unborn child at exposure levels that would be harmless to an adult.

Chapter 5

The current safety assessment procedure

In the Netherlands, the Board for the Authorisation of Pesticides (CTB) is responsible for deciding whether the agricultural use of a pesticide represents a potential hazard to consumer health. To perform this task, the board requires two types of information: data on the toxicity of the substance and information about the likely patterns of exposure. In this context, distinction has to be made between risks arising out of prolonged exposure to pesticides and risks arising out of brief exposure or single exposure incidents. This chapter accordingly has a section dealing with each of these two categories of risk. The Committee's description of the procedure that is currently used to estimate the nature and the size of the potential risks to the consumer and to keep such risk within acceptable bounds is based mainly on the extensive commentary given in the *Handleiding voor de Toelating van Bestrijdingsmiddelen* (*Pesticide Approval Handbook*) published by the CTB (CTB02). Several summarised descriptions of the procedure have appeared in the academic press (Dor02b, Lan02, Wal98, WHO97).

5.1 Risks associated with long-term exposure

5.1.1 Estimating toxicity

A pesticide's toxicity can be expressed in the form of an intake level that is considered to be safe. The apparently safe dose for prolonged consumption is the so-called Accept-

able Daily Intake, generally abbreviated to ADI*. The term was originally coined by the JECFA** for use in relation to food additives, such as dyes, fragrances, and flavourings, but has since been adopted for pesticides as well. The ADI is defined as 'an estimate of the amount of a substance in food or drinking-water, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation' (WHO97). The concept is based on the assumption that the toxicity of a substance has a threshold value: the organism has some capacity to prevent the substance causing any harm; health is adversely affected only if this capacity is exceeded.

Calculation of an ADI is a two-stage procedure. First, it is necessary to characterise the potential hazards posed by the substance, in both qualitative and quantitative terms. To this end, the manufacturer is required to conduct a series of toxicity studies with laboratory animals – usually rats, mice, rabbits or dogs. These studies are intended to shed light on the critical effect of the substance in question: the harmful effect that occurs first (i.e. at the lowest exposure level). The tests that have to be carried out are listed in Table 7. If the results of these studies or insight into the substance's mechanism of action suggest it is necessary, the manufacturer is expected to carry out additional problem- specific studies.

Acute toxicity, expressed in the form of an ED_{50} or LD_{50} (the doses at which, respectively, a specified effect or mortality is induced in 50 per cent of laboratory animals), is not generally very useful in the context of exposure via food. It is more relevant in the context of occupational exposure. Where consumer health is concerned, what normally matters most is any effect associated with more prolonged exposure to lower doses. The findings of studies that look at such exposure are used to calculate so-called No-Observed-Adverse-Effect Levels (NOAELs). An NOAEL is the highest concentration or dose used in a study that induces no observable (i.e. statistically significant) harmful effect. So a series of toxicity studies generates a set of NOAELs. The lowest of these NOAELs is an exposure level that is not quite sufficient to induce the critical effect or any other effect. Unless there are good reasons for proceeding otherwise, the lowest established NOAEL is used to calculate an ADI. In most cases, that means using the NOAEL from the chronic toxicity study, the reproductive toxicity study or the development toxicity study. It is generally accepted that one cannot have an NOAEL for a genotoxic*** carcinogenic substance, and that no safe dose can therefore be calculated. Hence, such compounds cannot be authorised (CTB02, Dor02b).

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* In the USA, the (chronic) Reference Dose ((c)RfD) is the established measure. This is conceptually identical to the ADI, but the values assigned to the two can differ, sometimes considerably (Sch00a).

** JECFA: FAO/WHO Joint Expert Committee on Food Additives.

*** Genotoxic: capable of causing genetic damage

Table 7 Required toxicity studies.

Metabolism and toxicokinetics
Acute toxicity
Subacute and semi-chronic toxicity
Chronic toxicity
Carcinogenity
Genotoxicity
Delayed neurotoxicity (for organophosphates and carbamates only)
Reproductive toxicity
Developmental toxicity

The second step of the procedure is to calculate an ADI from the selected NOAEL. This involves dividing the NOAEL by a certain factor, typically 100. The purpose of introducing this factor is to provide a safety margin by way of compensation for various uncertainties. For this reason, the factor is referred to as a safety factor or uncertainty factor. It is the product of two component factors of 10. The first component factor of 10 is included to compensate for the fact that the toxicity data actually relates to laboratory animals. Where data on the substance's toxicity for humans is available – from epidemiological research or research with volunteers, say – it may be felt that a smaller component factor offers sufficient safety, or that there is sufficient certainty to dispense with this component altogether. The second factor of 10 is intended to allow for the fact that individuals can differ in their sensitivity to a substance. Protection should be afforded not only to people of average sensitivity, but also to those who are particularly sensitive due to hereditary characteristics, nutritional status, health status or age. Such people are often referred to as YOPIGs*. Where not all the necessary toxicity data is available, or there are reasons to believe that a substance may be toxic to embryos or foetuses, additional safety factors may be used to calculate a (provisional) ADI (Dou02, Ren95).

In the Netherlands, ADIs are officially set by the CTB. However, the policy for some years has been to seek harmonisation with internationally prescribed ADIs, such as those published by the EU or the JMPR**. Approved pesticides are reassessed at intervals of no more than ten years. In this context, the ADI is reviewed in the light of the latest research findings and developments in toxicology, and adjusted where necessary. An ADI is not therefore an absolute value that is 'set in stone' (Dor02b, WHO97).

* Young, old, pregnant, ill and genetically susceptible people
** FAO/WHO Joint Meeting on Pesticide Residues

5.1.2 *Estimating exposure*

The extent to which consumers are exposed to trace pesticides in food depends on what they eat, how much they eat and what the pesticide concentrations are in the foods concerned. Information about food consumption patterns in the Netherlands is available from the Dutch National Food Consumption Surveys (Voe98). Using the survey findings, it is possible to define a sort of 'national average diet'.

When considering the pesticide concentrations, the worst case situation is initially assumed, i.e. a situation in which the produce being consumed contains the maximum permitted concentration of pesticide, the so-called Maximum Residue Limit (MRL) (Dor02b). A provisional MRL value is initially assigned on the basis of field tests. The manufacturer treats the growing crop with the maximum recommended frequency at the maximum recommended dosage (both values are recommended by the manufacturer in question), then determines how much pesticide remains in the produce after a given safety period. The findings of these tests have to be included in the dossier that the manufacturer is required to submit when applying for product approval. To give farmers and other growers the reassurance that their produce will not contain excessive levels of pesticide if treated in accordance with the instructions, the provisional MRL is generally set at a level two or three times higher than the average concentration detected in the field tests. In practice, therefore, actual concentrations will almost always be lower than the provisional MRL, particularly given that effective pest control does not necessarily require use of the maximum dosage or treatment frequency, and that concentrations in harvested produce are liable to decline during storage.

It will be apparent from the foregoing that an MRL is an agricultural, rather than a health-based standard. It is defined with a view to achieving efficient pest control. However, before the provisional value acquires definitive status, it is necessary to check that it is not inconsistent with a safe human exposure level, namely the ADI. To this end, national dietary data and the provisional MRL are used to calculate a National Theoretical Maximum Daily Intake (NTMDI). The procedure used makes allowance for the fact that a pesticide may be used on several different crops. The CTB works out one NTMDI for the Dutch population as a whole and another for children between one and six years old. If the NTMDI proves to be lower than the ADI, the provisional MRL is adopted as the definitive MRL. If it is higher, steps are taken to arrive at a more realistic exposure estimate, the National Estimated Daily Intake (NEDI), by working from the median residue levels measured in the field tests and allowing for the fact that some parts of the crop are not eaten and for the way residue levels are likely to be influenced by food preparation (peeling, squeezing, boiling, etc). If the NEDI calculations also give a higher figure than the ADI, the maximum recommended pesticide dosage or treatment

frequency has to be adjusted, insofar as this is consistent with effective pest control. If the various requirements cannot be reconciled, the pesticide is not approved, at least not for use with the relevant crop.

Where possible, MRLs are nowadays set on the basis of international consultation, in order to avoid creating trade problems. In practical terms, therefore, they are defined by the Codex Alimentarius Commission*, acting on the advice of the Codex Committee on Pesticide Residues (CCPR).

5.2 Risks associated with brief exposure or single exposure incidents

5.2.1 Estimating toxicity

Until recently, it was assumed that any traces of pesticide in food could not cause acute symptoms of poisoning. However, about ten years ago, it became clear that the concentrations in individual units of vegetables or fruits may vary considerably. In some cases, a unit of produce can contain pesticide in a concentration well above the MRL or what might be expected from ordinary monitoring data (which is always obtained using mixed samples of produce). Consumption of such heavily loaded units – sometimes referred to as 'hot' – can occasionally result in levels of exposure in excess of what was previously believed to be possible. This is significant mainly in relation to pesticides whose acute toxicity for humans is high and in relation to produce that is eaten in 'unit form', such as apples, pears, cucumbers and carrots (Har00, Mar00). In order to protect the public against peak exposures, a measure known as the Acute Reference Dose (ARfD) has been devised. This is 'an estimate of the amount of a substance in food and/or drinking-water, normally expressed on a body-weight basis, that can be ingested in a period of 24 hours or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation' (JMPR02). With a few exceptions (see, for example, JMPR01), ARfDs are always equal to or higher than the corresponding ADIs. Since an ADI is a limit for chronic exposure, brief consumption of slightly excessive quantities need not cause an immediate health risk, provided that the average daily intake over a longer period is not higher than the ADI (Her00, Mar00, Mor00). An ARfD is an expression of the safe ceiling for exposure peaks (Mor00).

An ARfD is calculated by dividing an NOAEL for an acute toxic effect by a safety factor, typically 100. This can be problematic, because the required toxicological tests are not yet really geared to ARfD calculation (Bil00, Dew00b, Raa01, Raa02). Of par-

* The inter-governmental committee responsible for running the United Nations' FAO/WHO Food Standards Programme. The aim of this programme is, while protecting consumer health, to remove obstacles to trade by developing food standards (Eck95).

ticular relevance are neurotoxicity and effects on the developing organism (Bil00, Har00, Her00, Mor00, Raa01, WHO97). In the Netherlands, ARfDs are set by the CTB, albeit in line with the levels defined by international bodies such as the EU and the JMPR where possible.

5.2.2 *Estimating exposure*

The ARfD is compared with an acute consumer exposure estimate, the National Estimated Short-Term Intake (NESTI). This figure is based on information regarding short-term food consumption patterns (the size of a generous portion, the size of individual units of fruit and vegetables) taken from the Dutch National Food Consumption Survey and data on residual pesticide concentrations in produce obtained from the pesticide manufacturer's field tests. Unlike a chronic exposure estimate, an acute exposure estimate needs to take account of the variations in residue concentrations that exist between individual units of fruit or vegetables. In most cases, however, the only data available relates to mixed samples. So the residue concentration figures from mixed samples are multiplied by a selected standard value to estimate the maximum residue concentration of a single unit. Like the NTMDI, the NESTI is calculated both for the population as a whole and for children between one and six years old.

The outlined calculation method is deterministic and assumes a worst case scenario: a high residue concentration combined with a generous portion. For each population group (the Dutch population as a whole and children aged twelve months to six years), this yields a single figure for the intake of a pesticide. In recent years, however, there has been increasing interest in probabilistic calculation methods. Such methods take account not only of variation in the quantity of produce consumed, but also variation in the residue concentration. This results in a probability distribution reflecting all possible levels of intake with the likelihood of each level occurring. Any chosen percentile* within the intake range can then be compared against the ARfD. One major advantage of such an approach is that it provides much more information (Boo02, Boo03a, Lóp03). If the NESTI value calculated by deterministic means is higher than the ARfD, the CTB, like its counterpart in the United States, the EPA (Suh00b), nowadays applies the more sophisticated probabilistic calculation method. Then, if the 99.9^{th} exposure percentile proves to exceed the ARfD, it is concluded that changes need to be made in the way the pesticide is used.

ARfDs are used not only in the context of pesticide approval, but also to estimate the health risks in specific cases, where agricultural produce with excessive pesticide

* Percentile: the n^{th} percentile is the value (in this case the level of intake) that is greater than n per cent of the entire range of possible values and less than (100-n) per cent of the possible values.

concentrations (i.e. concentrations higher than the MRL) is discovered to be in circulation (Raa02).

It is only in the last few years that scientists have been estimating the risks associated with brief exposure to relatively high doses of pesticide in food. Consequently, there are only a few substances for which ARfDs have been calculated, or concerning which it has been concluded no ARfD is needed. Efforts are currently being made to bring about international methodological harmonisation (Har00), but numerous points remain to be agreed (JMPR01). These include the length of the exposure period, criteria for determining whether an ARfD is needed for a given substance, which toxic effects are most relevant in the context of ARfD determination and what safety factors should be used. The RIVM has recently published guidelines on the calculation of ARfDs, in which various issues, including those mentioned here, are addressed (Raa01). These guidelines have been discussed in the relevant international forums. Another RIVM report outlines recent national and international developments relating to ARfDs and acute exposure to pesticides (Raa02; see also JMPR02).

5.3 Conclusions

Before a pesticide is allowed onto the market, its safety is thoroughly assessed. One of the focuses of this assessment is the potential risk associated with the consumption of produce that has been treated with the pesticide. To this end, the amounts of pesticide that consumers are likely to ingest in a single day and over a longer period are estimated, and the figures compared with data on what is considered safe in the context of, respectively, acute and chronic exposure. The latter information is derived from extensive toxicological research with laboratory animals. A pesticide is approved for use only if the anticipated levels of consumer exposure are within the bounds of what is considered safe. The guiding principle is that protection should be afforded to all population groups, including children. For this reason, the required toxicological testing includes addressing the possibility that the pesticide under examination might adversely affect development. Furthermore, the methods used to estimate exposure take account of children's specific food consumption patterns.

Chapter 6

Adequacy of the current safety assessment procedure: toxicological testing

It has already been explained how children differ from adults in terms of exposure, toxicokinetics and toxicodynamics. In the following chapters, the Committee considers whether the current procedure for assessing the safety of pesticides takes sufficient account of these differences. In particular, the following topics are addressed:

1 The critical effect (chapter 6)
2 The uncertainty factors that should be applied (chapter 7)
3 The likely levels of exposure (chapter 8).

The first two topics are pertinent to the determination of safe levels of exposure for all consumers, as expressed in the form of ADIs and ARfDs. The third topic is significant for comparison of the anticipated levels of exposure – as based on the proposed MRL – against the levels of exposure that are regarded as safe. In this chapter, the Committee looks at identification of the critical effect.

6.1 The sensitivity of children

Physiological differences exist between developing individuals and adult individuals (see chapters 3 and 4). These differences are greatest at the start of the development process and gradually become smaller. As a result, the effect of a substance on a developing individual can differ in both qualitative and quantitative terms from the effect on an adult. The findings of toxicological studies on adult individuals cannot necessarily be used to extrapolate conclusions regarding developing individuals, therefore. The only

way to ensure that proper protection can be afforded to developing individuals is to take them into account in toxicological testing. For this reason, great emphasis is placed on the developing organism in the toxicological testing that is required in support of an approval application. The necessary information is obtained mainly in the developmental toxicity study and in the multi-generation reproductive toxicity study, which together cover all stages in the development of an organism.

Evaluations of toxicological data on a large number of substances (Koe83; Dou02; Mid03), including pesticides, have shown that, with some substances, the critical effect is an effect on the developing individual. In such cases, it is during development that an organism is most sensitive to the effects of the substance (Koe83; Dou02; Mid03). Where other substances are concerned, the critical effect is an effect on the adult individual. In these cases, it is in adulthood that an organism is most sensitive to the effects of the substance. The question 'Are children more vulnerable to chemicals than adults?' (see for example Jub03) cannot therefore be answered with a simple 'Yes' or 'No'. Each substance has to be considered individually.

Since the whole population, including all age groups, requires protection, the principle is generally applied that safe intake levels (ADIs and ARfDs) should be based on the most sensitive effect and the most sensitive age group (see, for example, Lar98). However, the point has been made that, if this principle is applied to the calculation of ARfDs, the resulting figures can be unnecessarily conservative in some cases. The reason being that, if the critical effect is an effect on foetal development, this is very important for women of childbearing age, but not for other adults or for children. Under such circumstances, the JMPR believes that separate ARfDs should be calculated for the different population groups (JMPR02). The European Union considers such an approach undesirable, however (EU03). The Committee considers this to be essentially a matter of policy.

6.2 Are the existing toxicological testing requirements sufficient?

Although the prescribed developmental toxicity study (teratogenicity study) and multi-generation reproductive toxicity study are capable of revealing many possible effects on developing organisms, the Committee does not believe that either study is capable of detecting every effect that a substance might have on an organ or organ system in a developing organism. There is a particular danger of effects on the developing nervous system or the developing immune system being overlooked. The Committee feels that effects associated with endocrine disruption might also go undetected; less certainty exists regarding this danger, however, since a very thorough multi-generation reproductive toxicity study might be sufficient to highlight any problems. Opinion on this point differs (Fos02, Gra01, McI00, NTP01). Those who doubt the efficacy of modern multi-

generation reproductive toxicity studies in this regard make the point that various endocrine-disrupting substances not have not been identified as such by this kind of study. It is functional effects that result from exposure during development but manifest themselves only in adulthood that are most likely to go unnoticed. Indeed it is possible that some effects become apparent only at an advanced age, when the resilience of various systems begins to decline or when the organism is exposed to other stressors. The potential for problems of this kind has been recognised internationally (EU98, Lar98).

There are various reasons for regarding the present toxicity studies as inadequate. In most studies, animals are not exposed to pesticide until they have been weaned. Before birth and when suckling, the animals do not come into contact with the substance under investigation, so at important developmental stages there is no exposure. If a substance is capable of interfering with development processes during these phases, a study designed along standard lines will not reveal the potential impact. In developmental toxicity studies, it is normal for animals (the so-called F1 generation) to be exposed in the womb and sometimes when suckling, but they are then killed immediately after weaning at the latest. As a result, the opportunity to gather information about any possible long-term effects is lost. Only in multi-generation reproductive toxicity studies are F1 animals that have been exposed in the womb allowed to reach adulthood and produce a second generation (F2). Even in studies of this type, however, insufficient importance is attached to the information that could be gleaned from F1 animals exposed in the womb. Neither immune system functionality (resistance) nor central nervous system functionality (behaviour, learning ability, memory, motor skills) are thoroughly investigated, if they are investigated at all. Similarly, no routine attention is given to parameters that could indicate subtle disruptions of the hormone balance. Furthermore, F1 animals are studied only in small numbers, so low-incidence hormone-related effects can remain undetected (Gra01, McI00, Fos02, NTP01).

It is possible that an effect which is overlooked in this way could in fact be the critical effect of a substance. Such an oversight would have implications for calculation of safe exposure levels. Indeed, there is evidence to suggest that more thorough study of functional parameters in F1 animals can lead to lower NOAELs, and thus to lower ADIs or ARfDs. Examples have come to light mainly in the field of developmental neurotoxicology. A retrospective analysis of nine developmental neurotoxicity studies of pesticides submitted to the EPA for approval (Mak98) revealed that, for two of the nine pesticides (carbaryl, emamectin), the NOAEL for developmental neurotoxicity was lower than or equal to the NOAEL for developmental toxicity, reproductive toxicity and acute and subchronic neurotoxicity. Middaugh *et al*. recently evaluated the contribution made by behavioural neurological study of F1 animals to the toxicological characterisation of substances (Mid03). They observed that, with 15 per cent of the 174 substances examined (more than 80 per cent of which were medicines), F1 behavioural parameters

along with other F1 parameters defined the no-effect level in F1 animals. Where 2.6 per cent of the investigated substances were concerned, F1 behavioural parameters were the most sensitive F1 parameters and therefore the sole NOAEL determinants in relation to F1 animals. With 3 per cent of the substances, F1 behavioural parameters were found to be influenced even though no maternal toxicity was detected. Finally, the data presented in Annex C provides further evidence that targeted research into functional and other parameters of the nervous system of F1 animals can reveal effects that occur at exposure levels below the lowest levels to induce effects in the standard tests. It seems reasonable to suppose that more thorough investigation of possible immunotoxic and hormonal effects on F1 animals might also lead to lower NOAELs being defined for a number of substances. This would have implications for the calculation of both ADIs and ARfDs, since it is believed that, during sensitive periods of development, developing organisms can be affected even by isolated exposure incidents (Bil00; EPA98, Har00; Mor00; Raa01; WHO97).

6.3 Proposed modifications to toxicological testing procedures

6.3.1 Standard tests

The Committee believes that the present toxicological testing procedures should be modified in order to improve the detection of potential effects on developing individuals. In the USA, the 10x Toxicology Working Group at the Environmental Protection Agency (EPA) has recommended adding three further types of study to the standard series that manufacturers are required to carry out (see Table 8): one to look for acute or subchronic neurotoxicity in adult laboratory animals, one to look for immunotoxicity in adult animals and, finally, one to look for developmental neurotoxicity (Kim01a, Kim01b, Sch00b, Sch00c; see also Fen01). The two additional studies with adult animals are important to enable the identification of organs which might be targets in young individuals, and which therefore warrant closer study, particularly if antenatal exposure or exposure shortly after birth is likely. The third additional study is intended to allow better characterisation of developmental toxicity.

Table 8 Recommended additions to the list of studies required for all pesticides in the USA (Kim01a).

Acute/Subchronic neurotoxicity
Immunotoxicity
Developmental neurotoxicity

In the Netherlands (and other EU countries), pesticides whose chemical structure is such that they are expected to have a neurotoxic effect already have to undergo neurotoxicological testing as a matter of course (CTB02). For reasons of efficiency, the Committee does not favour adding to the list of required studies. The Committee would, however, like to see additional test parameters incorporated into the existing standard studies. The aim should be to enhance the screening sensitivity of the standard tests, so as to reduce the risk of effects on developing organisms being missed. The OECD* guidelines for twenty-eight-day oral toxicity studies was revised a few years ago with a view to enhancing the detection of immunotoxic effects, and further revisions are proposed (Bar02). However, only young adult animals are exposed in studies of this kind. In order to identify substances that interfere with the development of the immune system, the Committee would like to see immune parameters incorporated into the multi-generation reproductive toxicity study (see Col99, Ric02, Hol03, Lov03). The additional parameters should include both structural parameters (histopathology of lymphoid organs, numbers and types of lymphocytes in the blood) and functional parameters (such as DTH response) (Lov03). The Committee further recommends the incorporation in this study of functional, neurological parameters, in order to provide an initial indication as to whether the substance might be harmful to development of the nervous system. A recent update of the OECD guidelines on two-generation reproductive toxicity studies already includes a recommendation that parameters such as motor activity, sensory functions and reflexes should be investigated in F1 animals (OECD01). Other relevant parameters that might be examined include those listed in the guidelines for developmental neurotoxicity studies (see below). The OECD is currently working on revisions of its guidelines for developmental toxicity studies and multi-generation reproductive toxicity studies, with a view to enhancing detection of the more subtle consequences of hormone disruption (Tir02). In this context, it has been recommended that greater numbers of first-generation offspring should be allowed to reach adulthood and reproduce, in order to increase the likelihood of low-incidence abnormalities being detected (Gra01, McI00, Fos02, NTP01).

6.3.2 *Follow-up research*

The findings of the extended standard studies should subsequently be carefully assessed to determine the need for so-called second-tier studies – detailed follow-up research focusing on particular effects. To this end, it is very important that good, validated testing methods are developed and defined in guidelines. The EPA working group referred to above has identified a number of study types for which guidelines are required (Table 9).

* Organisation for Economic Co-operation and Development

Table 9 As yet undeveloped second-tier studies, which should be performed only where necessary (Kim01a)

Toxicokinetics
Direct neonatal exposure
Specialised developmental neurotoxicity
Developmental immunotoxicity
Developmental carcinogenity
Endocrine disruption

The Committee endorses the working group's recommendations, except in that the Committee would prefer to see the developmental neurotoxicity study, which the EPA working group has suggested should be compulsory for all pesticides (see Table 8), added to the list of second-tier studies. EPA guidelines on this type of study have been available for ten years and are currently due for review (Kim01a, Kim01b, Cla00, Sch00c). Draft OECD guidelines on this topic were also published several years ago (OECD99). Both sets of guidelines identify the functional endpoints that should be measured and allow a degree of freedom in the selection of test methods. However, some of the methods suggested in the guidelines have yet to be developed and validated sufficiently to enable effective neurotoxicological evaluation (Has03; Kau03). Neuropathological techniques still require refinement, as does the methodology for studying learning-related and memory-related effects during neurotoxicity screening.

A review of the carcinogenity study (with *in utero* exposure, as sometimes performed in the USA) is not yet being considered in Europe (EU98). With regard to the carcinogenic significance of exposure during the early phases of life, the Committee refers the reader to the final EPA report on this subject, which is due for publication shortly (see EPA03 and Sch03). Once this report is made available, it will be possible to assess the need for a separate Health Council committee to advise the government on this topic.

It is very important that the modification of existing study protocols and the development of new ones take place in the context of international consultation. The reason being that internationally accepted toxicity studies are a prerequisite for internationally accepted safety standards. Numerous developments are in progress that promise to be of significance in this regard. The Committee believes that such international initiatives warrant energetic support and would like to see the Netherlands play a full part in the contribution of expertise.

6.3.3 *The use of laboratory animals*

More thorough toxicological study could lead to an increase in the number of laboratory animals needed for research purposes, which in itself would be undesirable. The Committee believes that the most efficient possible use should be made of laboratory animals. To this end, consideration should be given to combining studies where possible (see also Has03, Kim01a). So, for example, F1 animals not required for breeding the F2 generation in a multi-generation reproductive toxicity study could be used for neurotoxicological or immunotoxicological research.

6.4 Conclusions

Developing organisms, including human children, are more sensitive to some substances than adults and less sensitive to others. The standard toxicological testing presently required for pesticide approval purposes is not sufficiently comprehensive to enable all possible effects on a developing organism's organs and organ systems to be detected. Effects on the development of the nervous system and immune system and possibly effects on endocrine-dependent development processes are most likely to go undetected. Such effects can be important for the calculation of safe exposure levels (ADIs and ARfDs). The Committee does not believe it is necessary to add to the list of compulsory toxicity studies. It would prefer to see additional parameters incorporated into the existing standard studies in order to enhance detection of the types of effect referred to. Where the standard study findings suggest there is a need, targeted second-tier studies should be carried out. The Committee considers it very important that appropriate study protocols are developed through international consultation.

Chapter 7

Adequacy of the current safety assessment procedure: use of uncertainty factors

Safe exposure levels, expressed in the form of the ADI and ARfD, are calculated for a substance by applying a number of uncertainty factors to the NOAEL obtained through toxicological research. Three of these factors are discussed in this chapter, with particular consideration for the protection of children. The factors in question are those designed to compensate for the uncertainty associated with:

- Extrapolation from animals to humans
- Variation within the human population
- Lack of necessary toxicity data.

A future Health Council committee will shortly look more closely at the use of uncertainty factors for the calculation of safe exposure levels.

7.1 Uncertainty factor relating to extrapolation from animals to humans

In order to study the developmental toxicity of pesticides, it is necessary to carry out tests using (unborn) young animals. However, the extrapolation of the findings of such tests to humans is particularly difficult, since the various stages of development take place at different times in different species (Den98, Gin04, Lar98, Øst98). In humans, for example, the brain growth spurt occurs in the third trimester of the pregnancy, while in rats and mice it is in the first three to four weeks after birth. The enzyme systems that detoxify and break down xenobiotic substances also develop earlier in humans than in

rodents (Lar98, Ren98a). Such differences need to be taken into account when designing tests and interpreting the results.

The availability of data on humans – from epidemiological research or from clinical pharmaceutical research, for example – can reduce the uncertainty associated with extrapolation from animals to humans (Bur99). Furthermore, manufacturers have in recent years started testing pesticides on human volunteers – although not until extensive toxicity tests have been performed on laboratory animals, of course (EWG98). By doing so, the manufacturers hope to remove the need to apply an extrapolation uncertainty factor, or at least the need to apply such a large factor. However, doubts have been expressed, particularly in the USA, as to whether this would actually lead to better risk assessment and whether it is ethical to deliberately administer low doses of potentially toxic substances to people (EWG98, EPA00b, NRC04). The Committee would point out that such research can at best make only a small contribution to improved assessment of the risk to (unborn) children, since the volunteers involved are necessarily adults. Nor is there any prospect of neurotoxic substances being deliberately administered to immature subjects in the United States, as this would definitely be considered unethical (NRC04) because of the unknown risks (EPA00b). Extrapolation from the findings of research with young laboratory animals will therefore remain necessary.

7.2 Uncertainty factor relating to variation within the human population

Questions have been raised concerning the adequacy of the uncertainty factor of 10 that is typically used to compensate for inter-individual variation, given the great differences that exist between people, and particularly between children and adults. Renwick argues that 10 is a suitable default value, although a higher figure should be used in certain cases (Ren98b). He suggests breaking the factor down into one element to allow for toxicokinetic differences (4.0 or 3.2) and another to allow for toxicodynamic differences (2.5 or 3.2) (Ren98a, Ren98b). Renwick regards a higher uncertainty or safety factor for weaned children to be unnecessary on the grounds of age-related toxicokinetic differences. He points out that children are often able to expel xenobiotic substances from the body more quickly than adults, and suggests that this may actually compensate, at least partially, for any increased organ sensitivity during development (Ren98a). Many scientists share this view (Bru00, Bur99, Dou02).

However, recent studies have indicated that the application of a factor of 3.2 to compensate for toxicokinetic differences may in some cases afford insufficient protection to neonates or, occasionally, to older children (Dor01a,b; Dor02a; Dor03a,b). Kalberlah and Schneider take the view that the factor of 10 traditionally applied for differences within the human population is probably sufficient to compensate for toxicokinetic differences between healthy adults (Kal98), although they also make the point that it is

unclear how far the (large) variation resulting from genetic polymorphisms in the enzymes that detoxify xenobiotic substances is accommodated. They conclude that an unknown proportion of vulnerable groups, such as children, the elderly and the infirm, are not protected. They also argue that the factor of 10 makes little allowance for toxicodynamic differences, and point out that very little quantitative data on such differences has been published in the academic press. Accordingly, they argue that a factor of 25 would normally be more appropriate, being the approximate product of a factor of 8 to allow for toxicokinetic differences and a factor of 3 to allow for toxicodynamic differences. In exceptional cases, however, they suggest that an even higher factor may be needed to cover the toxicodynamic differences between children and adults. Where lead is concerned, for example, they say a component factor of 6 is needed because of the sensitivity of the developing nervous system.

Another Health Council committee previously made the following point regarding inter-individual variation (GR85):

> It may nevertheless be said that a good toxicity study for the purpose of defining a NOAEL should involve the exposure of pregnant animals, suckling animals, young and old animals. This aspect is covered by the variation, so that in humans it is not normally necessary to treat pregnant women, infants or children as special risk groups (…).

The Committee in question thus took the view that allowance for development-related differences in vulnerability should be made in the toxicological database, rather than in the uncertainty factor relating to variation within the human population. The present Committee endorses this view. However, it also believes that, because of the previously highlighted shortcomings in standard toxicological testing in the fields of developmental neurotoxicity, developmental immunotoxicity and endocrine disruption, toxicological databases rarely make sufficient allowance at present. This Committee also takes the view that an inter-individual variation safety factor of 10 is generally sufficient to afford protection to children, provided that adequate research is conducted into developmental and reproductive toxicity. If data on these matters is lacking, it cannot be assumed that an inter-individual variation safety factor of 10 provides sufficient compensation, even though this factor is regarded as making proper allowance for age-related differences in sensitivity. The reason being that the safety factor is applied to an NOAEL that relates to one particular effect that is deemed to be critical. So if, for example, the critical effect is liver damage, the factor serves to compensate for the fact that some people suffer such damage at lower levels of exposure than others. However, it cannot be assumed in the absence of specific data that, say, the developing nervous system or immune system will not be harmed in the event of exposure at a level ten times lower than the NOAEL for liver damage.

7.3 Uncertainty factor relating to gaps in the data set

7.3.1 The FQPA factor used in the United States

In the United States, the 1996 Food Quality Protection Act (FQPA) requires that an enhanced level of protection is afforded to children; specifically, the EPA has to apply a further safety factor of 10 (known as the 'FQPA safety factor'), in addition to the factor of 100 that has traditionally been applied (Lan04). The value of 10 assigned to the FQPA factor is based upon the extra factor of 10 that the EPA had always used in cases where a pesticide appeared to be harmful to the foetus. In effect, therefore, what the Act does is extend the application of this extra safety factor to protect children against disruption of their development after birth as well (NRC93). The choice of 10 as the value for this factor may have been influenced by the observation of the US National Research Council that vulnerability differences between children and adults can normally be covered by a factor of no more than 10 (NRC93). However, this observation related only to quantitative differences in sensitivity – the possibility that the same effects occur in children and adults at different exposure levels – and not to qualitative differences in sensitivity – the possibility that children and adults are affected in different ways. The Act allows the EPA to apply a lower factor (as low as 1, where appropriate), if it is apparent from the available toxicity data that this is sufficient to protect the safety of children (EPA02a). An EPA working group has recently concluded that, on scientific grounds, it is not necessary to apply an FQPA safety factor for children if a pesticide has been thoroughly tested – in other words, if it has undergone the standard tests and any additional tests that the original data set might suggest are appropriate (Kim01a, see also Sch00a, Lan01). It is then important that all types of effect and the most sensitive test parameters are taken into account, along with information regarding bioaccumulation, the gradient of the dose-response curve and the mechanism of action. Only by taking account of all such factors can one be sure that all population groups, including children, are properly protected. The FQPA safety factor for children used in the USA should therefore been regarded as a factor designed to compensate for the data set being incomplete with regard to the risks for children. As such, it is a temporary emergency measure, for which there is no need anymore once the risks that a substance poses for children have been thoroughly investigated (Fen01).

The EPA is currently reviewing the adequacy of all pesticide dossiers with regard to the risks to children. In cases where the available data is not sufficient to allow confident estimation of the risks to children, an FQPA safety factor of more than 1 but no more than 10 is being applied on the basis of a weight-of-evidence approach. Account is taken of the size of the available database and the quality of the studies conducted. In addition,

the EPA tries to estimate the likelihood that any missing data might be materially relevant to characterisation of the substance's toxicity, and thus to the outcome of the risk assessment as a whole. In practice, what this amounts to is that the FQPA safety factor is being reduced to 1 – which effectively amounts to its exclusion – wherever the standard studies (supplemented where appropriate by neurotoxicity studies with adult animals) have been conducted properly and no evidence has been found to suggest increased sensitivity during development (see EPA98, EPA02a). Where there are gaps in the data – where, say, there is no information about neurotoxicity in adult animals, so that it is not really possible to determine whether an additional developmental neurotoxicity study is needed – an FQPA safety factor of 3 is typically applied. Where the standard study findings or published scientific literature include definite evidence of developmental toxicity, for which proper provision is not made in the ADI (or, more precisely, its US equivalent, the cRfD) or ARfD (EPA98, EPA02a), the statutory factor of 10 is retained. The EPA has now specified FQPA safety factors for numerous organophosphates (CU01). Where thirty-six of the forty-nine substances are concerned, the FQPA factor has been dropped, for a further seven it has been reduced to 3, and for the remaining seven it has been left at 10 (see Table 10). The EPA is now considering how, pending the availability of good studies and study data, allowance should be made for the lack of data on endocrine disruption and developmental immunotoxicity (Gol00). It has recently been decided that, where the endocrine-disrupting substance vinclozolin is concerned, the FQPA safety factor should remain at 10 (EPA00c).

Application of the FQPA safety factor has brought about a situation where the United States has different standards for certain population groups, namely children and women of childbearing age. This is because it is felt that an FQPA safety factor is necessary only for these population groups. Hence, the ADI (cRfD) and the ARfD are divided by the FQPA factor only in relation to these population groups. This results in new safe intake levels, referred to respectively as the chronic and acute Population Adjusted Doses (cPAD and aPAD) (EPA02a).

Table 10 Organophosphate pesticides for which the EPA has retained a value of 10 for the *Food Quality Protection Act* safety factor, or has reduced the value to 3 (CU01).

FQPA factor 10	FQPA factor 3
Chlorpyrifos	Cadusafos
Chlorpyrifos-methyl	Dichlorvos
Dicrotophos	Isofenphos
Mevinphos (chronic toxicity)	Methamidophos
Parathion-methyl	Mevinphos (acute toxicity)
S,S,S-tributyl phosphorotrithioate	Phorate
Trichlorfon	Pirimiphos-methyl

7.3.2 *The European approach*

Among European researchers and risk assessors, the prevailing view is that no special uncertainty factors for children are necessary. Unusually high uncertainty factors should be reserved for cases where the toxicological database is considered insufficient for adequate assessment of the health risks, including those faced by children (Kui00, Lar98, Nie01, Raa01, Ren00, Wol02). However, it is not clear when a database should be considered insufficient. Some years ago, the EU's former Scientific Committee for Food (SCF) recommended investigating whether all the data that now has to be provided by a manufacturer is available for all pesticides assigned an ADI by the JMPR prior to 1977 and for all substances for which the JMPR has never set an ADI. It was suggested that, where gaps were found in the data, consideration should be given to corrective action on a case-by-case basis. The SCF did not have an immediate solution for the problem referred to above, namely that some substances can induce developmental neurotoxic, developmental immunotoxic or reproductively toxic effects that are not brought to light by standard studies currently undertaken. The SCF accordingly recommended obtaining further advice from appropriate experts (EU98).

7.3.3 *The Committee's view*

The Health Council Committee takes the view that, in the fields referred to, there are indeed important gaps in the required toxicological data set, even though pesticides are among the most thoroughly investigated substances. It may be said, in fact, that the available data set is incomplete, as a result of which full evaluation of the risks to the developing individual is not always possible. The best way of making allowance for the associated uncertainty should, the Committee feels, be left for experts to decide on a case-by-case basis (see also Das04). If, on the basis of all the available toxicological data, there is a reasonable suspicion that the critical effect occurs in the developing organism, and if adequate (second-tier) research has not been undertaken, the Committee considers it appropriate to apply a further uncertainty factor when setting the ARfD and ADI, in addition to the factor of 100 traditionally applied.

Where approved pesticides are concerned, suspicion may reasonably be based on scientific data available in the public domain, since it may be that increased vulnerability during development is discovered in the context of research into parameters not addressed by the standard tests. If allowance is not made for such increased sensitivity in the NOAEL and ultimately in the ADI or ARfD, the application of an additional uncertainty factor may be called for.

Data in the standard dossier may also indicate heightened sensitivity during development. The findings of the multi-generation reproductive toxicity study are particularly likely to be revealing in this regard. First generation offspring might, for example, exhibit abnormalities in brain or thymus weight following exposure in excess of a certain level. Under such circumstances, it would be reasonable to suppose that other neurological or immunological parameters not addressed by the standard tests, such as functional parameters, could be affected at lower dosages. In a case of this kind, the application of an additional uncertainty factor would be appropriate.

If the findings of a subacute or semi-chronic study with young adult animals suggest that the immune or nervous system is among the most vulnerable organ systems to the substance under investigation, the influence of the substance on the development of these systems should be investigated more closely. As long as data regarding any such possible influence is unavailable, an additional uncertainty factor is required.

Finally, suspicion that there may be increased sensitivity to a given substance during development may reasonably also be based on data concerning closely related substances.

However, if the standard tests have been carried out in full and in accordance with the current guidelines, and if neither the standard test findings nor data in the public domain nor structural similarities suggest heightened sensitivity during development, the Committee sees no reason to apply an additional uncertainty factor.

Where it is felt that an additional uncertainty factor is needed, it may often be the case that the available toxicological data provides an inadequate scientific basis for assigning a value to the factor. Under such circumstances, it would seem appropriate to follow the practice of using a factor of 10 where relevant research data is lacking (Dou02; EPA02a). Alternatively, one might adopt the US Food Quality Protection Act values of 10 or 3.

The Committee regards the introduction of an additional uncertainty factor as a temporary measure. As soon as the necessary additional research data becomes available, consideration should be given to adjusting the ADI and ARfD in the light of the new information. International consultation is essential at all stages, since it is undesirable for different countries to set different ADIs and ARfDs.

The Committee recommends that priority be given to the evaluation of pesticides to which the US authorities have assigned an FQPA factor of more than 1 (for a list of such products, refer to CU01; see also Table 10). Priority should also be given to pesticides where there is only a narrow margin between the calculated or measured levels of acute or chronic exposure and the intake levels believed to be safe (i.e. the ARfD and the ADI, respectively).

7.4 Conclusions

Laboratory animals and humans differ in the timing of their development. This makes it difficult to extrapolate research data from animals to humans. Such differences need to be taken into account when designing studies and interpreting the results. There is limited scope for conducting research with (adult) human subjects, and the research that can be performed is not capable of yielding all the toxicological data needed for a proper assessment of the risks associated with a substance. This is particularly true with regard to the risks to (unborn) children. For this and other reasons, research with laboratory animals, and with young laboratory animals in particular, remains necessary.

Provided that the possibility of developmental and reproductive toxicity has been properly investigated, the Committee believes that the value of 10 normally assigned to the inter-individual variation uncertainty factor is sufficient to afford protection to children. However, if there are gaps in the data set – if, for example, not enough is known about the influence of a substance on the development of the nervous system, on the development of the immune system or on endocrine-dependent development processes – it cannot be assumed that an inter-individual variation factor of 10 provides sufficient compensation. The reason being that the factor of 10 is applied to a NOAEL determined for a different effect. Without specific data, there are insufficient grounds for believing that the developing nervous system or immune system will not be harmed in the event of exposure at a level ten times lower than the NOAEL for this other effect.

The Committee recommends that the authorities in Europe follow the US lead and consider on a case-by-case basis how best to make allowance for gaps in the standard toxicological research data. The use of a further uncertainty factor when setting the ARfD and ADI, in addition to the factor of 100 traditionally applied, is considered appropriate if there is a reasonable suspicion that the critical effect occurs during development, and if this effect has not been adequately investigated in the standard or second-tier studies. In this context, suspicion may reasonably be based on the findings of the standard toxicological tests, on scientific data in the public domain, or on information regarding closely related substances. Where it is felt that an additional uncertainty factor is needed, it may often be the case that there is inadequate scientific basis for assigning a value to the factor. Under such circumstances, a pragmatic approach is required. The Committee regards the introduction of an additional uncertainty factor as a temporary measure, which should be reviewed as soon as the necessary additional research data becomes available.

Chapter 8

Adequacy of the current safety assessment procedure: the estimation of exposure

The risk assessment procedure involves comparison of the anticipated level of exposure – assuming that the product is approved for use as requested – against the level of exposure that is considered to be safe. In this chapter, the Committee considers whether the process of estimating the anticipated levels of consumer exposure takes sufficient account of children's specific food consumption and behaviour patterns. Consideration is also given to simultaneous exposure to several pesticides and to exposure to a single pesticide with various applications.

8.1 Food consumption and residue data

The Dutch National Food Consumption Surveys provide reliable data on food consumption by people more than one year old in various population groups in the Netherlands (VCP98). From this data, it is apparent that children between one and six years old eat four to five times as much fruit per kilogram bodyweight as adults and roughly twice the amount of vegetables, potatoes and cereal products (see Table 1). Since children in this age band eat only small quantities of special infant/toddler food products (Voe02), which under EU law are not allowed to contain pesticide residues (EU98, EU99), it must be assumed that exposure of this group to pesticide residues is correspondingly high compared with that experienced by adults (see chapter 2). The Committee therefore endorses the policy of the Board for the Authorisation of Pesticides (CTB), which now makes a separate assessment of the risk to children of between one and six years old, based on the specific food consumption patterns of this population group. Given that the

food consumption patterns of population groups can change over time, the Committee believes that continued systematic monitoring of such patterns is important.

Children less than one year old have not so far been included in the Dutch National Food Consumption Surveys. However, recent research with toddlers has revealed that children aged nine months eat three to four times as much fruit per kilogram of body-weight as children of between one and six years old (Voe02, see chapter 2). Since special baby foods account for much of the fruit consumed by the younger children, exposure to fruit-borne pesticides at nine months is probably only about one and a half to two times as high as it is between one and six years old. Nevertheless, the Committee believes that this highlights the importance of systematically collecting data not only on the eating habits of adults and older children, but also on the diets of children aged of six to twelve months. Without such data, it is not possible to make an informed decision as to whether separate risk-evaluation is necessary for this age group. Since eating habits tend to change considerably in this phase of life, the first step should be to seek to establish the best way of monitoring fruit and vegetable consumption within the relevant age group.

With regard to research into the presence of pesticide residues in agricultural produce, the Committee would point out that sampling programmes are often aimed at ensuring compliance with the law, in the shape of the MRL. Consequently, there is a bias towards the investigation of suspect batches, making the resulting data unsuitable for use in the estimation of actual exposure levels in different population groups. No protocols are currently available for more representative sampling systems. Other complications include lack of data on the influence that the preparation of food has on residue concentrations, or on variations in residue concentrations between individual units of fruit or vegetables (Kro02). The reliability of the risk assessment procedure would be enhanced by making good these gaps in knowledge.

8.2 Exposure levels

In order to check whether exposure to approved pesticides is actually within safe limits (ADI and ARfD) in practice, so-called ‘total diet studies’ are sometimes performed, both in the Netherlands and in other countries. In these studies, the food that consumers eat in a single day is prepared in the same way as the consumers prepare it at home, then analysed to determine its pesticide content. There are various types of total diet study, in which complete daily diets, foodstuff groups or individual foods are analysed. These studies can relatively quickly provide a general picture of the consumption of a wide range of substances, provided that the analyses are based on food samples that are representative in terms of the season, the age and sex of the consumer and other relevant characteristics. When data on individual foods is linked to food consumption data, it is also

necessary to take account of the limitations of the food consumption database. A number of older Dutch and US studies indicated that the average and maximum levels of exposure to pesticides in both children and adults were well below the relevant ADIs (Bau99, Goe87, Gre87a,b,c, Gre88a,b, Gun95a,b). However, these studies were not suitable for determining whether the levels of exposure experienced by consumers whose intake was (chronically) well above average (consumers on the 95th, 99th or 99.9th percentile, for example), were also safe.

Using total diet study methods, Boon *et al.* (Boo03a) and López *et al.* (Lóp03) sought to establish how much food-borne pesticide was consumed on a daily basis by children of eight to twelve months old in, respectively, the Netherlands and the Basque country. In both studies, each of which looked at six pesticides, it proved that the 99.9th percentile consumption of all pesticides was well below the relevant ARfDs. The measured exposures were lower than those forecast using probabilistic methods, which in turn were lower than corresponding worst case deterministic calculations. It would therefore seem that the calculation methods used in the safety assessment of pesticides do not underestimate actual acute levels of consumer exposure.

In recent years, consumer exposure to pesticides has also been investigated by means of urine analysis (Apr00, Bar04, CDC03, Cur03, Heu01, Heu04). The studies carried out to date have focused mainly on (metabolites of) organophosphates. Some of these urine analyses have provided a less favourable picture of the exposure situation than the food analyses (Cur03, Heu01, Heu04, Sch04). However, interpretation of the data is difficult (CDC03). For one thing, various organophosphates with very different ADIs can account for the presence of a given metabolite in the urine. The possible contribution of several pesticides means that intake of a particular pesticide cannot easily be estimated or compared with the corresponding ADI. Furthermore, it is not clear to what extent metabolites in urine were already present as such in the food and not therefore consumed in the form of pesticide (Heu01, Heu04, Kri03). On the other hand, the metabolites excreted in the urine do not represent the entire amount of organophosphate taken in (Cur03). Finally, sources other than food may contribute to the presence of metabolites in the urine; pesticides used for domestic pest control may also find their way into the body, for example. Further research is required to explain the discrepancies between the findings of food analyses and urine analyses.

Although intake in excess of the ADI is undesirable, brief incidental periods of excessive intake are acceptable, provided that average exposure over the longer term remains below the ADI; the point being that the ADI is a measure of the safe level of long-term intake (Lar99, WHO97). On the other hand, the Committee does regard any intake in excess of the ARfD as unacceptable. Intake above the ARfD is particularly undesirable where pregnant women and children are concerned, since a single exposure peak during a sensitive development window can cause permanent health damage. A

concurrent view has previously been expressed by the RIVM (Raa02). Hence, the Food and Consumer Product Safety Authority immediately removes from the market any products found to have a residue concentration above that legally permitted on the basis of the MRL, if consumption of such foodstuffs would lead to the ARfD being exceeded (VWA04). According to the WHO, slightly excessive consumption need not necessarily be immediately harmful, since the ARfD does incorporate a safety factor of 100 (WHO97). Nevertheless, the Committee believes that action should always be taken in the event of the ARfD being exceeded.

When a probabilistic estimate of exposure is used for risk assessment purposes, the CTB currently compares the 99.9th exposure percentile against the ARfD. This implies that a pesticide is considered acceptable if no more than one in a thousand Dutch people a day exceeds the ARfD for that pesticide. The selection of an appropriate percentile is in fact a policy issue. Nevertheless, the Committee recommends that consideration be given to the adoption of a higher percentile, since any exposure in excess of the ARfD is in principle unacceptable. The Committee recognises, however, that the uncertainty inherent in the calculations increases rapidly at higher percentiles. The Committee also recommends the practical implementation of probabilistic exposure estimation methods for both adults and children. Within the international scientific community, such methods are currently undergoing active development, but a number of design points have yet to be finalised. The Committee believes that it is important for the Netherlands to contribute to international debate in this field.

8.3 Cumulative and aggregated exposure

People are sometimes exposed more or less simultaneously to several pesticides with the same mechanism of action. This phenomenon is known as cumulative exposure and can occur with, for example, organophosphates (insecticides that all inhibit the enzyme acetylcholinesterase). In the event of cumulative exposure, the combined effect of the pesticides can be harmful, even if exposure to each of them is at a level that would be safe in isolation. This problem is not specific to children. However, with the risk to children's health in mind, the US National Research Council (NRC93) has highlighted the possible dangers of cumulative exposure. In response to the NRC's recommendations, the Food Quality Protection Act requires that the effects of cumulative exposure be taken into account when assessing the risks posed by pesticides. That can be done by assigning a so-called 'toxicity equivalence factor' (TEF) to every compound with the same mechanism of action. The TEF is an expression of a substance's toxicity relative to a given reference substance. Using the TEF, the exposure to each individual substance can be converted into a corresponding level of exposure to the reference substance. By aggregating the doses for all the substances with a common mechanism of action, one can cal-

culate an overall figure in terms of exposure to the reference substance. This can then be compared with the ADI or ARfD for the reference substance. This kind of approach has for some years been used around the world (including the Netherlands) when assessing the risk associated with dioxins, furans and dioxin-like PCBs (see, for example, Ber98). The EPA has now developed this method further and assessed the risks of cumulative exposure to organophosphates (EPA01a, EPA02b). Other bodies in the USA, Denmark and the Netherlands have made similar calculations for this group of pesticides, either on the basis of food analyses, or on the basis of urine analyses (Boo03b, Cas03, Coc02, Jen03, Lef00, Luij00, NRC93, Wil98, Wil99). These calculations have produced a varied picture. Some of the calculations suggest that a (small) number of the children and pregnant women may consume more acetylcholinesterase inhibitors than would seem appropriate on the basis of safe chronic or acute intake levels. By contrast, other calculations appear to indicate that cumulative exposure to acetylcholinesterase inhibitors does not represent a risk. The EPA intends to perform cumulative risk assessments for other groups of pesticides with common mechanisms of action, such as triazine herbicides (EPA02c).

People can come into contact with pesticides not only through their food, but also as a result of, for example, treating insect infestations in or round the house, killing weeds on paved areas or using fungicides. Furthermore, some pesticides are used as human or veterinary pharmaceuticals. These various applications all contribute to the overall level of exposure to a given pesticide – what is known as aggregated exposure. In the USA, the Food Quality Protection Act requires that all these exposure routes are taken into consideration when assessing the risk associated with a substance. Appropriate assessment techniques are currently under development (EPA01b).

However, so far as the Committee is aware, neither cumulative nor aggregated risk assessment currently form part of the approval procedure elsewhere. The JMPR has concluded that a number of issues need to be resolved before either form of assessment can be incorporated into the JMPR/CCPR process (Dor00). It is felt that aggregated risk is mainly a consequence of national circumstances and should therefore be addressed at the national level. Cumulative risk, on the other hand, is linked to substance properties and should therefore be addressed at the international level, i.e. by the CCPR.

A previous Health Council committee recommended that the risks associated with exposure to combinations of substances that have a common mechanism of action but do not influence one another's action, should be assessed using toxicity equivalence factors (TEFs) and dose addition (GR02**)**. A similar recommendation was recently made by the UK Food Standards Agency (FSA02). The present Committee endorses this recommendation, but attaches the qualification that a cumulative assessment of the risks to children is for the time being compromised by gaps in the scientific understanding of pesticides' mechanisms of action in relation to children. A pesticide's mechanism of

action is not necessarily the same in children as in adults, as research with chlorpyrifos has shown (see chapter 4). Nevertheless, the Committee believes it is important that assessment of the risk associated with an individual pesticide should take account of the possibility of simultaneous exposure to other pesticides and of the possibility of exposure via different routes (see also FSA02). The Committee accordingly recommends that the Netherlands should actively support international activities in this field where possible.

8.4 Conclusions

The Committee supports the performance of separate risk assessments by the CTB for children of between one and six years old, in order to take account of their specific food consumption pattern. In addition, because food consumption patterns are liable to change, the Committee would like to see the continued collection of data on the eating habits of children, including those aged six to twelve months. A suitable monitoring system still needs to be developed for children in this age category, however.

With a view to increasing the reliability of exposure calculations, it is important that the sampling systems used in the context of residue analysis are geared to obtaining a more representative picture of exposure in the population. More information is required about how the way food is prepared influences residue concentrations and about variations in residue concentrations between individual units of fruit and vegetables. Food consumption analyses suggest that the exposure of adults and children to approved pesticides is well below the corresponding ADIs and ARfDs, but urine analyses show a less favourable picture. Further research is needed in this area.

The Committee recommends further development of probabilistic methods for calculating exposure to pesticides in adults and children. It is considered particularly important that pregnant women and children do not exceed the ARfDs, since a single exposure peak during a sensitive development phase can cause permanent damage. The Committee accordingly recommends that consideration be given to changing the basis on which probabilistic acute exposure data is used to assess the acceptability of a pesticide; at present, the 99.9^{th} exposure percentile is normally used, but a higher percentile might be more appropriate.

The Committee also recommends that assessment of the risk associated with an individual pesticide should take account of the possibility of simultaneous exposure to other pesticides with the same mechanism of action and of the possibility of exposure via routes other than food intake (see also FSA02). The Committee would therefore like to see the Netherlands actively supporting international activities aimed at the development of appropriate methods.

Chapter 9

Conclusions and recommendations

In this final chapter, the Committee presents its conclusions and recommendations, which also form its answers to the Ministers' questions.

The Committee's findings and conclusions:

1 Evaluations of toxicological data on a large number of substances, including pesticides, have shown that, with some substances, the critical (i.e. most sensitive) effect is an effect on the developing individual, whereas with other substances, the critical effect involves the adult organism. In other words, children are more sensitive to some substances than adults, but less sensitive to others.

2 Developing organisms are physiologically different from adult organisms. The earlier the stage of development, the greater the difference. As a result, the effects of substances on developing organisms can differ from the effects on adult organisms in both qualitative and quantitative terms. Hence, the findings of toxicological research with adult organisms cannot necessarily be extrapolated directly to developing organisms. It is therefore necessary to address all stages of development in toxicological research.

3 Standard toxicological testing focuses explicitly on developing organisms, in the developmental toxicity study and in the multi-generation reproductive toxicity study. Together, these studies cover all stages of development. However, the parameters measured in standard studies are too limited to be sure of detecting effects on an all important organs or organ systems. Effects on the development of the nervous

system and immune system and possibly effects on endocrine-regulated development processes are particularly likely to go undetected. Most likely to be overlooked are changes resulting from exposure during development, which only manifest themselves later in life. Consequently, there is insufficient insight into what levels of exposure are safe for the developing nervous and immune systems, or for endocrine-regulated processes.

4 Information on the influence of pesticides on the development of the nervous system, the immune system and endocrine-regulated processes is important not only for the calculation of safe chronic exposure levels (expressed in the form of ADIs). It is also relevant for the derivation of safe short-term intake levels (ARfDs). The reason being that, where some substances are concerned, brief exposure during a sensitive period in the development of an organism can lead to permanent structural or functional damage.

5 If developmental toxicity data is lacking, it cannot be assumed that an inter-individual variation safety factor of 10 provides sufficient compensation. The reason being that the safety factor is applied to an NOAEL that relates to one particular effect that is deemed to be critical. So, if for example the critical effect is liver damage, it cannot be assumed in the absence of specific data that, say, the developing nervous system or immune system will not be harmed in the event of exposure at a level ten times lower than the NOAEL for liver damage. Thus, it is also unclear whether the present calculated safe intake levels (ADI and ARfD) always afford sufficient protection to young individuals in periods of increased sensitivity during development.

6 If the foetal phase is the most sensitive phase for short-term exposure to a given substance, and if the safe intake level (ARfD) is set at a level that affords protection to the foetus, this level may be more stringent than is really necessary to protect children and adults other than women of childbearing age. Whether separate (higher) ARfDs should be set for these other population groups, as some commentators have argued, or whether the ARfD for women of childbearing age should apply to all population groups, is a policy issue.

7 The present risk assessment rightly takes account of the specific food consumption patterns of children of between one and six years old.

8 There is presently no material evidence that the development of children is in practice harmed by the presence of pesticide residues in food. However, insufficient research has so far been performed to be sure that they are not being harmed. Furthermore, it would be difficult to detect any effects that there might be on, for example, behaviour, learning ability, motor skills, immunity or fertility. It has been scientifically demonstrated that some substances, such as PCBs, dioxins and lead, can adversely affect the development of children.

The Committee makes the following recommendations:

1 In view of the results of animal research, the Committee recommends that the toxicological research performed in the context of pesticide safety assessment should focus more on developing organisms. The number of toxicological studies that a manufacturer is required to perform as standard need not be increased. However, the existing research protocols should be improved. In particular, parameters relating to effects on development of the nervous and immune systems and on the hormone balance should be added to the protocol for the multi-generation reproductive toxicity study. If more first-generation offspring animals were allowed to reach adulthood and reproduce, it is more likely that low-incidence abnormalities resulting from hormone disruption would be detected.

2 If the existing research protocols were improved and the various toxicity studies combined wherever possible, with more efficient use of laboratory animals, it would be possible to obtain more relevant information regarding the toxicity of a pesticide without significantly increasing the testing burden.

3 Standard testing, including the (extended) multi-generation reproductive toxicity study, needs to serve as a screening tool. If any evidence is found to suggest that the development of organisms might be harmed by the substance under examination, additional problem-specific follow-up research should be performed. In effect, this is already a requirement, but it is important that good, validated research methods are developed for various types of follow-up study, such as developmental neurotoxicity studies and developmental immunotoxicity studies. There are international initiatives in progress with a view to drawing up research protocols. The Committee recommends Dutch support for these activities.

4 As long as the present study procedures have shortcomings in terms of their ability to reveal any harm that may be caused to developing individuals, the available data set on the toxicity of a pesticide may be regarded as incomplete. The best way of making allowance for the associated uncertainty should, the Committee feels, be left for experts to decide on a case-by-case basis. If, on the basis of all the available toxicological data, there is a reasonable suspicion that the critical effect occurs in the developing organism, and if adequate (second-tier) research has not been undertaken, the Committee considers it appropriate to apply a further uncertainty factor when setting the ARfD and ADI, in addition to the factor of 100 traditionally applied. International consultation is essential in this context, since it is undesirable for different countries to set different ADIs and ARfDs.

5 Where it is felt that an additional uncertainty factor is needed, it may often be the case that there is no adequate scientific basis for assigning a value to the factor.

Under such circumstances, it would seem appropriate to follow the established practice of using a factor of 10 where relevant research data is lacking, or to adopt the US policy of using a factor of 10 or 3, depending on the circumstances of the case.

6 The Committee regards the introduction of an additional uncertainty factor as a temporary measure. As soon as the necessary additional research data becomes available, consideration should be given to adjusting the ADI and ARfD in the light of the new information.

7 Pesticides for which, following reassessment by the EPA, the US authorities have retained an additional uncertainty factor for the protection of children (the so-called Food Quality Protection Act factor) should have priority in the Dutch/European reassessment of pesticides on the basis of their toxicity to developing organisms. Priority should also be given to pesticides where there is only a narrow margin between the calculated or measured levels of exposure and the exposure levels believed to be safe.

8 Food consumption patterns – including children's food consumption patterns – can change over time. The Committee therefore considers it very important that future Dutch National Food Consumption Surveys continue to focus on obtaining information on children's eating habits. It is recommended that food consumption data also be collected for children between six and twelve months old, since such data is required to determine whether a separate risk assessment is necessary for this age group. Since eating habits tend to change considerably in this phase of life, the first step should be to seek to establish the best way of monitoring food consumption within this age group.

9 The possibility of simultaneous exposure to several pesticides with the same mechanism of action, or of simultaneous exposure via various routes (food, water, use in and around the home) should be systematically addressed when assessing the risk associated with individual pesticides. However, the methods necessary to make this possible are still under development. The Committee accordingly recommends that the Netherlands lends its expertise to support international activities in this field where possible.

Pesticide policy, including policy with regard to the way the risks associated with these substances are assessed, is increasingly determined by the European Union. The Committee therefore urges the Dutch government to put these recommendations forward for discussion within the European Union (including the European Food Safety Authority, EFSA), where central policy can be formulated.

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Annexes

Annex **A**

Request for advice

On 2 August 1999, the Vice-President of the Health Council received a letter (reference GZB/VVB-993063) from the Minister of Health, Welfare and Sport, the text of which was as follows:

Dear Prof. Hautvast,

In 1993, the US National Academy of Sciences (NAS) published a report entitled *Pesticides in the Diets of Infants and Children*. In this report, it was recommended that when maximum residue limits (MRLs) were set for pesticides in food, explicit account should be taken of the possibility that children may be more sensitive to pesticides and of the higher levels of exposure to which children are subject. At the end of 1996, the law was changed in the US in line with the NAS's main recommendations, and the EPA began making appropriate changes to its procedures, including the introduction of an additional safety factor for children.

Although the issues involved here relate in principle to the ingestion of chemicals in general, the regulation of pesticide residues in food is of particular significance, partly because of the potential toxicity of such substances.

In the Netherlands and the EU, children were not until recently treated as a separate risk group when setting MRLs for pesticides; the assumption was that the ADI (the toxicologically safe limit defined for chronic ingestion) for a pesticide took sufficient account of the sensitivity of children and that the safety factors applied created a sufficiently large margin to ensure that the ADI was valid for all population groups and for average exposure over a lifetime. However, this approach is now under discussion within the EU, partly as a corollary to the definition of standards for processed baby formula and infant foods, which are based upon a strict precau-

tionary principle. When assessing the acceptability of ordinary MRLs for vegetable products, some countries already make explicit allowance for the eating habits of children or of abnormally large consumers of the products concerned. On the subject of ADIs, the Scientific Committee for Food (SCF) recently stated, when advising on the definition of standards for baby formula and infant foods, that the ADI for a pesticide should be valid for all population groups, and that the ADI figure should take account of any heightened sensitivity on the part of children. Thus, the (European) SCF has taken a different stance to that which is in principle favoured in the USA. The SCF made recommendations regarding various other matters, including the criteria for setting and re-evaluating ADIs and for investigating the acceptability of MRLs.

In addition, there has recently been a great deal of interest in the potential acute toxicity of some pesticides (i.e. toxicity in the context of a single ingestion incident).

On several occasions in the past, my ministry has been asked about the Netherlands' current response to the findings contained in the NAS report. Now that the US has actually implemented the report's recommendations by modifying its legislation, and with a debate underway in the EU, it is desirable for the Netherlands to consider the policy implications of the issues involved.

Little or no data is available concerning the possible heightened sensitivity of children to chemicals such as pesticides, and it is not clear what policy should be pursued, partly because the ADIs presently in force differ in their background, their evidential basis and their age.

It has been suggested that the procedure for investigating the acceptability of MRLs should take more account of the possible greater exposure of children to pesticide residues. However, doubts exist regarding the scientific availability, usefulness and relevance of data on extremes within the food consumption patterns of different population and age groups (also partly in relation to acute intake).

With regard to the procedure for investigating the acceptability of MRLs, in view of the considerations set out above, the State Secretary of Agriculture, Nature Management and Fisheries and I would like to hear the Health Council's views concerning the possibility of children being more sensitive than adults to xenobiotic substances in general and pesticides in particular, concerning the significance of any such heightened sensitivity for the values assigned to toxicological limits such as the ADI for (semi)chronic ingestion and the Acute Reference Dose (ARfD) for the brief (typically single-incident) ingestion of these substances, and concerning the most appropriate way of taking account of levels of exposure to these substances that differ significantly from the norm, as associated with the food consumption patterns of, for example, children.

Kind regards,
R. Bekker
Secretary General,
p.p. the Minister of Health, Welfare and Sport

Annex **B**

Membership of the Committee

- Professor PJJ Sauer, *Chairman*
  Professor of Paediatric Medicine; Groningen University Hospital
- Dr JH Brussaard
  Nutritionist; TNO Nutrition and Food Research, Zeist
- JW Dornseiffen, *consultant*
  Ministry of Health, Welfare and Sport, The Hague
- Professor VJ Feron
  Emeritus Professor of Toxicology; Zeist
- Dr HFP Joosten
  Toxicologist; Organon NV, Oss
- Dr JHCM Lammers
  Neurotoxicologist; TNO Nutrition and Food Research, Zeist
- Professor D Lindhout
  Paediatrician, Professor of Medical Genetics; University Medical Centre, Utrecht
- Professor H van Loveren
  Professor of Immunotoxicology; University of Maastricht; also RIVM, Bilthoven
- Dr PWJ Peters, *Consultant*
  Former Professor of Teratology; Chief Inspector, Food and Consumer Product Safety Authority, The Hague
- Professor IMCM Rietjens
  Professor of Toxicology; Wageningen University & Research Centre

- Dr HFG van Dijk, *Scientific Secretary*
  Health Council, The Hague

Annex **C**

Developmental toxicity

In this Annex, the Committee looks more closely at the sensitivity of developing organ systems to toxic substances. The points are illustrated by reference to the nervous system, the immune system and the reproductive system. These organ systems are characterised by prolonged and complex development, which continues until well after birth, and to some extent even into adolescence. This is in fact the case with various other organ systems, such as the respiratory system and the cardiovascular system, which could have served as examples equally well.

Substances that are toxic to the nervous system

The development of the nervous system is a precisely regulated process, in terms of both timing and siting (Ric00a). There is ample evidence from animal studies to show that exposure to pesticides during development can disrupt this process, thus leading to permanent changes in the nervous system (EPA00, IEH96). Table 11 lists a number of examples of animal studies with pesticides of various classes (organophosphates, carbamates, pyrethroids) in which effects on the developing nervous system have been observed at levels of exposure below those necessary to induce effects on the mature nervous system.

Eriksson and his team have performed various studies with pesticides whose mechanism of action involves interference with the sodium channels in the nerve cell membrane (pyrethroids, DDT). It was found that oral administration of the pyrethroid bioallethrin to mice on day ten to day seventeen after birth has an influence on the cho-

linergic system. This induces changes in the density of muscarine receptors in the cerebral cortex, influencing motor activity both during development and in adulthood (Ahl94, Eri91). The neonatal brain is sensitive to low dosages of these pesticides, which are not sufficient to induce permanent effects in adult animals. The effects observed could not be replicated in a similar study involving inhalation exposure to the pyrethroid d-allethrin (Tsu02).

Neonatal exposure to DDT causes increased sensitive to pyrethroids in adulthood. This means that interference with the normal development of the brain can lead to permanent changes, which manifest themselves as soon as adult animals are exposed again (Eri97). In mice, it was found that a short period of exposure to low doses of various pesticides during the neonatal period was sufficient to increase the sensitivity of the adult animal to renewed exposure to doses insufficient to induce permanent effects in adult animals that had not been exposed as neonates (Eri00). On the basis of the Eriksson team's findings and other evidence, a report published by the UK's Institute for Environment and Health (IEH96) concluded that exposure to low dosages during the period when rapid development of the nervous system was taking place could lead to irreversible changes in the functions of the mature nervous system.

A similar situation exists with other classes of substance, such as the organophosphates. The Hazard Identification and Assessment Review Committee of the US EPA concluded on the basis of data from animal experiments that there was sufficient evidence of increased neonatal sensitivity to chlorpyrifos (EPA98). The difference in sensitivity between young and adult animals is a function of the dose, the duration of exposure, the timing of exposure and effect measurement, and of the selected toxicological endpoints. At the LD_{10} or MTD,* newborn animals are up to five times more sensitive than adult animals to cholinesterase inhibition in the brain and the blood (the best-known effect of organophosphates), and to clinical and behavioural effects. Cholinergic receptors, which play an important role in the normal development of the nervous system, are reduced in number at much lower acute doses in pups than in adult animals. Repeated exposure has not been found to increase cholinesterase inhibition in young animals, with a single exception: the ED_{50} for cholinesterase inhibition in seven-day-old pups is two thirds of the ED_{50} in adult animals. At daily doses equal to 3 mg/kg/day or above, weight and behaviour were found to be affected in neonates at doses three times lower than those needed to induce such effects in adults. In the Toxicology Chapter for Chlorpyrifos (EPA00a), the US EPA again concludes that there is evidence that foetuses and young animals are particularly sensitive to biochemical, morphological or behavioural changes brought about by exposure during development of the nervous system. According to the Chapter, young animals exhibit greater sensitivity to cholinesterase

* MTD: Maximum Tolerated Dose

inhibition than adult animals, even at relatively low dosages. Following acute exposure, young and adult animals differ in their response by a factor of between two and five. Following repeated exposure, young animals were up to nine times more sensitive, but on the level of the LD_{10} and MTD.

In a review of the effects of exposure to low dosages of organophosphates, the Institute for Environment and Health (IEH02) states that foetuses and young animals are more sensitive to the effects of organophosphates because the immature nervous system does not respond in the same way as a mature nervous system and can therefore undergo irreversible changes. Some organophosphates (e.g. chlorpyrifos) affect the behaviour of adult animals that have been exposed during development to doses, the equivalent of which are insufficient to induce such effects in adult animals. The changes brought about sometimes involve mechanisms unrelated to the main effect of organophosphates, namely acetylcholinesterase inhibition. The EPA's Toxicology Chapter for Chlorpyrifos (EPA00a) cites various studies in the public domain (Cam97, Roy98, Son97, Whi95) and a developmental neurotoxicity study (Hob98), which may indicate that chlorpyrifos affects the early development of the nervous system by means of mechanisms other than cholinesterase inhibition (e.g. changes in synapse development, changes in DNA, RNA and protein synthesis, inhibition of cell division and disruption of the structural architecture of the brain), which can result in permanent changes in the structure and/or function of the nervous system. In a number of other studies, many of them performed at the same laboratory, effects were observed at low doses on endpoints unrelated to cholinesterase inhibition. These included changes in the synthesis of macromolecules, changes in cell signalling, a reduction in the number of muscarine receptors and morphological changes in the brain (Cam97, Gar02, Gar03, Lev02, Qia02, Qia03, Slo01, Slo02, Son97). From these studies, it appears that chlorpyrifos interferes with various processes during the development of the nervous system and that some of the effects of this manifest themselves in adulthood. There is presently insufficient data to say whether these conclusions are valid for all organophosphates (IEH02).

Sensitivity differences between young and adult animals may result not only from differences in mechanisms of action (toxicodynamic differences), but also from differences in the way in which substances are dealt with by the body (toxicokinetics). So, for example, age-related differences in sensitivity are much greater with chlorpyrifos than with methamidophos (Pad00). This is probably attributable to differences in the detoxification mechanisms associated with the two pesticides. Chlorpyrifos is detoxified using carboxyl esterases and A-esterases – enzyme systems that are not well developed in young animals. Methamidophos is detoxified by a different route.

Age-related differences in the sensitivity to deltamethrin have also been reported (She94). Young rats (twenty-one days old) and adult rats (seventy-two days old) both exhibited a 50 per cent reduction in the acoustic startle response at a dose of 4 mg/kg.

However, this dose was associated with a higher concentration in the brains of young rats than in the brains of adult animals, indicating lower sensitivity to low doses on the part of young animals. On the other hand, the LD_{50} for young animals proved to be much lower than the LD_{50} for adult animals. The internal concentrations were roughly equal, however, suggesting a toxicokinetic difference rather than a difference in the sensitivity of the target organ.

When the sensitivity of organisms is determined using LD_{50} values, it appears that neonatal rats are roughly nine times more sensitive than adult animals to certain organophosphates and twenty times more sensitive to certain pyrethroids (She00). This is because the enzyme systems involved in detoxification of these substances are not properly developed in young animals. However, the data concerned cannot be used for evaluating the risks associated with pesticide residues in food, and it is necessary to determine the relative sensitivity of young animals to lower doses.

Sheets (She00) examined data from studies on organophosphates, from which it is apparent that the NOELs for cholinesterase inhibition are much lower for adult animals than for young animals. He attributes this observation to the fact that the adult animals studied were directly exposed to the relevant dose, whereas the young animals were exposed indirectly via their mothers to only a fraction of the dose. Studies involving four pyrethroids and direct oral exposure of rats on day 21 and day 72 found that young animals were no more sensitive than adult animals.

The dosages used in the studies listed in Table 11 are external exposure concentrations. Furthermore, in many studies, the young animals were indirectly exposed via their mothers. The dosage data therefore says little about the levels of internal exposure in the target organ, or about the sensitivity of the developing individual to the (neuro)toxicity of a given substance. After exposure to chlorpyrifos, the concentrations found in rats' milk were five times higher than those found in the blood of the mother animals (Mak98). By contrast, after exposure to aldicarb, a very low concentration was detected in the animals' milk, suggesting that the offspring ingested very little of this substance. Proper evaluation of the perinatal neurotoxicity of the substance requires direct dosing of the animals immediately after birth. Toxicokinetic data is therefore important when estimating the actual exposure levels experienced by the offspring.

Age-related differences in sensitivity are clear from the no-observed-effect levels (NOELs) determined for the various age groups. NOELs can be based on various effects. In a retrospective analysis of nine developmental neurotoxicity studies with pesticides for which approval was requested from the EPA (Mak98), the NOELs determined in the developmental neurotoxicity studies proved to be lower than the NOELs for foetuses determined in the prenatal toxicity studies for eight of the nine pesticides, and equal where the ninth was concerned. Compared with the two-generation reproduction study, the NOEL from the developmental neurotoxicity study was lower for six of

the nine pesticides and equal for another one. The NOEL for developmental neurotoxicity was lower than or roughly equal to that for acute and/or subchronic neurotoxicity for six of the nine pesticides. Taking all the studies together, where two of the nine pesticides were concerned (carbaryl, emamectin), the NOEL for developmental neurotoxicity was lower than or equal to that for all the other forms of toxicity referred to.

From various publications, it is apparent that, with some pesticides, effects on the nervous system are discernible following exposure during development to dosages that are lower than the NOELs on which the ADI or ARfD for the relevant pesticide are based. Where chlorpyrifos is concerned, for example, effects were observed in young and adult animals when the lowest studied dosage (1 mg/kg/day) was administered prenatally via the mother or directly during the postnatal period (Lev02, Qia02, Qia03, Slo01, Slo02, Son97) (see Table 11). No NOAEL has been established for these effects. In a number of other studies with chlorpyrifos, effects were similarly observed at the lowest studied dosage, so that no NOAEL could be established (Gar02, Whi95). In other studies again, one of which was a developmental neurotoxicity study (Hob98), the NOAEL was found to be 1 mg/kg/day (Cam97, Gar03).

Pyrethroid-group pesticides have also been found to affect the developing nervous system at dosages lower than the NOELs on which the ADI and ARfD are based. Deltamethrin was observed to have various effects during development (Pat97, She00) and in adulthood (Eri90, Eri91, Laz01) following exposure to very low dosages (see Table 11). Both similar effects and other effects have been reported for bioallethrin (Ahl94, Eri90, Eri91), permethrin (Ima02), cypermethrin (Mal93, She00) and fenvalerate (Mal93). For each of these substances, although effects were observed at somewhat higher dosages, no NOAEL was established, so it is not known whether the actual NOAEL is lower or higher than that on which the ADI is based.

Maternal exposure to carbaryl, a carbamate, was found to induce morphological changes in the brain of the offspring (Rob97). Here again, the NOAEL for this effect was lower than the NOAEL used to establish the ADI and ARfD. In the retrospective study of nine developmental neurotoxicity studies referred to above (Mak98), carbaryl was named along with emamectin as a substance whose NOEL for developmental neurotoxicity was lower than or equal to the NOEL established in other toxicity studies.

Table 11 Pesticides that can affect the development of the nervous system in animals.

Pesticide	Species	Exposure	Route	Doses	LOAEL Dev. Neuro-tox.	NOAEL Dev. Neuro-tox.	Effect	NOEL adults Neurotox.	NOEL used to calculate (incl. effect)		Ref.
									ARfD	ADI	
Organophosphates											
Chlorpyri-fos	Rat	GD6-PND11	Maternal, gavage	0.3, 1, 5 mg/kg/day	5 mg/kg/day	1 mg/kg/day	AChE inhibition, modified brain morphometry	15 mg/kg/day (rat, 90 days) (JMPR 99)	1 mg/kg/day (AChE inhibition in erythro-cytes, human, single dose) (JMPR99)	1 mg/kg/day (AChE inhibition, rat, 2 years, diet) (JMPR99)	Hob98 (In: Mak98)
Chlorpy-rifos	Rat	GD9-12, GD17-20	Maternal, subcuta-neous	1, 2, 5, 10, 20, 40 mg/kg/day	1 mg/kg/day		Modified protein/DNA ratio, elevated ChAT in foetal brain				Qia02
Chlorpy-rifos	Rat	GD17-20	Maternal, subcuta-neous	1, 5 mg/kg/day	1 mg/kg/day		Reduced neuronal activity, elevated ChAT in postnatal brain				Qia03
Chlorpy-rifos	Rat	GD17-20	Maternal, subcuta-neous	1, 5 mg/kg/day	1 mg/kg/day		Long-term changes in cognitive performance (juvenile, adult)				Lev02
Chlorpy-rifos	Rat	PND1	Subcuta-neous	2 mg/kg	2 mg/kg		Inhibition DNA and protein synthesis				Whi95
Chlorpy-rifos	Rat	PND1-4, PND11-14	Subcuta-neous	1, 5, 25 mg/kg/day	5 mg/kg/day	1 mg/kg/day	Cell loss in brain				Cam97
Chlorpy-rifos	Rat	PND1-4, PND11-14	Subcuta-neous	1, 5 mg/kg/day	1 mg/kg/day		Cholinesterase inhibition, modified adenylyl cyclase signalling cascade				Son97
Chlorpy-rifos	Rat	PND1-4, PND11-14	Subcuta-neous	1, 5 mg/kg/day	1 mg/kg/day		Cholinergic synaptic activity				Slo01
Chlorpy-rifos	Rat	PND1-4, PND11-14	Subcuta-neous	1, 5 mg/kg/day	1 mg/kg/day		Modified catecholamine turnover				Slo02
Chlorpy-rifos	Rat	PND11-14	Subcuta-neous	5 mg/kg/day	5 mg/kg/day		Change in GFAP concentration				Gar02
Chlorpy-rifos	Rat	GD17-20, PND1-4, PND11-14	Subcuta-neous	1-40 mg/kg/day (GD17-20); 1 mg/kg/day (PND1-4); 5 mg/kg/day (PND11-14)	5 mg/kg/day	1 mg/kg/day	Changes in neuro-protein markers for oligodendrocytes, neurons and axons				Gar03

Chlorpyrifos	Mouse	PND1- 4, PND11-14	Subcutaneous	1, 3 mg/kg/day	1 mg/kg/day		Cholinesterase inhibition, ↑ motor activity, ↑ agonistic behaviour				Ric03
Carbamates											
Carbaryl	Rat	GD6-PND10	Maternal, gavage	0.1, 1, 10 mg/kg/day	10 mg/kg/day	1 mg/kg/day	Modified morphometry in forebrain and/or cerebellum (PND11,60)	1 mg/kg/day (rat, 90 days) (JMPR 01)	3.8 mg/kg/day (dog, 5 weeks, AChE inhibition in plasma) (JMPR01)	<15 mg/kg/day (mouse, 2 years, carcinogenity) (JMPR01)	Rob97 (In: Mak98)
Pyrethroids											
Bioallethrin	Mouse	PND10-16	Gavage	0.21, 0.42, 0.7, 42 mg/kg/day	0.21 mg/kg/day		↑ MAChR density in cortex (PND17); ↓ MAChR, ↑ motor activity (4 months)				Ahl94
Bioallethrin	Mouse	PND10-16	Gavage	0.7 mg/kg/day	0.7 mg/kg/day		↓ MAChR density in cortex, ↑ motor activity (4 months)				Eri90; Eri91
Deltamethrin	Mouse	PND10-16	Gavage	0.7 mg/kg/day	0.7 mg/kg/day		↑ motor activity (4 months)	2.5 mg/kg (rat, 90 days) (WHO 90)	5 mg/kg (rat, single dose neurotoxicity) (JMPR 00b)	1 mg/kg/day (rat, 2 year, diet, general toxicity) (JMPR 00b)	Eri90; Eri91
Deltamethrin	Rat	GD6-15	Maternal, gavage	0.08 mg/kg/day	0.08 mg/kg/day		Reduced immobility latency when swimming, reduced open field locomotion (*M*, PND60); increased dopaminergic activity (*M*, PND140)				Laz01
Deltamethrin	Rat	PND21	Gavage	1, 2, 4 mg/kg	1 mg/kg		Reduced acoustic startle response				She00
Deltamethrin	Rat	PND9-13	Intra peritoneal	0.7 mg/kg/day	0.7 mg/kg/day		Retarded cytogenesis and morphogenesis in cerebellum; damage to blood vessels in brain				Pat97
Permethrin	Mouse	PND1-PND7, 14, 21	Maternal	7.7, 120, 1200 µg/day, drinking water	7.7 µg/day, drinking water		c-fos mRNA in cerebellum depressed	15.5 mg/kg (rat, 90 days) (FAO99)		5 mg/kg/day (rat, long-term toxicity) (FAO99)	Ima02
cis-Permethrin	Mouse	PND1-21	Maternal, gavage	1 mg/day	1 mg/day		c-fos mRNA in cerebellum depressed				

Cypermethrin	Rat	PND21	Gavage	9, 19, 38 mg/kg	9 mg/kg		Reduced acoustic startle response	37.5 mg/kg (rat, 4 wks) (WHO 89)		7.5 mg/kg/day (rat, 2 years, bodyweight, liver toxicity) (JMPR81)	She00
Cypermethrin	Rat	GD5-PND21	Maternal, gavage	15 mg/kg/day	15 mg/kg/day		Changes in AchE, Na^+K^+- ATPase; changes in dopaminergic and cholinergic receptor density in striatum				Mal93
Fenvalerate	Rat	GD5-PND21	Maternal, gavage	10 mg/kg/day	10 mg/kg/day		Changes in MAO, AchE, Na^+K^+-ATPase; changes in dopaminergic and cholinergic receptor density in striatum	200 mg/kg (rat, acute) (WHO 96b)		3.5 mg/kg/day (mouse, diet) (JMPR84)	Mal93
Antibiotic derivatives											
Emamectin	Rat	GD6-PND20	Maternal, gavage	0.1, 0.6, 3.6/2.5 mg/kg/day	0.6 mg/kg/day	0.1 mg/kg/day	↓ motor activity (*F*, PND17)		0.075 mg/kg/day	0.075 mg/kg/day	Wis97

GDx: gestational day x; PNDx: postnatal day x; ↑ : elevated; ↓ : reduced; *M*: in male animals; *F*: in female animals.

Substances that are toxic to the developing immune system

The Committee has not attempted to produce a full list of substances that have been shown to be harmful to the developing immune system. Comprehensive lists can be found in various reviews (Bar96, Die00, Hol94a, Hol00, Lus03 and Lov03). Here, the Committee merely presents a number of characteristic examples. The substances whose harmfulness to the developing immune system is best documented include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), hexachlorobenzene, chlordane, benzo[*a*]pyrene, lead, organotin compounds and a number of pharmaceutical products. Very little research has been done into the possibility of modern pesticides affecting development in this way (Table 12).

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

TCDD is a highly toxic chlorinated hydrocarbon found as a contaminant in some commercial products. The substance attacks the developing thymus, in particular the epithelial cells in the cortex (Vos89). It also interferes with the maturation of thymocytes into the various types of T-lymphocyte. There is evidence that stem cells in the bone marrow, from which the thymocytes originate, may be damaged as well (Fin89, Lus79).

Vos and Moore (Vos74) demonstrated that in young rats and mice various T-lymphocyte-dependent immune functions, such as the rejection of skin transplants, the so-called graft-versus-host (GVH) response and the response to PHA and ConA – substances that trigger T-lymphocytes to multiply – are inhibited if the mother animal is exposed during gestation or lactation to several TCDD doses of 5 µg per kg bodyweight. By contrast, direct exposure of four-month-old mice to several TCDD doses of 25 µg per kg bodyweight had no influence on the response to PHA, or on the GVH response. From these findings, Vos and Moore (Vos74) concluded that exposure during development of the immune system appeared to be a condition for inhibition of the said immune functions.

Administration of several TCDD doses of 5 µg per kg bodyweight to mother rats on various days during gestation and lactation led to young rats exhibiting loss of tissue in the thymus cortex and an abnormal thymus tissue structure (Fai77). In animals that were exposed via the mother both before and after birth, the damage to the thymus was still visible at the age of 145 days. By contrast, the thymus of animals exposed during lactation only had returned to normal by that age. At the age of 145 days, both groups of animals exhibited a reduced delayed-type hypersensitivity reaction (DTH) – an immune function based on the action of T-lymphocytes and macrophages. This is notable, since the animals that were exposed only after birth seemed to have normal thymuses at that time. The consequences of the exposure via the mother appeared to be prolonged (at least four months) and, moreover, were more serious where exposure had taken place before birth.

More recently, Gehrs and Smialowicz (Geh99) reported that a single TCDD dose of 0.1 µg per kg bodyweight, administered to pregnant female rats on gestation day 14, was sufficient to suppress the DTH in male offspring fourteen months old. The young were suckled by the exposed mother animals for a further four weeks. Suppression of the same immune function in female offspring of the same age required a dose three times as high.

Animal research has shown that exposure to TCDD before or immediately after birth influences the T-lymphocyte-dependent immune functions almost exclusively. The functions that depend on B-lymphocytes are virtually unaffected. This is not the case with the exposure of adult animals, in which both T- and B-lymphocyte-dependent functions are disrupted (Bar96).

Research among Dutch children has demonstrated that there is a correlation between perinatal exposure to dioxins and PCBs and increased susceptibility to infectious diseases and a reduced vaccination response (Wei00b).

Taken together, these research findings indicate that effects on the immune system are greater or occur at lower dosages and persist for longer periods, if exposure takes place during the development of the system, and especially if exposure takes place

before birth. Furthermore, the nature of the effects appears to depend on the timing of exposure.

Hexachlorobenzene (HCB)

HCB is a substance with a variety of industrial applications, which in the past was used as a fungicide. In rats, the substance stimulates immune system activity, particularly B-lymphocyte-dependent functions and some T-lymphocyte-dependent functions. The immune system is especially sensitive during development. This was demonstrated by two studies in which young rats were exposed to HCB from conception until after their birth, first via the mother, then via the mother's milk and finally directly via their feed, in dosages varying from 4 to 150 mg HCB per kilo of feed (Vos79b, Vos83). Even at an exposure level of 4 mg HCB per kilo of food, young rats injected with tetanus toxoid produced more M and G antibodies against the antigen. The DTH response to the xeno-biotic protein ovalbumin also appeared to be stimulated at this dosage. By contrast, the exposure of weaned rats (three to four weeks old) to the much higher dosage of 1000 mg HCB per kilo of feed had no influence on the DTH and stimulation of the production of antibodies against tetanus toxoid was less pronounced (Vos79a). However, it was found that in these animals the weight of the popliteal lymph gland and the number of neutro-philic granulocytes – a particular type of white blood cell – increased at dosages of more than 500 mg HCB per kilo of feed. It nevertheless appears that the developing immune system is more sensitive to this substance than the mature system.

In developing mice, by contrast, HCB appears to suppress immune system function, as evidenced by a reduced DTH response (Bar87). No satisfactory explanation has yet been found for this difference (Bar87, Vos86). Among Inuit children, Dewailly *et al*. (Dew00a) found a positive correlation between prenatal exposure to HCB and p,p'-DDE (a metabolite of DDT) and the prevalence of otitis.

Chlordane

Chlordane is an organo-chlorine compound that in the past was used as an insecticide. Daily exposure of pregnant mice throughout gestation to dosages of between 4 and 16 mg chlordane per kilo bodyweight led to a reduced DTH response in the offspring (Spy82, Bar85a,b). The reduced response was probably attributable to damaged mac-rophages (Bar90a). By contrast, mice exposed to 8 mg of chlordane per kilo bodyweight at an age of between six and seven weeks did not exhibit a clearly reduced DTH response (Joh86).

Intrauterine exposure to 4 and 8 mg of chlordane per kilo maternal bodyweight also resulted in a reduction in the number of stem cells in the bone marrow and foetal liver,

from which the macrophages and granulocytes originate (Bar90a,b). Furthermore, the number of more primitive, pluripotent stem cells capable of re-colonising the spleen of irradiated mice was diminished. Some 200 days after birth, the number of stem cells was still unusually low. Exposure of adult mice to comparable quantities of chlordane had no influence on the number of stem cells in the bone marrow. With this substance too, therefore, the immunity implications of perinatal exposure appear to be more serious and more permanent than the implications of exposure in adulthood.

Benzo[a]pyrene

Benzo[*a*]pyrene is a polycyclic aromatic hydrocarbon. Exposure of pregnant mice to 150 mg/kg bodyweight led to the offspring exhibiting strongly reduced immunity, with both B-lymphocyte-dependent functions and T-lymphocyte-dependent functions affected (Urs80, Urs82, Urs84). The observed functional effects were still apparent eighteen months after birth. In addition to reduced immunity, an increased prevalence of tumours was detected in the exposed mice. The precise nature of the effects appeared to depend partly on whether the benzo[*a*]pyrene was administered in the middle of the gestation period or towards the end (Urs82). Postnatal exposure of mice to equivalent doses of the substance had barely any effect on antibody formation by B-lymphocytes or the development of tumours.

Benzo[*a*]pyrene appears to interfere with maturation and differentiation of thymocytes in the various types of T-lymphocytes and, like TCDD, to lead to atrophy of the foetal thymus. It is also associated with a decline in stem cell numbers, the cells from which T- and B-lymphocytes originate (Hol94). It is clear that the substance is harmful to the immune system mainly during development and that it causes a more or less permanent reduction in immunity.

Lead

Lead was for some years added to petrol as an anti-knock agent on a large scale. It was also used in various types of paint. Old water pipes were often made of lead, leading to the metal's presence in drinking water. Luster *et al.* (Lus78) demonstrated that lead was toxic to the developing immune system of the rat. They subjected young rats to prolonged exposure to lead in their drinking water (25 and 50 ppm lead). The young were initially subjected to intrauterine exposure through their mothers' drinking water; indirect exposure continued after birth via their mothers' milk, and finally the animals were directly exposed via their own drinking water. The young animals were found to produce fewer antibodies against red blood cells taken from sheep and had lower concentrations of the antibody IgG in their blood serum. The research findings showed that the

reduced antibody formation probably resulted not from interference with the B-lymphocytes that produce the antibodies, but from interference with certain T-lymphocytes that assist the B-lymphocytes. The lead concentrations in the young rats' blood were found to be comparable with those often found in children growing up in urban areas. Follow-up research involving a similar exposure regime found that exposure to the metal resulted in reduced thymus weight, reduced T-lymphocyte response to substances that are supposed to trigger their division, and reduced DTH response (Fai79).

More recent research (Che99, Mil98) has shown that intrauterine exposure of rats via their mothers' drinking water (100 to 500 ppm lead) induced a shift in the balance between certain types of T-lymphocytes, the so-called Th1- and Th2-lymphocytes. These are distinguished by the substances they produce (cytokines). Normally, Th2-lymphocytes dominate prior to birth, since Th1-lymphocytes are liable to attack maternal tissue. After birth, the balance shifts more towards the Th1-side under the influence of contact with antigens in the outside world. In recent years, it has become clear that children in whom this natural shift does not take place are at increased risk of developing atopic conditions, such as allergies, hay fever, eczema and asthma (see, for example, Kay01). Thirteen-week-old adult rats that had experienced prenatal exposure to lead exhibited inhibited Th1-lymphocyte activity and stimulated Th2- lymphocyte activity. These animals had notably low concentrations of lead in their blood and bones and the researchers concluded that even brief intrauterine exposure to lead could lead to permanent disruption of the immune system. They speculated that lead might have contributed to the recent increase in asthma and other Th2-related chronic childhood conditions. In the mother animals, the same exposure caused no changes to the immune system, or temporary changes at worst.

The nature and seriousness of the effects of prenatal exposure to lead depend partly on the timing of exposure (early or late in gestation) and on the sex of the offspring; these facts have been demonstrated by research with rats and chickens (Bun00, Bun01a, Bun01b, Lee01a, Lee02). In this regard, Dietert *et al.* (Die02) point out that exposure to lead during the early embryonic phase does not lead to reduced Th1-functions in the offspring, but exposure during the late embryonic phase does cause such a reduction. This observation is consistent with the belief that the elements of the immune system that provide functional Th1 capacity develop only towards the end of gestation.

It is clear that lead is much more harmful to the developing immune system than to the mature system. It is one of the few substances in connection with which the relationship between the precise timing of exposure and the nature of the immunotoxic effect has been studied in some detail.

Organotin compounds

Organotin compounds have a wide range of industrial and commercial applications, including use as pesticides in agriculture, in antifouling paints for ships' hulls and as stabilisers in plastics. Direct exposure of unweaned rats of between three and twenty-four days old, to ten doses of di-*n*-octyl tin chloride (DOTC) (5-15 mg/kg bodyweight on each occasion) resulted at the age of ten weeks in a temporary reduction in the ability of T-lymphocytes from the spleen to react to substances that normally trigger their multiplication (Smi88). Two weeks later, the response had returned to normal. Exposure of eight-week-old rats according to the same regime had no effect on the described immune response four weeks after the last administration of DOTC. The researchers concluded from these findings that the developing immune system was more sensitive than the mature system. Exposure via pregnant or lactating mother animals had no discernible influence on the immune system of the offspring. The researchers speculated that the substance passed through the placenta in insufficient quantities and was barely excreted in the mother rats' milk.

Smialowicz *et al.* (Smi89) investigated the effect of another organotin compound, tributyl tin oxide (TBTO), on the developing immune system. In adult rats of nine weeks old, all the observed effects of TBTO (ten doses of up to 20 mg/kg bodyweight on each occasion) on the immune system disappeared within three weeks of the last exposure. By contrast, in animals that were exposed at between three and twenty-four days old (ten doses of 10 mg/kg bodyweight on each occasion), the ability of T-lymphocytes from the spleen to multiply in response to a trigger substance was reduced at the age of ten weeks. This effect was not apparent at the ages of six and twelve weeks, however. Furthermore, the researchers point out that exposure resulted in reduced bodyweight in the animals and that there may therefore have been toxic effects on organ systems other than the immune system.

Prenatal and postnatal administration of tributyl tin chloride to rats (from gestation day 8 to postnatal day 30) in dosages of 0.025, 0.25 and 2.5 mg/kg bodyweight per day had no clear influence on humoral immunity, although incidental shifts were discernible in antibody concentrations in the serum in adulthood (Try04). At the highest dose, atrophy of the thymus was observed. In addition, at the middle and highest dosages, shifts were noted in both the absolute and relative numbers of various types of T-lymphocyte in the spleen, but the picture was not constant over time. The numbers of NK cells (natural killer cells, a type of lymphocyte) were elevated at the highest dosage. Furthermore, the activity of the NK cells increased with the size of the tributyl tin chloride dose administered, and was significantly higher at all dosages than in the control group. The DTH response to oxazolone at the two lowest dosages was elevated relative to the control group, while at the highest dosage it was reduced. The authors concluded that prena-

tal and postnatal administration of tributyl tin chloride to rats adversely affected several specific and non-specific immune parameters, particularly in older rats (sixty to ninety days after birth) and that some effects occurred even at a dose of 0.025 mg per kilogram bodyweight per day (i.e. a dose equal to the NOAEL on which the current TDI* is based).

Modern pesticides

Very little data is available on the influence of 'modern' pesticides on the developing immune system. The Committee was only able to find a small amount of information about the insecticides diazinon, carbofuran and chlorpyrifos and the herbicides 2,4-D and atrazine. Prenatal exposure of mice to diazinon and carbofuran resulted merely in slight temporary disruption of antibody concentrations in the blood serum (Bar80).

In rats that were administered chlorpyrifos (1 mg/kg bw/d) for four consecutive days immediately following birth, the T cells showed no reduced response at the end of the period to Concanavalin A, a substance which triggers division in such cells (Nav01). However, when the animals reached adulthood, the T cells response did appear to be inhibited. Exposure to chlorpyrifos at the age of ten to fourteen days had the same outcome in adulthood. The researchers postulated that this phenomenon was due not to any direct effect of chlorpyrifos on the T cells, but to a neurological effect. They suspected that the 'programming' of the T cell function, which normally takes place under the influence of the nervous system during the foetal and neonatal periods, had been disrupted. When adult rats were exposed to chlorpyrifos for four weeks (twice 5 mg/kg bw/w), there was an immediate reduction in the response of T cells to Concanavalin A (Bla99).

A single administration of the herbicide 2,4-D to pregnant mice midway through gestation had no influence on their six-week-old offspring's ability to make antibodies against the red blood cells of sheep. Also, the response to LPS and Con A, substances that trigger B and T cells, respectively, to start dividing, was unaffected (Bla86). This could, however, simply reflect the fact that the experiment involved only a single dose of a substance that quickly leaves the body (Bar96). Lee *et al.* (Lee01b) administered a commercial 2,4-D formulation to pregnant mice in their drinking water (up to 650 mg/kg/d) from day 6 to day 16 of gestation. At the highest dosage, the T cells of the offspring exhibited a reduced response to Con A, and there was a relative increase in B cells and a relative reduction in cytotoxic or suppressor T cells in the spleen. No effect was observed on the production of antibodies to the red blood cells of sheep, or on

* TDI: Tolerable Daily Intake; similar to the ADI, the former being used for food contaminants, the latter for food additives and pesticides.

phagocytosis by peritoneal macrophages. The observations were made seven weeks after birth of the young. From these findings, the authors concluded that prenatal exposure to 2,4-D appeared to lead to permanent changes in cells of the immune system.

In the male offspring of female rats exposed during pregnancy and lactation to the herbicide atrazine (35 mg/kg/d from gestation day 10 to postnatal day 23), the primary antibody response (IgM) to red blood cells from sheep eight weeks after birth was less vigorous than in the offspring of unexposed females (Roo03). The DTH response to Bovine Serum Albumin (BSA) nine and twelve weeks after birth was similarly reduced in male offspring exposed via their mothers. Six months after the birth, these observed differences in humoral and cellular immune functions had disappeared. The immune functions of female offspring did not appear to be affected by atrazine administration to the mother animals. The findings neither proved nor disproved the hypothetical link between the immunotoxicity and the endocrine-disrupting properties of atrazine.

Medicines

Stahlmann *et al*. (Sta91) treated pregnant rats with the virus inhibitor acyclovir (three injections of 100 mg/kg bw) on gestation day 10. Eleven days later, some of the foetuses were found to have developed no thymus, or to have developed it in the wrong place. In other foetuses, the weight of the thymus was low compared with the control group. Similar effects were observed in adult offspring. The weight of the liver also appeared to be diminished. The weight of the spleen, on the other hand, was greater relative to overall bodyweight. Infection tests with *Trichinella spiralis* indicated that the morphological changes were accompanied by reduced antibody production and reduced resistance to these parasitic worms (Sta91, Sta92).

Bakker *et al*. (Bak00) treated rats on a single occasion in the first week after birth with a clinically relevant dose of dexamethasone. Induction of experimental allergic encephalomyelitis, an autoimmune model for multiple sclerosis, by sensitisation with myelin had quicker and more serious effects on treated animals than on untreated animals. The increased sensitivity proved to be a lifelong phenomenon.

Table 12 Substances that can affect the development of the immune system in laboratory animals.

Chemical compound	Species	Exposure	Dosages	Route	LOAEL Devel. immuno-tox.	NOAEL Devel. immuno-tox.	Effect Developm. immunotox.	LOAEL adult	NOAEL adult	Effect adult	Ref.
Hexa-chloro-benzene	Rat	GD1/3-PND35	0-50-150 mg/kg feed	Via mother, mother's milk, feed	50 mg/kg feed		Histopathology, increased IgG response to tetanus toxoid				Vos 79b
Hexa-chloro-benzene	Rat	GD1/3-PND35/77	0-4-20-100 mg/kg feed	Via mother, mother's milk, feed	4 mg/kg feed		Histopathology, DTH elevated, IgG and IgM response to tetanus toxoid elevated				Vos83
Hexa-chloro-benzene	Rat	PND21/28-PND42/49	0-500-1000-2000 mg/kg feed	Via feed				500 mg/kg feed	1000 mg/kg feed	Histopathology, increase in weight of popliteal lymph gland, increase in neutrophilic granulocytes DTH unmodified	Vos79a
Hexa-chloro-benzene	Mouse	GD1-18	0-0.5-5 mg/kg bw/d	Via mother, (+ milk)	0.5 mg/kg bw/d		DTH reduced				Bar87
Chlordane	Mouse	PND42/49-PND56/63	0-0.1-1-4-8 mg/kg bw/d	Oral gav-age					>8 mg/kg bw/d	DTH response to KLH	Joh86
Chlordane	Mouse	GD1-19	0-0.16-8 mg/kg bw/d	Via mother	8 mg/kg bw/d	0.16 mg/kg bw/d	DTH response to oxazolone reduced				Spy82
Chlordane	Mouse	GD1-19	0-4-16 mg/kg bw/d	Via mother	4 mg/kg bw/d		DTH response to oxazolone reduced				Bar85a
Chlordane	Mouse	GD1-19	0-4-8-16 mg/kg bw/d	Via mother	8 mg/kg bw/d		DTH response to influenza A virus reduced				Bar85b
Chlordane	Mouse	Pups: GD1-19, Adults: PND80-98	0-4-8 mg/kg bw/d	Pups via mother (+ milk); adults oral	4 mg/kg bw/d		GM-CFU reduced, CFU-S reduced		>8 mg/kg bw/d	GM-CFU, CFU-S	Bar90
TCDD	Rat, Mouse	Rat pups: PND0, 7, 14 Adult mice: PND120-155	0-5 µg/kg bw/d 6x 0-1-5-25 µg/kg bw	Pups via mothers' milk, Adults oral?	5 µg/kg bw/d		PHA response spleen cells diminished, GVH activity spleen cells reduced		(>)25 µg/kg bw	PHA response spleen cells, GVH activity spleen cells	Vos74
TCDD	Rat	Group 1: GD18, PND0, 7, 14	Group 1: 0-4x5 µg/kg bw/d	Group 1: via mother, via mother's milk	Group 1: 5 µg/kg bw/d		Group 1: histopathology thymus >PND145, DTH inhibited >PND145				Fai77

		Group 2: PND0, 7, 14	Group 2: 0-3x5 µg/kg bw/d	Group 2: via mother's milk	Group 2: 5 µg/kg bw/d		Group 2: histopathology thymus <PND39, DTH inhibited >PND145				
Benzo[*a*]-pyrene	Mouse	Group 1: GD11-13	Group 1: 150 µg/kg bw	Group 1: via mother	Group 1: 150 µg/kg bw		Group 1: reduction in anti-SRBC PFC (19S) >78w; more tumours				Urs82
		Group 2: GD16-18	Group 2: 150 µg/kg bw	Group 2: via mother	Group 2: 150 µg/kg bw		Group 2: reduction in anti-SRBC PFC (7S) >78w; more tumours				
		Group 3: PND7	Group 3: 3-150 µg/kg bw	Group 3: ip injection				Group 3+4: 150 µg/kg bw		Group 3+4: tumours significantly fewer than in Groups 1+2 and anti-SRBC significantly higher	
		Group 4: PND112	Group 4: 3-150 µg/kg bw	Group 4: ip injection							
Pb	Rat	pups: prenatal, throughout gestation; mothers: throughout gestation + 10 d prior	0-100-250-500 ppm in drinking water	Via mother's drinking water	100 ppm		Increase in IgE in serum		500ppm	IgE in serum	Mil98
					250 ppm	100 ppm	Increase in TNF-α		500ppm	TNF-α	
					250 ppm	100 ppm	Increase in nitrite		500ppm	Nitrite production	
					500 ppm	250 ppm	Reduction in IFN-γ		500ppm	IFN-γ	
					250 ppm	100 ppm	Reduction in DTH (all at age 13w)		500ppm	DTH	
Pb	Rat	Pups: prenatal, entire gestation	0-250 ppm in drinking water	Via mother's drinking water	250 ppm	Reduction in DTH					Che99
					250 ppm	Reduction in IFN-γ					
					250 ppm	Increase in IL-4					
					250 ppm	Increase in TNF-α					

Chlor-pyrifos	Rat	PND1-4	0-1 mg/kg bw/d	Subcutaneous injection	1 mg/kg bw/d		Reduction in Con A T cells respond on PND60, not on PND5				Nav01
Chlor-pyrifos	Rat	5-7 w old, 4w duration, 2x/w	0-5 mg/kg bw on each occasion	Oral gavage				5 mg/kg bw on each occasion		Immediate reduction in T cells' Con A and PHA response, reduction in production of antibodies to SRBC/10^6 spleen cells, not per spleen, shift in lymphocyte subpopulations	Bla99
Atrazine	Rat	GD10-PND23	0-35 mg/kg bw/d	Via mother and mother's milk	35 mg/kg bw/d		Reduction in IgM to SRBC; reduction in DTH to BSA				Roo03
Acyclovir	Rat	GD10	0-1x100-3x100 mg/kg bw	Subcutaneous injection	3x100 mg/kg bw 1x100 mg/kg bw		Thymus malformation and lower thymus weight on GD21, reduction resistance to Trichinella; lower thymus weight on PND105				Sta91

GDx: gestational day x; PNDx: postnatal day x.

Substances that affect sexual development by endocrine disruption

An increasing number of substances are now known to affect the hormone balance and thus the processes that these signalling substances regulate, such as gender differentiation and the reproductive cycle. Known endocrine-disrupting substances include a number of pesticides. By way of illustration, several of these substances are discussed below (see Table 13).

Vinclozolin

Vinclozolin is a fungicide, approved for use in the USA and twelve of the fifteen EU states, including the Netherlands. In the EU/Norwegian monitoring programme of 1996-98, vinclozolin proved to be one of the pesticides that were most frequently detected in agricultural produce (Tir02). In 2001, the Inspectorate for Health Protection and Veteri-

nary Public Health found the substance in sixty-six of the 2600 samples analysed. In three of these cases, the MRL was exceeded. In 2002, forty-nine out of 3200 samples tested positive and four were over the MRL (Inspectorate for Health Protection and Veterinary Public Health, oral communication). Vinclozolin has been shown to have an anti-androgenic effect (Gra94), which probably involves two metabolites that are formed in the body and attach themselves to androgen receptors, thus blocking them (Kel94). Prenatally exposed male rats exhibited disrupted development of the reproductive organs and quasi-female characteristics: a reduction to female proportions of the distance between the anus and the genital opening (anogenital distance, AGD), extra nipples and areolae, a divided penis with the opening of the urethra on the underside of the penis (hypospadia), undescended testicles, formation of a vagina-like structure, granulomas* in the epididymises and under-sized or absent sex accessory glands (Gra94; Hel00; Gra01). Barely any abnormalities were found in female rats. Wolf *et al*. showed that male rats exhibited abnormalities mainly if they were subjected to intrauterine exposure between gestation day 14 and gestation day 19 (Wol00). Exposure on days 16 to 17 appeared particularly effective. Exposure on gestation days 12 to 13 or 20 to 21 had barely any effect. According to the authors, the affected tissues are most sensitive during or shortly after the appearance of androgen receptors in a tissue known as the mesenchyme. Once they leave the mesenchyme and migrate elsewhere (primarily to the epithelium), malformations can no longer be induced (Wol00). Some anti-androgenic effects, such as reduced AGD, were detected at 3.125 mg/kg bw/d, the lowest test concentration used in the study (Gra99a). The dose-effect curves for some effects (reduced AGD, areola induction and reduced ventral-prostate weight) appear to have no threshold and to take a linear form in the low dose segment (Gra01). Young male rats that underwent direct exposure to vinclozolin (as opposed to exposure via their mothers' milk) in the immediate postnatal period also exhibited abnormal play habits, more akin to those of female rats (Hot02). Prepubertal administration to young male rats retarded pubertal maturation and the growth of the epididymis and sex accessory glands at doses of 30 mg/kg bw/d and above (Mon99).

Procymidone

Procymidone is a fungicide closely related to vinclozolin. Like the latter, it is anti-androgenic, albeit to a slightly lesser extent (Ost99). Administration of procymidone to pregnant and lactating rats (GD14-PND3) caused male offspring to exhibit reduced AGD, additional permanent nipples, reduced androgen-dependent organ weight and malforma-

* Granuloma: granular swelling; a swelling formed from granular connective tissue with a high concentration of blood vessels, ordinarily triggered by infection, which later shrivels, leaving scar tissue

tions of the sex organs. These effects occurred at the lowest test dosage used (25 mg/kg bw/d). From studies undertaken by Hosokawa *et al*., it appears that procymidone has very little effect on the reproductive organs of the adult male rat, if administered for two weeks at a high dosage (6000 ppm in the feed) (Hos93a,b). Temporary disruptions to hormone levels have been observed, however. This led Ostby *et al*. to suggest that the observation of subtle endocrine changes in adult males must raise concerns about the possibility of more far-reaching effects on developing foetuses (Ost99).

Procymidone and vinclozolin share a common mechanism of action: they attach themselves to androgen receptors, thus blocking them. Their effects appear to be additive both *in vitro* as *in vivo* (Gra01, Nel03).

Linuron

The herbicide linuron is also known to have endocrine-disrupting properties (Gra99b, Lam00, McI00). Its effect may involve other mechanisms, in addition to attachment to and blocking of androgen receptors (Gra99b, Lam00). Administration of linuron to pregnant female rats between GD12 and GD21 in dosages of 0, 12.5, 25 and 50 mg/kg bw/d had no influence on AGD of the male offspring, but did lead to dose-dependent nipple/areola retention. Underdevelopment of the testicles and epididymises was also observed in the adult offspring at all dosages. Partial agenesis (non-formation) of the epididymis occurred only at the highest dosage (McI00). According to Gray *et al*., the anti-androgenic effects of linuron and p,p'-DDE (another anti-androgen) on androgen-dependent organ weights following exposure of adult males were detectible only at clearly toxic dosages (Gra01).

Atrazine

Atrazine is a widely used weed-killer belonging to the triazine group. Its use is still permitted in some EU member states, but it is now banned in others, including the Netherlands. The European Union has recently decided to withdraw approval throughout the union. In the USA, it remains approved for use. Exposure to atrazine can induce tumours in the mammary glands of female rats (EPA02). This is probably due to interference with the hypothalamic-pituitary-gonadal axis. Atrazine inhibits the excretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus. GnRH is the hormone that triggers the pituitary to release luteinising hormone (LH), which under normal circumstances gives the signal to the ovaries that ovulation should take place. Under the influence of atrazine, however, the LH level in the blood serum remains too low to trigger ovulation. This results in continuous production of oestradiol and prolactin. The hormonal environment thus created promotes the development of the tumours referred to

above. Since the working of the human hypothalamic-pituitary-gonadal axis is quite different from that of the rat axis, this effect is probably not relevant for humans (EPA02). However, the hormonal disruptions that induce tumours appear to also play a role in some effects on the development of rats, which may be relevant for humans. The administration of atrazine to pregnant female rats, for example, has been found to result in abortion and delayed parturition (Nar01). Furthermore, the administration of atrazine to female rats in the prepubertal phase has been shown to retard pubertal development (Law00). There is evidence to suggest that the hypothalamic-pituitary-gonadal axis in peripubertal female rats may be less sensitive to disruption than in adult female rats (Ash02).

Atrazine can also delay pubertal development in male rats (Sto00). What is more, exposure to atrazine around puberty appears to inhibit testosterone production by the Leydig cells in the testis (Fri02). Administration of atrazine to lactating mother rats depressed prolactin release, resulting in an increase in the prevalence and seriousness of prostate inflammations in the male offspring (Sto99).

Other triazine-herbicides, such as simazine and propazine, as well as a number of metabolites of these substances can disrupt the hormone balance in a similar way (EPA02).

Table 13 Pesticides that have an endocrine-disrupting effect on laboratory animals.

Chemical compound	Species	Exposure	Dosages	Route	LOAEL Developm. reprotox.	NOAEL Developm. reprotox.	Effect Developm. reprotox.	LOAEL adult	NOAEL adult	Effect adult	Ref.
Vinclozolin	Rat (m)	GD14-PND3	0-3.125-6.25-12.5-25-50-100 mg/kg bw/d	Via mother (milk)	3.125/6.25 mg/kg bw/d	(<)3.125 mg/kg bw/d	Reduced anogenital distance, extra nipples/areolae, reduced ventral prostate weight				Gra99a
Vinclozolin	Rat (m)	PND22-54	0-10-30-100 mg/kg bw/d	Oral (gavage)	30 mg/kg bw/d	10 mg/kg bw/d	Delayed puberty (opening of praeputium retarded)				Mon99
Vinclozolin	Rat (m)	PND2-3	0-200 mg/kg bw/d	Injection	200 mg/kg bw/d	<200 mg/kg bw/d	Reduced male play on PND36-37				Hot03
Procymidone	Rat (m)	GD14-PND3	0-25-50-100-200 mg/kg bw/d	Via mother (milk)	25/50 mg/kg bw/d	(<)25 mg/kg bw/d	Reduced anogenital distance, extra nipples, reduced ventral prostate weight				Ost99

Procymidone	Rat (m)	2 weeks duration	0-6000 ppm	In feed						Very little effect on reproductive tract, increase in LH in serum and pituitary	Hos93b
Linuron	Rat (m)	GD12-21	0-12.5-25-50 mg/kg bw/d	Oral (gavage)	12.5 mg/kg bw/d 50 mg/kg bw/d	<12.5 mg/kg bw/d 25 mg/kg bw/d	Histopathological abnormalities in testicle and epididymis, increased nipple/areola retention, partial agenesis of epididymis				McI00
Atrazine	Rat (f)	6 months duration						3.65 mg/kg bw/d	1.8 mg/kg bw/d	Fall in LH	Morseth, 1996 in EPA02
Atrazine	Rat (f) F344 SD LE	GD6-10	0-25-50-100-200 mg/kg bw/d	Oral (gavage)	mg/kg bw/d 50 200 200	mg/kg bw/d 25 100 100	Abortion				Nar01
Atrazine	Rat (f) F344 SD LE	GD6-10	0-25-50-100-200 mg/kg bw/d	Oral (gavage)	mg/kg bw/d 100 100 >200	mg/kg bw/d 50 50 200	Delayed parturition				Nar01
Atrazine	Rat (f)	PND 22-41	0-12.5-25-50-100-200 mg/kg bw/d	Oral (gavage)	50 mg/kg bw/d	25 mg/kg bw/d	Delayed puberty (opening of vagina retarded)				Law00
Atrazine	Rat (f) Wistar SD	PND 21-46	0-10-30-100 mg/kg bw/d	Oral (gavage)	mg/kg bw/d 100 30	mg/kg bw/d 30 10	Delayed puberty (opening of vagina retarded)				Ash02
Atrazine	Rat (m)	PND 1-4	0-6,25-12.5-25-50	Via mother's milk	50 mg/kg bw/d	25 mg/kg bw/d	Increased incidence and seriousness of prostatitis				Sto99
Atrazine	Rat (m)	PND 23-53	0-12.5-25-50-100-150-200 mg/kg bw/d	Oral (gavage)	12.5 mg/kg bw/d	<12.5 mg/kg bw/d	Delayed puberty (opening of praeputium retarded)				Sto00
Atrazine	Rat (m)	PND 46-48 PND 22-48	0-50 mg/kg bw/d	Oral (gavage)	50 mg/kg bw/d 50 mg/kg bw/d	<50 mg/kg bw/d <50 mg/kg bw/d	Testosterone level in serum and testicle reduced				Fri02

GDx: gestational day x; PNDx: postnatal day x.