Exposure to combinations of substances: a system for assessing health risks

brief

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To

the Minister of Housing, Spatial Planning and the Environment

the Minister of Health, Welfare and Sport

the State Secretary of Social Affairs and Employment

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Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Contents

Executive summary

Every day, people are exposed to all kinds of substances, through swallowing and inhalation among other things. In this advisory report, a Committee of the Health Council of the Netherlands considers the possible adverse health effects of this type of combined exposure.

The Committee first sketches a methodological framework for determining the harmfulness (toxicity) of random combinations of substances, in aid of risk assessment. Risk assessment is intended to determine the nature and size of (potential) health impairment. This advisory report describes how the risk assessment for combinations of substances relates to that for separate substances. The framework the Committee outlines provides a starting point when ascertaining the number of recommended exposure limits that allow the consequences of exposure to be determined. It also helps in the derivation of recommended exposure limits and working with them. The Committee also assesses the degree to which research results can be applied to the circumstances under which combined exposure actually occurs, and the consequences for the way in which recommended exposure limits are derived and applied. Finally, the Committee presents a method for ranking combinations according to the severity of the health impairment.

The Committee discussed its ideas at a working conference with an international group of specialists in the field. The insights obtained at this conference have been incorporated into this advisory report.

Combined effect

The methods for expressing combined effect in figures have not essentially changed since 1985, when the Health Council's first advisory report examining the problem of exposure to combinations of substances was published. However, it is now possible to express the combined effect in figures for more substances. The Committee presents a proposal for a structured approach to combination issues and links it to the 1985 advisory report's classification system based on biological criteria and adapted to current scientific knowledge. This system, which examines substances in pairs, amounts to the categorization of the combined effect into four types, on the basis of the working mechanism. It is based on whether or not the toxic effects correspond and whether or not interaction occurs. Calculation rules exist for the similar and dissimilar effects without interaction, known as dose and response addition. These rules can be used to derive the effect of the combination from the effects of the separate substances and make it possible to determine the degree to which the recommended exposure limits for separate substances provide protection. There is no universal rule for the similar and dissimilar effects in the case of interaction. The system's successful application depends on clarification of the working mechanism. Although this knowledge is available for an increasing number of substances, its collection in routine toxicological studies of substances does not take place as a matter of course. The Committee calls for an improvement in this situation, in line with its recently published advisory report on taking a more efficient approach to research into the health risks of substances. Given the quantification problems, the Committee also recommends arranging for research to be conducted with a view to establishing a well-founded relationship between the nature of the combined effect and the type of calculation rule. This could remove the existing knowledge gap in this field.

The framework presented by the Committee is not a detailed protocol but leaves the interpretation of data in specific situations to specialists. It can be seen as an attempt to give a structure to risk assessment for combinations of substances.

Combinations

For practical reasons, the Committee distinguishes between mixtures and specified (or defined) combinations of substances. A characteristic of mixtures is that the exposure to the components occurs simultaneously and via the same route(s). Information about the composition of mixtures varies. For example, many details are known about the composition of paint fumes whereas only limited data is available on polluted air. The term 'specified combination' means that all substances are known, regardless of

whether they occur together. In the case of a specified combination different exposure routes may exist and the exposures may be independent of one another. An example is the combination benzene and dioxin: exposure through inhalation and food respectively. Although some combinations fit into both categories, it is useful to distinguish between mixtures and specified combinations for the risk assessment. Different approaches are generally considered for the two types in the risk assessment. The best approach in a given case depends on the available data on composition and toxicity.

Substance combinations can be treated as a single entity or as if composed of fractions or separate constituents. In this context ‘fraction’ refers to a group of substances with similar physico-chemical properties. The different methods are shown alongside each other in a flow chart. The Committee recommends going through the entire chart and, rather than making a choice in advance, only making it once the assessment indicates the most suitable approach. To enable the best choice, it is important to examine all of the information together. If required data on one or more methods is incomplete or missing, fewer possibilities will obviously be available. Theoretically, the aforementioned approach can be taken for any combination and for any toxic effect. Determining for the end result is the knowledge available on the nature and toxicity of the substances concerned.

In principle, all three approaches are appropriate for risk assessment. Experience should show how reliable they are. The Committee calls for the component-based method to be tested with combinations of increasing complexity. This will make it possible to determine whether it is scientifically sound and practical.

Interpretation of toxicity data

One of the most difficult parts of assessing the consequences of combined exposure is estimating the magnitude of the combined effect for specified substances under real-life exposure conditions. For example, the combined effect of realistic concentrations in the air generally has to be derived from observations on (much) higher concentrations. Since 1985, more empirical data has been gathered about the consequences of exposure to such ‘low’ concentrations of substances in combination. The scarce findings are in line with the recommended calculation rules and the Committee believes they do not give grounds for assuming that commonly used recommended exposure limits provide insufficient protection. The Committee therefore sees no reason for changing the derivation of recommended exposure limits and the way the numbers are dealt with in the risk assessment of combinations. However, the scope of the research was limited. Moreover, the analyses concerned

short-term exposure to a small number of substances. Therefore, the Committee believes additional research is necessary.

Setting priorities

The Committee also presents a prioritization method for risk assessment. This enables situations involving combined exposure to be systematically compared and ranked according to how problematical they are.

The hazard quotient for each substance in the combination is determined by dividing the actual exposure by an exposure limit. The quotients are then combined to produce a hazard index. In the case of a similar effect, this is done by adding the quotients. In the case of a dissimilar effect, the highest quotient is chosen. The level of risk increases as the resulting number increases.

The hazard index does not take into account the possibility of interaction. Taking this into account refines the index. Up to now, this refinement – correction with weight-of-evidence scores – has only been worked out for similar actions. For each pair of substances the data that (may) indicate interaction is divided into categories and this information is qualitatively weighed according to evidential value. Then, all possible interactions are placed in a matrix, together with the alphanumerical weight-of-evidence. In this matrix each cell relates to a pair of substances. Subsequently, the qualitative weight factors are replaced with numbers and the cells are weighed together. At this stage, the actual exposure is also taken into account. The reason for concern increases in proportion to the final result.

The method can be used for all kinds of combination issues. The variant that is most suitable depends on the purpose and the required accuracy. However, increasing accuracy also means increasing the laboriousness of the procedure. Practice should determine the feasibility of the method in its various forms. Further validation is necessary, as is a sensitivity analysis.

Chapter 1

Introduction

Every day, people are exposed to all kinds of environmental factors that may be harmful to health, such as substances and various types of radiation and noise. Examples of the first group are the dioxins (in food), benzene and particulate air pollutants (in outdoor air) and welding fumes and wood dust (in the air in certain industrial premises). A combination of exposures can present extra health hazards, as proved in the case of asbestos and tobacco smoke (GR88). The government takes various measures, among them the setting of standards, to prevent or limit the harmful consequences. The setting of standards is preceded by risk assessment in which, barring some exceptions, the environmental factors are examined separately. Risk assessment is generally carried out on an ad hoc basis, although risk comparisons could be helpful when weighing measures against one another.

In fact, all causes of diseases should be listed and the role of each cause in the diseases' total impact should be determined. However, a lack of information about the causes of many diseases makes this impossible. On the one hand, determinants (causes of disease) often play a role in the development of various diseases. On the other hand, a disease generally has various causes ('multi-causality') (Rot76). For example, smoking plays a role in the development of lung cancer and coronary diseases. It is regarded as being a cause of lung cancer because smokers are more likely to get lung cancer than non-smokers. However, it is not the only determining factor, as not all smokers get lung cancer. There is insufficient knowledge about the other causes of lung cancer to take a disease-based approach. This example illustrates why, in the practical aspects of the policy on substances, one is restricted to assessing the effect of

a substance (or other determinant) that is assumed to play a dominant role while the other factors are seen as the (blameless) background.

1.1 Recommended exposure limits

A toxicology-based recommended exposure limit is established for exposure to a substance. It is the highest level of exposure to that substance for which the likelihood of a particular undesirable health effect (in a particular population group) is not reasonably expected to exceed a specified figure (GR96a). In this report, it is further referred to as the 'recommended exposure limit'. The term health-based recommended exposure limit is used in cases in which the likelihood of an undesirable effect is considered to be zero. In practice, this type of recommended exposure limit is considered to be applicable to most substances. It is assumed that the harmfulness only manifests itself above a certain concentration, known as the threshold. Recommended exposure limits in cases in which the likelihood of undesirable effects occurring is considered not to be zero – *i.e.* those not belonging to the 'health-based' category – are far less common. They exist for substances with carcinogenic and genotoxic properties that are theoretically assumed to lack a threshold. Depending on the field of policy concerned, a recommended exposure limit may be, for example, a concentration in the open air (environment) or in the air of industrial sites (place of work), or an amount per day (*e.g.* of a substance such as a food preservative that is intentionally added to foods). Such a recommended exposure limit only applies to exposure to the substance in question. It does not take into account the background of environmental factors to which people are exposed, such as a range of substances in food and in the air. Thus far, this has been the only approach that is generally applicable and adequately substantiated scientifically.

1.2 Combinations of substances

In 1985, the Health Council looked into the harmful (toxic) effects of substance combinations and the degree to which the details can be incorporated in recommended exposure limits (GR85). At the time, they could only be taken into account in certain cases. In the other cases, the 1985 advisory report indicated that there is a possibility that recommended exposure limits do not provide the intended protection. Adopting larger margins (*i.e.* lower recommended exposure limits) would offer improved protection. In 1985, it was not yet possible to calculate margins of this kind in a scientifically sound manner, as the available knowledge of the combination effect was too fragmentary.

Since then, a Committee set up by the President of the Health Council has looked into the risk assessment of substances on several occasions, focusing on the general principles and procedures that should be applied. In 1996, the programmatic advisory report on toxicology-based recommended exposure limits (GR96a) was published, in which the Committee said it wanted to address a number of subjects. One of these was dealt with in last year's publication on taking a more efficient approach to research into the health risks of substances (GR01). Another advisory report is in preparation on the so-called benchmark dose method for calculating recommended exposure limits. The present advisory report, which was drawn up after the Committee was augmented by a number of specialists (see annex B), deals with the third topic that was formulated at the time - how exposure to other substances and co-factors such as noise and heat affect recommended exposure limits - and carries on from the work done in 1985 (see annex A).

1.3 Content and composition of the advisory report

The Committee decided to confine itself to multiple exposures to substances. Combinations that include other environmental factors that can damage health have been ignored, as knowledge about this subject was extremely scarce, especially with regard to realistic levels of exposure. This applied to ionizing, ultraviolet and electromagnetic radiation, as well as noise (except in combination with solvents), odours and vibrations.

The Committee defines combination exposure as exposure to more than one substance, via one or more of the following routes: the alimentary canal, the skin and the respiratory tract, the exposures occurring simultaneously or so shortly after each other that they cannot be toxicologically considered as exposures to individual substances. The latter means that non-simultaneous exposure may also result in a combined effect. Even exposures to two substances (very widely) separated in time may result in combination toxicity, when, for example, the first exposure concerns a substance not readily broken down in the body. Annex C contains a summary of the main terms the Committee uses.

Owing to the complexity of the material, the Committee drafted the advisory report so that it is in line with the substance-based approach generally taken in toxicology. The Committee focuses on two main subjects: assessing the harmfulness of random combinations of substances and recognizing the combinations that can affect public health most severely.

Since 1985, there has been a considerable increase in the amount of information available on mechanisms that form the basis for combined effects. Nevertheless, there

are still not many possibilities for expressing combined effects in figures. The Committee has therefore adopted the categorization of combined effects into four types and the quantification possibilities recommended in the 1985 advisory report. It has primarily concentrated on other, more procedural and methodological aspects of determining the health risks of combined exposure, aspects not dealt with in the 1985 advisory report.

The advisory report presents a guideline for taking a structured, systematic approach to combination issues for risk assessment. It does not contain completely worked out methods or protocols; this is precluded by the current level of scientific knowledge. The Committee views it as an attempt to streamline the risk assessment of substance combinations.

Before finishing the advisory report, the Committee discussed a draft version at a working conference with an international group of field specialists; the results were incorporated into the final version. The programme, the list of participants and the results of the working conference are provided in annex D, E and F respectively.

The advisory report's composition is as follows. Chapter 2 describes the terminology and the biological categorization of combined effects into four classes, together with the existing, limited possibilities for expressing the effects in figures. In chapter 3, the Committee presents a proposal for a structured approach to combination issues, which results in the use of this classification system. Insofar as relevant to the setting of standards, chapter 4 discusses data interpretation. Finally, chapter 5 describes how combinations can be ranked in advance according to the (actual or potential) health impairment.

Chapter 2

Terminology

Exposure to a substance may result in the substance's uptake in the body. The main routes into the body are through the alimentary canal, the respiratory tract and the skin. Transport and biotransformation processes mean that the substance and its metabolites reach organs and then participate in or affect biochemical and physiological processes. Any disruption of these processes may adversely affect health and may even be fatal in the worst case.

The transport and biotransformation processes together determine where the substance and its metabolites finish up in the body and how long they remain there. This is known as the kinetic phase of the exposure-effect chain (figure 1). The interaction with biochemical and physiological processes in the dynamic phase then determines the consequences of this. The terms health effect and response are used to indicate the consequences of exposure. A health effect is a particular reaction to the exposure, e.g. a halving of renal functioning. The term response concerns the percentage of the exposed population that displays that reaction. For the sake of clarity, the Committee uses the term 'result' (or 'effect') of the exposure and, wherever misunderstanding is possible, it indicates whether the individual 'health effect' or the 'response' is meant.

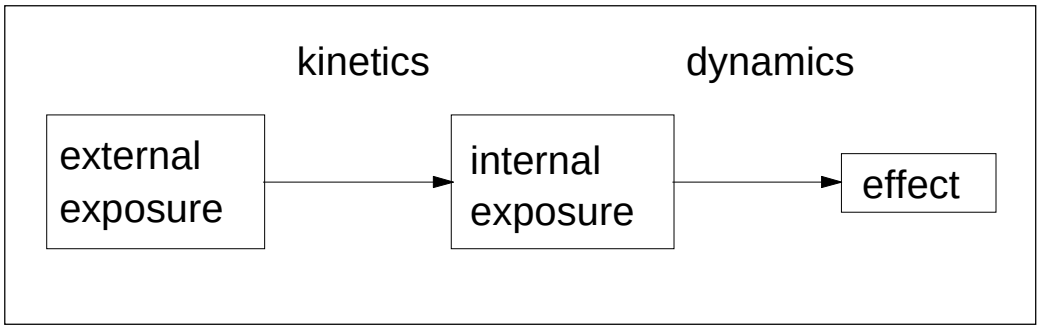

Figure 1 Diagram of the exposure-effect chain for the effect of substances on an organism.

2.1 Combinations of substances

A practical distinction is made between specified combinations and mixtures for determining the consequences of exposure to combinations of substances. All the substances in a 'specified combination' are known, regardless of whether or not they occur as a mixture. A simple example involving three substances is exposure to the air pollutants benzene and sulphur dioxide (SO_2) via the respiratory tract and to a food preservative via the digestive system. This example involves various exposure routes and independent exposures. The exposures may, but do not necessarily, overlap in time.

A characteristic of mixtures is the simultaneity of the exposure to the constituents, as a result of their joint occurrence. Some mixtures belong to the category 'specified combination of substances'. These are intentionally formed mixtures with a known composition, such as paint. However, most intentionally formed mixtures contain some unknown components and do not therefore fit in the aforementioned category. Nevertheless, their composition is reproducible to some degree (for example: the cracking products of mineral oil). Unintentionally produced mixtures often also contain numerous unknown or partially known components (diesel exhaust fumes, tobacco smoke). Major differences may therefore exist in the knowledge about a mixture's composition. Complete characterization of a mixture involves more than identifying the substances; their concentrations and any variations in them must also be known. However, for the sake of simplicity, these aspects are not considered here.

On the basis of the exposure scenario and composition, combinations of substances may therefore be categorized as mixtures and specified combinations of substances, categories that partially overlap. The exposure scenario for mixtures is relatively simple, whereas the common feature of specified substance combinations is a relatively well-defined composition. In spite of the overlap, the aforementioned distinction is useful for risk assessment because combinations require different methods of working, depending on the available composition and exposure data.

Below, the system for characterizing and – as far as possible – estimating the extent of combined effects is discussed, after which the method of working and the system are brought together in chapters 3 and 4.

2.2 Combined effect according to Plackett and Hewlett

The size of any harmful effect of exposure to a combination of substances is generally assumed to be related to the levels of exposure. It is essential to find a suitable calculation rule. Pioneering work that laid the foundation for the present system and possibilities for expressing the combined effect of substances in figures dates back to the nineteen thirties (see for example Bli39). Groups of laboratory animals were exposed to two substances separately or simultaneously in combination. The statistical analysis of, amongst other things, mortality percentages formed the basis for distinguishing between combination effects of various kinds and, as far as possible, for the associated quantification. Later, Plackett and Hewlett expanded on this research by working out the details of the mathematical and physiological basis and by categorizing the combination effects into four different types: similar and dissimilar effects, each with and without interaction (Pla52). Various other systems exist (see for example Gre85, Gre92, Koo81, Pie97, Rot76 and Sei87). Ideally, the type of combined effect would determine the calculation rule to be used to estimate the size of the combined effect. However, it is often not possible to apply the aforementioned categorization of the combined effect because of a lack of data that forms the basis for doing so. The Committee prefers the Plackett and Hewlett system because it is relatively simple and often used for analysing combined effects. The Committee knows of no widely accepted alternative. This choice is in line with the Health Council's 1985 advisory report. The Committee also recommends that research be conducted with a view to establishing a well-founded relationship between the nature of the combined effect and the type of calculation rule, in order to get rid of the existing knowledge gap in this area.

Similar action without interaction

When two substances can each cause the same health effect, renal damage for example, through the same physiological mechanism, but they do not influence each other's effect, the action is called 'similar without interaction' (synonymous with: simple similar action and simple joint action). There is a constant ratio between the doses of both substances which produce equivalent responses. This enables one to derive the response to two doses (one of each substance) together from the responses to the two substances separately, i.e. by multiplying the dose of one by the constant and adding it to that of the other. This rule is currently called 'dose addition'

(synonymous with ‘concentration addition’); the constant mentioned is the toxicity equivalence factor. The Committee refers to handbooks for the method of calculation (e.g. Fin71). The most familiar example is that of the dioxins, which are capable of acting on cells by binding to the intracellular aromatic hydrocarbon receptor (GR96b*).*

Dissimilar action without interaction

If two substances can cause dissimilar health effects (e.g. one damages the liver and the other the pancreas) or can cause the same effect through different mechanisms (e.g. both causing liver damage but through a different mechanism), but they do not influence each other’s response, the term used is ‘dissimilar action without interaction’ *(*synonymous with: simple dissimilar action, independent action, independent joint action and dissimilar independent action). Here, the consequence of combined exposure – in the first example the fraction with an effect (damage to the liver and/or pancreas) – can be determined by adding the single responses in accordance with the independence principle of the probability theory (Fin71). This is known as ‘response addition’ (Nie96, Yan94).

Interaction

This covers similar and dissimilar action with interaction. Plackett and Hewlett speak of interaction when two substances increase or decrease each other’s concentration in the body, or reinforce or weaken each other’s effect, or do both (Pla52). The Committee also uses this term to cover the concentrations of relevant metabolites. Unlike when there is no interaction between the substances, the combined toxicity when there is interaction cannot be ascertained from just the dose-effect curves of the individual substances, but only from the results of research into the toxicity of dose combinations of the two substances.

Plackett and Hewlett did not work out the similar and dissimilar action with interaction separately, because the knowledge at that time did not provide the adequate starting points for doing so. Physiological explanations are now available for the existence of both. To illustrate this, the Committee refers to the combination of di-ethyl ether with styrene and that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) with benzo (a) pyrene. In both cases the interaction has its origins in the kinetic phase of the reaction.

Di-ethyl ether has hepatotoxic (and neurotoxic) properties and styrene is carcinogenic (Lew96). Di-ethyl ether can increase the concentration in the body of cytochrome-P450-2E, the enzyme that converts styrene into its carcinogenic metabolite. The two substances therefore form a pair with dissimilar action (in the dynamic phase: hepatoxicity *versus* carcinogenicity) and interaction (in the kinetic phase: di-ethyl ether induces an enzyme, which leads to a reduction in the body’s

concentration of styrene and increases the concentration of its carcinogenic metabolite).

TCDD and benzo (a) pyrene form a pair with similar action and interaction (GR90, GR96b, Lew96). Both bind to the aromatic hydrocarbon receptor (the similar action, in the dynamic phase). Benzo (a) pyrene can cause lung cancer. On its own, this substance is not carcinogenic but it can be converted into a carcinogenic metabolite by the enzyme cytochrome-P450-1A. Moreover, benzo (a) pyrene can increase the body's concentration of this enzyme. This requires binding to the aforementioned receptor. TCDD binds much more strongly to that receptor and much more powerfully increases the concentration of the aforementioned enzyme. Thus, exposure to TCDD and benzo (a) pyrene together, in comparison with exposure to benzo (a) pyrene only, leads to increased conversion of benzo (a) pyrene, which then results in an increase in this substance's carcinogenity. TCDD and benzo (a) pyrene therefore display interaction, as described above.

2.3 Application

In principle, the Committee considers the classification system described above to be suitable for all adverse health effects and all substances.

Chapter 3

Analysing combinations

This chapter describes the lines the Committee believes the risk assessment for mixtures and specified combinations of substances could take. A summary is shown in Figure 2. Three approaches can be considered for combinations of both types, they are discussed in the order in which they are presented in the chart. In order to achieve the best results, the risk assessment should be conducted along all three lines simultaneously until it becomes clear which method is best. The Committee recommends against choosing a method in advance, as all information counts. A given option is not used in cases in which this working method is not feasible or not useful, e.g. when data is lacking or insufficient. The methods differ in the number of recommended exposure limits used to assess the consequences of exposure. Various simplifications have been made in the description of the process and problem. This is because the advisory report is intended as a guideline. One such simplification is the application of paired assessments to specified combinations. Another example is the characterization of combinations on the basis of the nature of the substances only. Their concentration, stability and volatility are not considered, yet, in practice, these characteristics obviously should be involved in the assessment.

3.1 Mixtures

If a mixture's composition is (largely) known or at least reproducible and stable (e.g. fumes from paint or asphalt), there are three possibilities for drawing a conclusion about the implications of exposure to the mixture and for determining whether one or

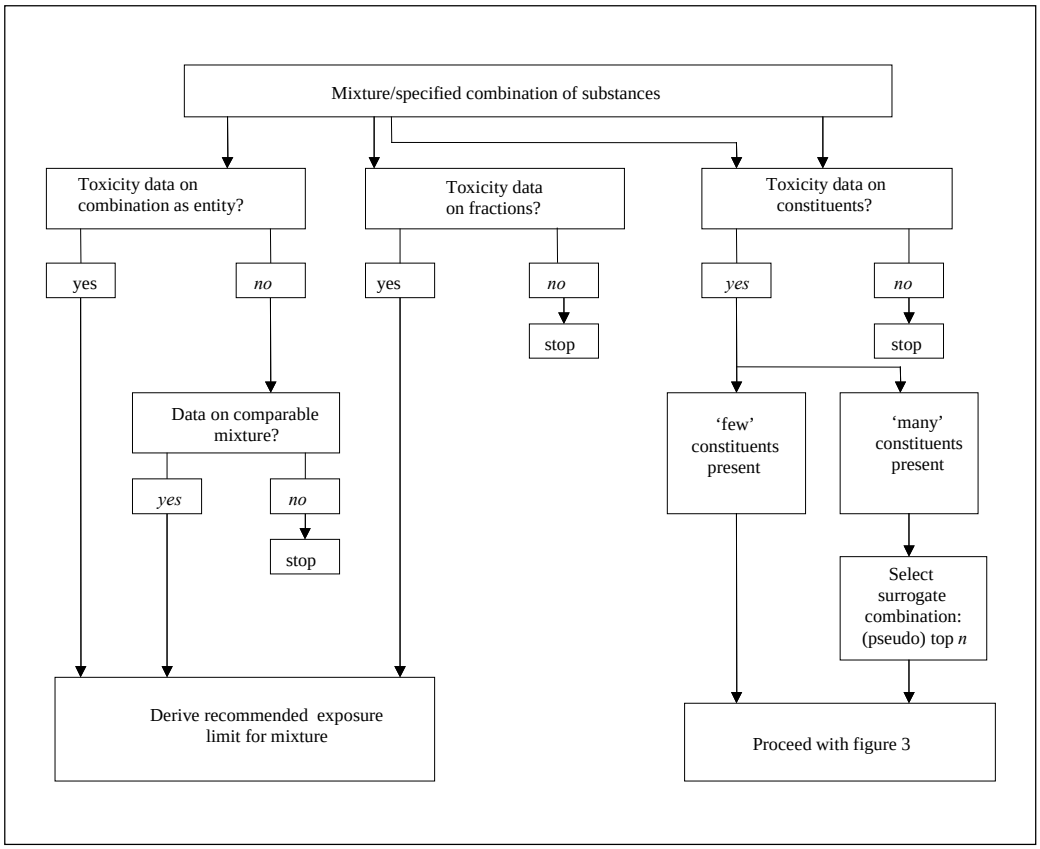

Figure 2 Flow chart for analysing the nature of the combination problem and for determining the harmfulness of a combination, as well as for working out recommended exposure limits.

more than one recommended exposure limit is required to enable assessment of the consequences of exposure. The mixture can be treated as a single entity. Alternatively, an approach based on fractions or constituents can be taken. In the first two situations one recommended exposure limit suffices, in the third situation it does not.

Mixture as single entity

Once the levels at which the mixture is harmful and not harmful have been empirically established, the possibility of deriving a recommended exposure limit from the data can be examined. A recommended exposure limit may also be derived if the mixture has been the subject of epidemiological research into its harmfulness, provided the researchers have determined the level of exposure. In theory, a recommended exposure limit can, in both cases, be derived with the method used for individual substances (GR96a). This is not possible when the mixture contains a component that inhibits the toxicity of the rest and when this inhibitory effect increases less sharply with the concentration than that toxicity.

In the situation described above, the mixture is treated as a single substance. In the case of particulate air pollutants with an aerodynamic diameter of less than 10 µm epidemiological data was available and formed the basis for a recommended exposure limit for the mixture as such (GR95).

It is routine practice in the EU to accept toxicity data on a mixture as an entity, although the intention is not to set standards. 'New substances' (possibly with contaminants) and preparations (mixtures intentionally created) may be marketed on the basis of data of this kind (EU67, EU88). Exceptions are made for, amongst others, substances classified as carcinogenic, mutagenic or toxic to reproduction or the developing human organism.

If the health effects of the mixture have not been investigated but those of a 'mixture with a similar composition' have, one may consider basing the risk assessment on the latter findings. In the simplest situation, such a similar mixture may be identified without the use of any aids; the data can then be adopted directly for the mixture concerned. Pattern-recognition techniques, which enable statistical analysis of the relationship between mixture composition and mixture toxicity, are at the other end of the range of possibilities.

Pattern-recognition techniques may be applied to identify similar mixtures for which toxicity data are available in files containing composition and toxicity data on mixtures (Wol99). Using one of these techniques, principal component analysis, the mixture can be physico-chemically compared with other mixtures. However, comparisons of this kind require the data in the file to be collected in a standardized manner. This means either in the form of 'finger prints' of mixtures (obtained using gas chromatography/mass spectrometry for example) or by means of a qualitative and quantitative description of all constituents. The dioxin composition of sludge from European estuaries has been compared using this technique (Eve93, Kje95).

Pattern recognition also enables the toxicity of the mixture in question to be extrapolated from that of other mixtures. This introduces the second variant of pattern recognition: partial least squares projections to latent structures (Wol99). This technique can be applied to derive the toxicity of a mixture from its physico-chemical data and from the physico-chemical and toxicity data on other mixtures. It was used to analyse the toxicity of diesel exhaust fumes, for example (Eid98, Ost97).

Fractions

If a mixture has not been tested but has been divided into fractions according to its chemical or physical properties and the toxicity of those fractions has been studied, there is, at least theoretically, still the option of calculating a recommended exposure limit through conversion of a recommended exposure limit for the most harmful

fraction (if there is a large difference in harmfulness vis-à-vis the other fractions) (Nai96, Ost97, Ver99). As far as the Committee is aware, this has not yet been done. When taking this approach, one needs to be prepared for changes in toxicity as a result of chemical changes that fractionation involves. Moreover, the separation into fractions may not divide the constituents according to structural or toxicological analogy. Some consolation is possible with regard to this by verifying the recommended exposure limit obtained. This can be done by, for example, recombining the various fractions in all kinds of combinations and also testing the resulting submixtures (Eid98, Ost97). Some mixtures appear to be well-suited to this approach, among these diesel exhaust fumes (Hen94). The particulate matter fraction appears to be entirely responsible for the inflammation of the respiratory tract and lung cancer discovered in animal tests; neither the free constituents nor the gaseous adsorbed constituents contribute to these symptoms. If none of the fractions stands out in terms of harmfulness, the Committee sees no possibility for arriving at a recommended exposure limit for the mixture.

Constituents

The third option for assessing the health risk of exposure to a mixture is to take the constituents as the starting point, as in the case of a combination of specified substances.

3.2 Combinations of specified substances

For combinations of specified substances and mixtures with known constituents, risk evaluation can largely follow the pattern outlined on the right of figure 2. Theoretically, there is no upper limit on the number of substances. The evaluation is fairly simple to make for combinations with a small number of constituents. However, for those with large numbers of constituents the situation has to be reduced to manageable proportions. In such cases, the Committee suggests that the risk evaluation should focus on the most harmful substances. These are substances for which the collective toxicity is considered to be representative of the entire combination, or at least to closely approximate it. The Committee believes choosing this surrogate combination is a job for experts.

At what number of substances does risk assessment require a surrogate combination? An earlier proposal was ten because the risk assessment was considered too difficult to perform in excess of that number (Fer98). The Committee believes that the number has to be decided case-by-case and therefore refers to the number n. Above n, a surrogate combination (of no more than n substances) has to be selected and assessed for toxicity. The best way of selecting the n substances depends on the

complexity of the combination problem. In the case of a not overly complex combination of more than *n* unrelated substances, it is best to select a 'top *n'*. On the other hand, in the case of 'many' substances that can be placed in no more than *n* (chemically or physiologically) related groups, the Committee believes a 'pseudo top *n'* approach is the best option. This means that the most relevant substance in each of the *n* groups is identified and these *n* substances (the 'pseudo top *n'*) are used instead of the total.

The Committee recommends testing this method with combinations of increasing size and complexity. The testing should first involve two or three substances and later deal with larger numbers and mixtures for which two or three (pseudo) substances form the surrogate combination. Finally, it should concern a gradually increasing total number of substances and a gradually increasing *n*. The combination issues must be realistic and the evaluation must extend to determining which health-protection measures are advisable. Working in this way, the upper limits for the total number of substances and the *n* figure that incorporate feasibility and scientific soundness can be determined. Past experience has shown that $n = 1$ is sometimes sufficient; in the case of polycyclic hydrocarbons, the Health Council ruled that benzo (a) pyrene could replace the total (GR90).

How should the composition of the surrogate combination be determined? This necessitates taking various interrelated decisions concerned with whether or not to form groups; selecting the most representative substance in a group; and selecting the *n* substances or groups of substances. The Committee believes that the choice of surrogate combination should be made on the basis of data on the (suspected) health risk, in other words, on the basis of the substance properties, the health effects, the exposure-effect curves *and* the exposure level. Without the latter, exposure-effect curves do not provide any information about the degree of toxicity.

A description has now been given of how the replacement method - with grouping - works for an aircraft fuel of which the constituents and the variation in their concentrations are known (Ver97). The article in question provides a general scheme and, although not all aspects are elaborated on to the same degree, it provides a good idea of what is possible with today's knowledge and computer techniques. It describes the division of the components into groups such as alkanes, alkenes and aromatics, and the type of data required for modelling the kinetics and dynamics of these groups.

Whether a mixture with partially known constituents qualifies for the replacement method depends on the information that is available about its composition. The less information available, the less suitable the method. One could consider requiring a larger percentage of known substances when exposure is higher.

3.3 Summary

The system the Committee recommends entails matching the risk evaluation of exposure to combinations of substances, whether or not occurring as a mixture, to the degree of knowledge about the constituents and the harmfulness of the combination (see figure 2). To this end, a combination is seen as a whole or as consisting of physico-chemically defined fractions or constituents. The different approaches should be followed alongside each other because each method involves advantages as well as disadvantages and all the information together contributes to the final choice. For practical reasons, the Committee believes the substance-based method requires an indirect approach in the case of combinations consisting of 'many' substances. This entails selecting no more than *n* substances to replace the combination, regardless of whether exposure to various substances independently or a mixture is concerned.

Chapter 4

Toxicity data and its interpretation

The previous chapter briefly discussed the major issues involved in the risk assessment of mixtures as a whole. In the case of such combinations, one possibility is to derive a single recommended exposure limit for the mixture, either directly (from the toxicity data on the mixture) or indirectly (from the toxicity data on a similar mixture or mixture fractions). The alternative based on the individual components is concerned with more than one agent and, therefore, more than one recommended exposure limit. This alternative is also suitable for combinations of specified substances that do not form a mixture.

In the component-based method, the risk assessment entails interpreting the data on the substances separately and in combination in a connected way. The available data differs in each case and specific solutions must therefore be found. The Committee consequently restricts itself to a few general statements about the interpretation of the data. It also highlights the latest developments in techniques for collecting and analysing data. With relevance for the setting of standards in mind, the Committee makes an exception in its reticence regarding the significance of research conditions for the actual situation. This mainly concerns the degree to which the effect of a given combination can be derived from data obtained under different conditions (routes, time-frames and levels of exposure). This is important for calculating recommended exposure limits, because these values often have to apply to situations that differ from those to which the data relates.

4.1 Data interpretation

First of all, it is necessary to determine, on the basis of the exposure routes and patterns together with the substance properties, whether combined exposure as defined in this advisory report occurs. If this is not the case, the exposures in question can be considered as exposures to individual substances for the purposes of the risk evaluation. The evaluation can then be carried out along the lines that are normally used.

The information will generally be fragmentary. The volume and quality of the data determine how complicated the risk evaluation is. If, for example, ten substances are involved, the data will probably be varied and different types of combination effects will occur alongside each other. Those different types of combined effects will generally be attributable to various pairs. However, it is conceivable that one pair causes more than one type of combined effect, in a single organ or in different organs. Consequently, even with only ten substances, the evaluation will be time-consuming and complex. There are numerous experimental, epidemiological, mathematical and statistical methods that can be used to obtain relevant findings. As adequate epidemiological findings are scarce, it is usually necessary to use data collected from laboratory tests with animals, cells, cell components and using models. The Committee only mentions a few techniques that have proven useful for directly determining combined effects or that have recently been described and sound promising.

Useful aids

A few methods that are particularly suitable aids in solving combination problems concerning specified combinations and which have meanwhile won their spurs are:

- physiologically based biokinetic (or referring to pharmacology, where they originate: pharmacokinetic) (pbbk*)* models: mathematical descriptions of the distribution of substances and their metabolites in the body across organs or groups of organs. Linking pbbk models for separate substances makes it possible to analyse, for example, interactions at the level of the kinetics (such as competition for an enzyme or a transport protein);
- isobolograms, figures with lines that link equally effective doses of two substances;
- comparison of exposure-response curves of a substance in the presence and absence of another;
- dose-response-surface modelling: statistical-analysis based mathematical description of the relationship between the doses of each of the substances involved and an effect;

- experimental techniques that can be used to determine the nature of the combined effect for more than two substances and for all possible pairs at once, without the need to test all doses in all possible combinations.

The main application of pbbk modelling in the context of this advisory report lies in quantifying the consequences of interaction. The other four can be used for empirically determining how the combined effect is expressed: through dose or response addition, or through falling short of or exceeding this. For details see the summary provided in Cas98.

The Committee believes a few comments are in order here. There are no tests or models that can be used universally for combination questions. The choice of experiments and models, as well as the way they are set up, will always have to be determined on a case-by-case basis. As has already been mentioned, the same therefore applies to the interpretation of the results. Computer analysis will be increasingly used for solving complex problems of the type covered in this advisory report (bio-informatics). The aforementioned pattern-recognition techniques and structure-oriented lumping illustrate the impact of bio-informatics (Yan00). The latter method is new to toxicology and can be used for obtaining missing data on the toxicity of substances separately and in combination. This can be done through extrapolation on the basis of data – in a databank – on the chemical structure and biological effects of a large number of substances separately and in combination. The idea behind this is that each substance can be described and represented by a limited number of structural characteristics, which determine the substance's biological (and therefore also toxic) properties. The structural and biological information of thousands of substances is stored in a matrix, in the form of units that indicate the presence or absence of each characteristic. The essential consideration for risk assessment is that gaps in the mosaic can be filled in by extrapolation, using models developed for this purpose.

Figure 3. Chart for assessing the collective toxicity of two substances.

Assessment of results in the light of standard setting

Figure 3 shows a chart for evaluating the risk posed by combinations of specified substances. The figure focuses on pairs of substances because with combinations of more than two substances it is necessary to assess the combination effect per pair, which also means for every possible pair. The chart can also be used for substances that form part of a surrogate combination and that represent groups of substances (pseudo top *n*).

Two steps are required; these entail determining whether the action is similar or dissimilar and whether interaction occurs. The upper part of the chart is concerned with dissimilar action, the lower part with similar action.

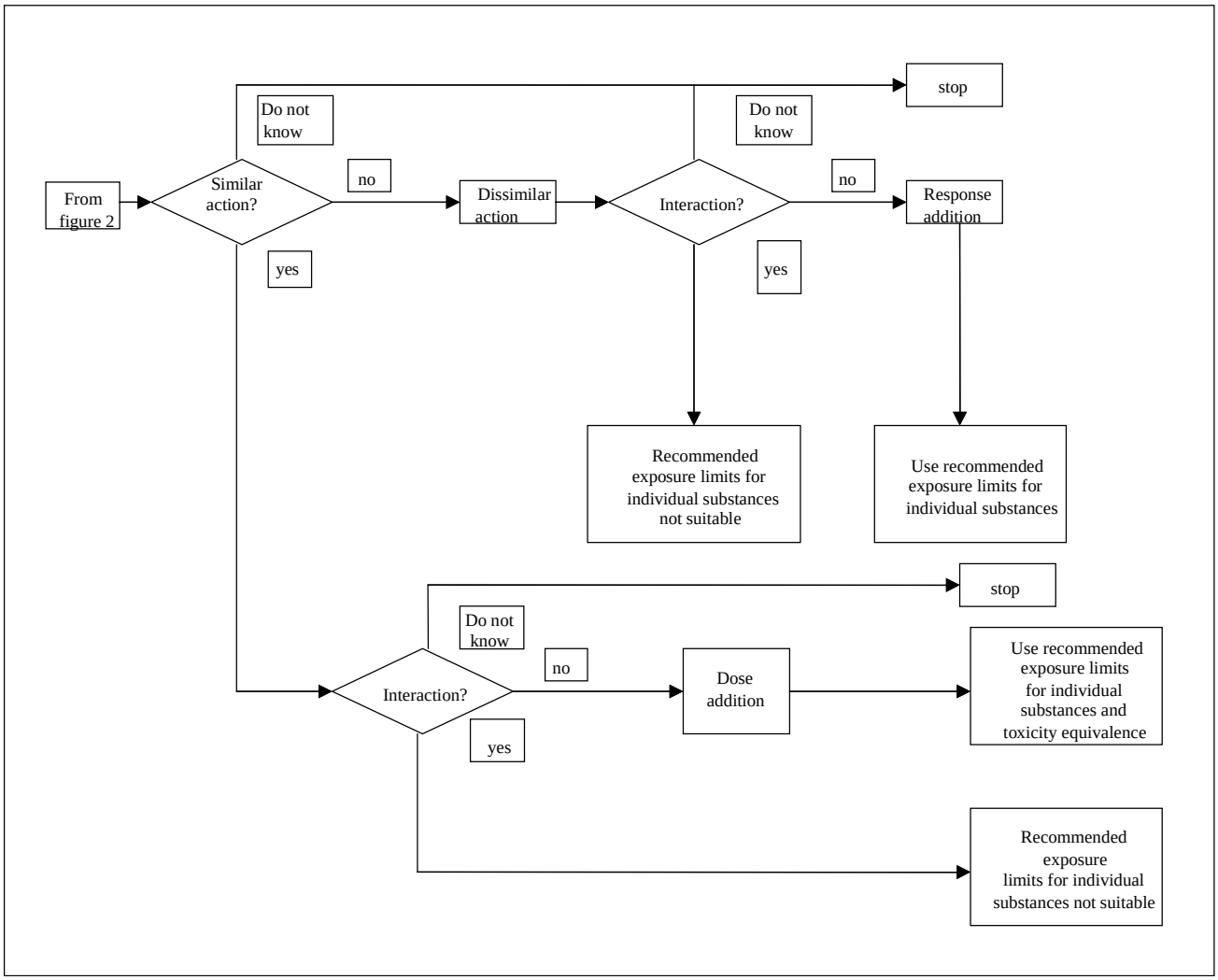

Figure 3 Chart for assessing the collective toxicity of two substances.

The chart begins with the working mechanism. This is followed first by the rule used for calculating the effect of exposure to the pair on the basis of exposure to each substance separately and finally, the consequence of this expressed in terms of recommended exposure limits. If the mechanism is not known, but the rule has been determined empirically, the chart can be followed from that point.

In the case of similar action without interaction, dose addition can determine the effect of combination exposure using empirically obtained exposure-effect data. In this situation, the levels of exposure of two substances not exceeding their respective recommended exposure limits can have an adverse effect greater than expected. This is true when the sum exposure figure calculated using their toxicity equivalence factor,

i.e. reduced to the figure for one of the two substances, exceeds the recommended exposure limit for that substance. This limit is therefore the reference point.

In the case of similar action with interaction, it may or may not be possible to draw quantitative conclusions, depending on the data. The Committee is unable to provide universal criteria for this. In theory, recommended exposure limits for the individual substances do not provide sufficient protection here, regardless of whether the result of the interaction can be expressed in figures, except when the result appears to be negligible. The use of uncertainty factors can be considered but there is no scientific basis for this. The options also depend on the exposure conditions. Adjusting recommended exposure limits in connection with each other could be a useful solution if the concentration ratio of the substances is fixed, as in the air of a factory. In the workplace, the temporary solution can also be considered of providing a warning about the recommended exposure limits, indicating the possibility of a potentially hazardous situation arising.

In the case of dissimilar action without interaction, response addition is the designated method for determining the combined effect. As long as the various concentrations do not exceed the respective recommended exposure limits, there is adequate protection. These limits therefore form the reference point. There is one exception: response addition in the case of substances of which the effect has no threshold (risk level zero). If the effect measure is an incidence (or likelihood) and the two substances act dissimilarly without interaction, but have health effects that can be treated in the same way (different types of cancer, for example), the combination toxicity can be calculated through response addition. In that case, cancer, not the type of cancer, is the reference point and combined exposure to the two substances, each below its recommended exposure limit, may exceed the acceptable risk level. The individual exposure levels must be reduced to maintain the level of protection.

In the case of dissimilar action with interaction, it is necessary, as with similar action with interaction, to examine whether the data permits quantitative conclusions to be drawn.

4.2 Testing in practice

The degree to which the results of empirical research confirm expectations about the toxicity of a combination (of specified substances) is mainly determined by the extent to which the working mechanism and kinetics of the constituents concerned are understood. The characteristics of exposure (level, route, duration, any interruption) form the second important factor. Within the scope of this advisory report, ‘low’ level exposure to substance combinations is particularly relevant. With ‘low’ the Committee means levels that do not exceed the recommended exposure limits. In the past, for

practical research reasons, among other things, studies were made in particular of levels at which the separate substances gave rise to (substantial) adverse effects. These levels were therefore (far) above those at which recommended exposure limits for exposure to individual substances lie or would lie. This raises a question about the degree to which the results apply to these much lower levels. In 1985, no results were available on research into the toxicity of combinations of specified substances at low doses. Now, such results are available, although not in large numbers. They provide answers to three questions. Are the combined effects at high and low exposure levels equivalent? Do current recommended exposure limits provide the anticipated protection? Finally, is a change needed in the way in which recommended exposure limits are derived or used?

Are the combined effects at high and low exposure levels equivalent?

Various findings confirm the effect of the exposure level on the extent of the combined effect; the combined effect is less at a lower dose (Cas94, Gro97, Jon93, Jon96). Therefore, the answer to the first question is negative. The Committee believes that this conclusion should also be expanded to cover the exposure pattern in the broader sense, by which it means that, for example, besides the level, the route along which exposure occurred should also be included. Results obtained under certain conditions cannot be simply applied to another.

Do current recommended exposure limits provide the anticipated protection?

The scarce research results support the hypothesis that similar action without interaction can manifest through health impairment below the (health-based) recommended exposure limits. The results indicate that the extent of the harm done can be determined by means of the toxicity equivalence factor (Jon96). They appear to also confirm that, for combinations of substances with dissimilar action without interaction, such recommended exposure limits provide protection against the consequences of exposure to the combination (Cas94, Gro97, Jon93, Jon96). Extrapolations with mathematical models that describe exposure-response curves of various combinations with dissimilar action without interaction also appear to confirm that, if the dose is sufficiently low, no significant combined effect will occur (Poe96). Furthermore, data obtained from pbbk modelling relating to a combination with interaction at the level of biotransformation reactions suggests that the effect of interaction at low exposure levels is negligible (Lea96, Lea97, Tar97). However, these conclusions are based on a small number of analyses of a likewise small number of substances. Moreover, the analyses were not conducted under conditions that are relevant for the setting of standards. It is true that realistic levels of exposure were analysed but during periods that correspond with considerably shorter exposure than

life-long, or otherwise long-term exposure, i.e. the situation on which the setting of standards largely concentrates. No conclusions can be drawn about the size of the combined effect of low levels of exposure from other research into the consequences of this kind of exposure. This is either because of a lack of information about the working mechanism (Cha89, Ger89, Hei94, Hei95, Hon92, Ito96) or because of shortcomings in the description of the data and its statistical processing (Nar95, Por99).

Is a change needed in the way in which recommended exposure limits are derived or used?

Thus, the data relating to the exposure range on which the setting of standards focuses does not provide any indications that the way in which recommended exposure limits are derived and used in risk assessment needs to be assessed. On the other hand, the data is too limited to allow for generalization of the conclusions. This creates a particular problem with regard to both dissimilar effects and interactions because of the question of whether, at low doses, the effects expected from a combination are different from those expected from individual substances. The answer is affirmative for similar action without interaction, which is in line with the existing consensus among toxicologists. In theory, regardless of the extent to which their working mechanism is known, substances may possess unknown possibilities for interaction. On the other hand, pbbk analysis suggests that, at low doses, interaction does not involve any significant consequences. More research is required to determine whether this is a general rule. The Committee recommends an expansion of research into the consequences of low-level exposure to substance combinations, especially with analyses of more long-term exposure and with more combinations capable of interaction. Substances that qualify for this are those for which the harmful properties and starting points for interaction are documented ‘relatively well’.

Chapter 5

Setting priorities

Risk assessments and measures should obviously focus on combination exposures known or assumed to pose a relatively great risk to public health. For the setting of priorities the Committee has examined the question of how combination situations can be ranked in advance on the basis of health impairment. Ranking of this kind requires data on the toxicity of the substances concerned and on the current or expected level of exposure. After all, low concentrations of extremely toxic substances may endanger health less than high concentrations of less toxic substances.

5.1 Methods

In 1986, the Environmental Protection Agency (EPA) in the United States proposed that situations involving exposure to mixtures of substances should be prioritized on the basis of the actual concentration and the health-based recommended concentration limit of the individual substances (EPA86). The ratio of these concentrations is known as the hazard quotient. To start, the quotient must be calculated for each substance in the analysed mixture. The quotients are then combined, to obtain the hazard index for the mixture as a whole. In the case of similar actions, the combination is the sum of the relevant hazard quotients (EPA99, Mum92, NRC89). The exposure limits must all concern the same health effect. Less stringent conditions were proposed later, such as limits based on various effects, for example the effects in different organs or different effects in one organ (EPA99). If the effect of the substances in the mixture differs, the

hazard index equals the highest hazard quotient. The greater the index, the higher the priority that has to be assigned to the exposure situation.

The aforementioned variants of the hazard index have in common that they ignore an interesting but complicating possibility: interactions between substances that increase or reduce the mixture's toxicity. The Committee has explained that there are sufficient indications that such interactions occur. The available information rarely enables toxicologists to precisely quantify the results of interaction. However, sometimes conclusions can be drawn about the probable direction of the interactions or about the degree of uncertainty involved. The Committee believes that Mumtaz and Durkin have proposed an interesting approach – the weight of evidence method – for getting more of a handle on this difficult issue (Mum92).

5.2 Refinement

Mumtaz and Durkin's approach involves detailed ranking of all available toxicological data and the allocation of qualitative and quantitative weight factors to the data categories thus obtained. Information on exposure can also be taken into account. This set of analytical instruments enables the qualitative and quantitative refinement of hazard indices (for substances with a similar effect). The Committee provides an outline of how this refinement works below.

Step 1: classification of binary interactions

Examine for each pair of substances in the mixture whether the data indicates interaction. The evidence scores for the assumed interaction increase with the knowledge of the mechanism (or mechanisms) and the degree to which the toxicological significance of that interaction has been demonstrated. Information about structurally related substances is given an interim position. Furthermore, information concerning a similar duration and route of exposure is assigned the heaviest weight. In addition, in vivo information is assigned a higher score than in vitro information. According to the alphanumerical system used, in theory, 72 categories of evidential value can be distinguished.

Step 2: qualitative matrix of interactions

Incorporate all possible binary interactions into a matrix. Each cell of the matrix corresponds with a binary interaction (What is the effect of substance a on substance b?) and contains an alphanumerical evidential value category (given sufficient data; otherwise the cell is left open). The authors recognize that interpretation of a qualitative situation-specific matrix of this kind is often far from clear. They say that it

is nevertheless possible to sometimes obtain an initial impression of the direction of the interactions. For example, in the case of (approximately) equal evidence scores, there may be more cells with interactions that strengthen the mixture's toxicity than cells with interactions that weaken it. This indicates that the toxicity is probably greater than could be anticipated on the grounds of the aforementioned hazard index. However, for a sharper picture of the situation, the matrix's cells must be weighed in relation to each other in some way. In other words, the qualitative matrix has to be converted into a quantitative matrix.

Step 3: quantitative matrix of interactions

Assign weight factors to the components of the alphanumerical evidential value categories in the qualitative matrix. Addition is first assigned the value 0, interaction that increases toxicity the value 1 and interaction that reduces toxicity the value -1. Mumtaz and Durkin then choose the weight factors so that, in theory, the highest degree of confidence in the direction of the interaction has the value 1 and the lowest the value 0.05. Each cell in the matrix is hereby given a number between -1 and 1. An 'open' cell in the qualitative matrix (insufficient data) is assigned the value 0. However, the level of exposure to the various substances in the mixture has still not been taken into account. This is erroneous as the toxicological significance of interactions increases with that level. Consequently, the researchers added a further refinement.

Step 4: matrix of interactions weighted according to exposure

Look at the specifics of the situation: the actual level of exposure to the individual substances in the mixture. The hazard quotients of those substances say something about the toxicological risks. Put differently, the cause for concern increases as the sizes of the quotients increase. The researchers introduce an interaction factor for each binary interaction: the product of the weight factor (obtained in the previous step) and the geometrical average of the hazard quotients of the two compounds concerned.

According to Mumtaz and Durkin, the matrix thus obtained makes the large amount of complex information practicable to work with. Adding all the interaction factors shows how strong the tendency of potential interactions in the mixture is in a certain direction. Standardizing this total score – that is dividing it by the score obtained if all binary interactions have the same direction and if maximum evidence exists in each case – also gives an idea of the level of confidence that can be attributed to the analysis results. The final outcome of the analysis is a hazard index corrected by this standardized total score (for precise details see Mum92).

As Mumtaz and Durkin point out, their approach is not suitable for an assessment of the absolute size of the interaction effects, to enable them, for example, to be taken

into account in exposure-effect relationships. The practical value lies in the possibility of systematically comparing exposure situations and ranking them according to how problematical they are. In addition, the researchers point out a few things that need to be considered when using their analytical method and mention aspects that could be improved. In connection with the alphanumerical system for example, they note that specialists may sometimes have reasons for weighing toxicological data in a way slightly different from that specified in the system. Therefore, in their view, ranking has to involve a 'story'. They also underline the necessity of carrying out the procedures for quantifying weight factors along more objective lines.

The Committee draws attention to various recent refinements of the weighting system. For example, a version has been developed that takes into account the ratio of the concentrations of the substances concerned; this ratio affects the size of the interaction effect (Dur00, EPA99). The Committee sees this as a major improvement. A 'biological' hazard index has also been proposed (Had99). This index involves working with measures of internal exposure, such as the concentration of the substances concerned in the blood. Pbbk models play a central role in the calculations. This has the advantage that extrapolation to lower doses (than in experiments) is possible. Nevertheless, there are still problems that have repercussions for the practical feasibility of analytical methods of this kind. The Committee briefly discusses these in the next section.

5.3 Application possibilities

With all the limitations and desiderata, the Committee wants to make clear that – with evidence scores corrected – hazard indices are, in principle, suitable for use as a prioritization instrument in risk assessment. The choice of most suitable variants depends on the purpose of the prioritization and the required accuracy. For example, if general ranking according to urgency is required for taking control measures, a qualitative approach will suffice (see for example ATS00, Poh99). If more precise information is required about the size of the risks, more recent quantitative variants are preferable. The advantage of greater accuracy must clearly offset the disadvantage of more work. In any case, the Committee believes that the methods described are not only appropriate for setting priorities in the remediation of locations with contaminated soil – the original field of application – but can also provide a basis for assessing other situations of combined exposure, such as the consumption of contaminated food or inhalation of polluted air. The Committee recommends testing their usefulness in the field.

Here too, the analytical methods discussed cannot stand up without validation. Although a start has been made on this (Mum98), so far, work has only been carried out on a small number of substances. The Committee believes more work in this area is necessary. More attention also needs to be paid to sensitivity analyses, which are used to obtain insight into the effect of all kinds of assumptions and model-based choices.

Finally, the Committee points out that still other criteria can be involved in the prioritization discussed here. It also referred to this in its advisory report on toxicity profiles (GR01). Criteria for consideration include the number of people exposed and the likelihood of exposure. It is up to policy makers to decide how any such criteria should be applied.

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Annexes

Annex **A**

The questions to the Committee

In 1996 the Committee published the advisory report 'Toxicology-based recommended exposure limits'. In its presentation letter accompanying that advisory report, the President of the Health Council of the Netherlands wrote that the Committee considered further elaboration to be possible on the basis of current knowledge. One of the topics that was considered for such elaboration was the effect of exposure to environmental factors in combination on toxicology-based recommended exposure limits. The report should be based on the answers to the following questions:

- What are the latest developments in toxicity research and the evaluation of the risk presented by mixtures?
- What role do similarities and dissimilarities between substances play with regard to target organs and working mechanisms?
- How should toxicity data on complex mixtures such as welding fumes, cigarette smoke, wood dust and novel foods, be extrapolated from tests on laboratory animals to make it applicable to humans or from high doses to make it applicable to low doses?
- Besides taking into account the combination effect of substances, is it also necessary to consider co-factors such as noise, odour, heat, vibrations and psychosocial factors? If it is, how should this be done?

Annex **B**

The Committee

- WRF Notten, *chairman*
  toxicologist; TNO* Prevention and Health, Leiden
- JH Beijnen
  professor of bioanalysis; University of Utrecht
- WFJPM ten Berge
  toxicologist; DSM, Heerlen
- BJ Blaauboer
  toxicologist; Institute for Risk Assessment Sciences, University of Utrecht
- VJ Feron
  professor emeritus of biological toxicology; University of Utrecht
- JP Groten
  toxicologist; TNO Nutrition and Food Research, Zeist
- JLM Hermens
  toxicologist; Institute for Risk Assessment Sciences, University of Utrecht
- PHM Lohman
  professor of radiation genetics and chemical mutagenesis; University of Leiden
- HME Miedema
  psychologist; TNO Prevention and Health, Leiden
- G de Mik
  toxicologist; National Institute for Public Health and the Environment, Bilthoven

* TNO is the Dutch acronym for Netherlands Organization for Applied Scientific Research

- WF Passchier, *consultant*
  Health Council of the Netherlands, The Hague
- MN Pieters, *consultant*
  National Institute for Public Health and the Environment, Bilthoven
- GMH Swaen
  epidemiologist; University of Maastricht
- RA Woutersen
  toxicologist/pathologist; TNO Nutrition and Food Research, Zeist
- JA van Zorge, *consultant*
  Ministry of Housing, Spatial Planning and the Environment, The Hague
- PW van Vliet, *secretary*
  Health Council of the Netherlands, The Hague

Secretarial assistance:
- M Javanmardi (till May 1, 2001)
- WY Lee (from May 1, 2001)

Lay-out:
- J van Kan
- M Javanmardi

Annex **C**

Terminology

combined exposure
: exposure to more than one substance, via one or more of the following routes: the alimentary canal, the skin and the respiratory tract, the exposures occurring simultaneously or so shortly after each other that they cannot be toxicologically considered as exposures to individual substances

combinations of substances
: every combination, whether or not in the form of a mixture

combination of substances, specified (or defined)
: whether or not a mixture, all substances are identified

dissimilar action with interaction
: two substances that show “dissimilar action without interaction” and “interaction” (q.v.)

dissimilar action without interaction
: two substances have dissimilar health effects or have the same effect through different mechanisms, but they do not influence each other’s response

dose addition

calculation rules for similar action without interaction. There is a constant ratio between the doses of the two substances which produce equivalent responses, so that the response to two doses (one of each substance) together can be calculated on the basis of the responses to the substances separately, namely by multiplying the effect of one by the constant and adding it to that of the other. The constant referred to is the so-called toxicity equivalence factor.

hazard

harmful properties

hazard index

a dimensionless variable that expresses the health risk of a specific combination and consists of either the sum of the hazard quotients of the separate substances or the highest of these quotients

hazard quotient

a dimensionless quantity that expresses the health risk of a substance and that is calculated by dividing the exposure concentration by its recommended exposure limit, or some other exposure limit

interaction

two substances increase or decrease each other's concentration (or the concentration of each other's metabolites) in the body, or reinforce or weaken each other's effect, or do both

mixture

combination of substances of which the simultaneous occurrence is characteristic

response addition

the chances of an effect (or the percentages of the population in which the effect can occur) are determined in accordance with the independence principle of the probability theory

similar action with interaction

two substances show "similar action without interaction" and "interaction" (q.v.), involving two consequences, for example in the kinetics and dynamics respectively

similar action without interaction

two substances can cause the same health effect, through the same physiological mechanism, but do not influence each other's effect

weight of evidence

method for assessing whether there are indications that interaction occurs and for determining the confidence in these indications and the direction of the interaction effect.

Annex **D**

Working conference programme

Working conference
'Exposure to combinations of substances: assessment of health effects'
Den Dolder, the Netherlands, 8-9 October 2000

October 8, 2000

13.00 - 14.00	*Reception*
14.00 - 14.10	*Wellcome address* JA Knottnerus, Vice-President Health Council
14.10 - 14.15	*Organizational aspects of the working conference* PW van Vliet
14.15 - 14.30	*Introduction to the work of the Council's Committee on Derivation of health-based recommended exposure limits - multiple exposure* WRF Notten

Session I. The decision tree approach I: categorization and concept of surrogate combination (draft report pp. 13-19).

Subjects: the ideas of subdivision into mixture and non-mixture combinations and selection of surrogate chemicals in complex situations; the contribution of pattern recognition techniques; the branching points; tree modification proposals*

Chair: RC Hertzberg
Rapporteur: GMH Swaen

14.30 - 15.00	*Introduction 1* RA Woutersen
	Introduction 2 HR Pohl
15.00 -15.45	*Discussion*
15.45 - 16.15	*Break*

Session II. The decision tree approach II: usefulness and feasibility as regards standard setting (draft report pp. 13-21).

Subjects: practical aspects; alternatives*

Chair: RC Hertzberg
Rapporteur: MN Pieters

16.15 - 16.45	*Introduction 1* PHM Lohman
	Introduction 2 AM Moses
16.45 - 17.30	*Discussion*
17.30 - 18.30	*Break*

* not limitative

Session III. The surrogate approach I: criteria and guidance for dealing with lack of insight into the chemicals involved (draft report pp. 13-19).

Subjects: selection criteria*

Chair: BJ Blaauboer
Rapporteur: JH Beijnen

18.30 - 19.00	*Introduction 1* VJ Feron
	Introduction 2 P Durkin
19.00 - 19.45	*Discussion*
19.45 - 21.30	*Dinner*

October 9, 2000

9.00 - 9.05	*Technical information*

Session IV. The surrogate approach II: criteria and guidance for dealing with lack of toxicity data (draft report pp. 19-23).

Subjects: assumptions, health risk below single-chemical exposure limits, impact of kinetic interaction*

Chair: BJ Blaauboer
Rapporteur: RA Woutersen

9.05 -9.35	*Introduction 1* J Groten
	Introduction 2 G Pöch
9.35 - 10.30	*Discussion*
10.30 - 11.00	*Break*

Session V. Improved mathematical base for assessment of toxicity of combinations of chemicals (draft paper by HME Miedema).

Subject: a general, unifying mathematical concept*

Chair: RSH Yang
Rapporteur: WFJPM ten Berge

11.00 - 11.30	*Introduction 1* HME Miedema
	Introduction 2 J Sühnel
11.30 - 12.15	*Discussion*
12.15 - 13.15	*Lunch*

Session VI. Priority setting (draft report pp. 24-26).

Subjects: criteria and guidance; simplifications*

Chair: AG Renwick
Rapporteur: JA van Zorge

13.15 - 13.45	*Introduction 1* JLM Hermens
	Introduction 2 H-G Neumann
13.45 - 14.30	*Discussion*
14.30 - 15.00	*Break*

Session VII. General discussion and conclusions

Chair: WRF Notten
Rapporteur: VJ Feron

15.00 - 16.55 *Discussion*

16.55 - 17.00 *Closing*

Annex **E**

List of participants at the working conference

Working Conference
'Exposure to combinations of chemicals: assessment of health effects'
Den Dolder, the Netherlands, 8-9 October 2000

Participants

- dr WFJPM ten Berge; DSM; Heerlen, the Netherlands
- dr JH Beijnen; Pharmacy Slotervaart Hospital; Amsterdam, the Netherlands
- dr BJ Blaauboer; Institute for Risk Assessment Sciences; University of Utrecht; the Netherlands
- mr N Burge; European Union; DG Enterprise; Brussels, Belgium
- dr P Daskaleros; European Union; DG Health and Consumer Protection/Product and Service Safety Unit; Brussels, Belgium
- dr P Durkin; Syracuse Environmental Research Associates, Inc.; Fayetteville, USA
- dr I Eide; Statoil Research Centre; Trondheim, Norway
- dr VJ Feron; Zeist, the Netherlands
- dr JP Groten; Division of Toxicology; TNO Nutrition and Food Research; Zeist, the Netherlands
- dr JLM Hermens; Institute for Risk Assessment Sciences; University of Utrecht; the Netherlands
- dr R Hertzberg; Waste Management Division; Office of Technical Support; US Environmental Protection Agency; Atlanta, USA

- dr PHM Lohman; Department of Radiation Genetics and Chemical Mutagenesis; Leiden University Medical Center; the Netherlands
- dr HME Miedema; Environment Division; TNO Prevention and Health; Leiden, the Netherlands
- dr AM Moses; Hartford, Northwich; United Kingdom
- dr HG Neumann; Institute of Toxicology; University of Würzburg; Germany
- dr WRF Notten; TNO Prevention and Health; Leiden, the Netherlands
- dr MN Pieters; Division Chemicals and risks; National Institute of Public Health and the Environment; Bilthoven, the Netherlands
- dr HR Pohl; Division of Toxicology; Agency for Toxic Substances and Disease Registry; Atlanta, USA
- dr G Pöch; Eggersdorf, Austria
- dr AG Renwick; Clinical Pharmacology Group; University of Southampton; United Kingdom
- dr C De Rosa; Division of Toxicology; Agency for Toxic Substances and Disease Registry; Atlanta, USA
- dr J Sühnel; Biocomputing; Institute of Molecular Biotechnology; Jena, Germany
- dr GMH Swaen; Department of Epidemiology; University of Maastricht; the Netherlands
- dr RA Woutersen; Division of Toxicology; TNO Nutrition and Food Research; Zeist, the Netherlands
- dr R Yang; Center for environmental Toxicology and Technology; Colorado State University; Fort Collins, USA
- dr M Younes; International Programme on Chemical Safety; World Health Organization; Geneva, Switzerland
- dr JA van Zorge; Directorate of the Environment, Ministry of Housing, Spatial Planning and the Environment; Den Haag, the Netherlands

Staff
- MFC van Kan
- M Kouwenberg
- dr WF Passchier
- dr PW van Vliet

Annex **F**

Summary of working conference

Working Conference
'Exposure to combinations of substances: assessment of health effects'
Den Dolder, the Netherlands, 8-9 October 2000

At the working conference the draft advisory report 'Exposure to combinations of substances' of the Committee on 'Derivation of health-based recommended exposure limits: multiple exposure' was discussed with an international group of suitably qualified experts. Beforehand, these experts had answered a number of questions concerning the draft, mainly to select the topics for discussion during the meeting. Additionally, a draft paper on mathematics by one of the Committee's members had been scheduled for discussion. Six topics had been selected as session subjects: the concept behind the approach chosen by the Committee, its practical consequences, criteria for dealing with incomplete insight into the chemicals involved, criteria for dealing with incomplete toxicity data, the mathematical base of combination effects and the setting of priorities for risk assessment. The invited international experts complimented the Health Council of the Netherlands as well as its committee on chemical mixtures for initiating a pioneering and courageous effort in Europe to deal with this very complicated and yet realistic and important issue.

The Committee's approach

The participants agreed that the approach of the Health Council of the Netherlands to standard setting for and risk assessment of exposure to combinations of chemicals should be regarded as a framework, rather than an elaborate protocol, that is aimed at managing the risk assessment process and priority setting for risk assessment. It is meant to provide guidance to experts. The external reviewers endorsed the approach as such, with subdivision into mixture and non-mixture combinations and selection of a number of (real or fictive) 'surrogate' chemicals for simplification of complex exposure situations. No decision points were found missing in the figures that together delineate the Committee's ideas. Each point needs to be covered by an explanatory text.

The suggestion to call the figures flow charts or schemes, rather than decision trees, and to explain the meaning of this designation in the text, met with general approval. It means that *all* relevant data should be taken into account at each step in the flow charts; they may concern mixtures, fractions thereof and individual components. Thus, all possible or reasonable approaches should be used rather than one. This would be most important when the data permitted a component-based, as well as a fraction- and/or whole-mixture-based assessment. In some cases it may be desirable to try more than one approach and compare the results. This should be mentioned in the accompanying text. Also, a discussion of the uncertainties to be anticipated at the various stages of the assessment should be added. The general feeling about the articulation of the schemes was that the present versions are to be taken as a first step toward a 'definitive' set. After experience has been obtained applying them, they can be and probably will need to be updated. Evaluation in a few years time was therefore recommended.

With regard to the number of chemicals to be selected, it was concluded that mentioning a default number, *i.e*. ten, detracts from the necessary flexibility, in spite of an accompanying explanation. A solution offered was to speak about the 'top *n'*. Furthermore the consensus opinion was that *n* should be kept small or at least manageable.

Practical aspects

Can the flow charts be applied generally? According to the meeting participants, the set of flow charts can be applied to all endpoints, for both hazard identification and risk assessment. In selecting compounds for the 'surrogate' or 'top *n'* approach experts

must ‘look at the whole picture’, in other words: invariably evaluate all the relevant data.

The ‘top *n’* may consist of any number, depending on the situation. For practical reasons, however, the number will have to be relatively low. In some cases it may even be as low as one - when one chemical, the ‘marker’ substance, represents (almost) all of a combination’s health risk. Experience gained by the U.S. Agency of Toxic Substances and Disease Registry from risk assessment of soil contamination sites had indeed taught that small numbers of chemicals, *i.e*. three to five, are often representative, and that this number is only occasionally higher, 15 for example. The workshop participants agreed that the report should include a recommendation that the schemes be tested iteratively: begin with a small ‘easy’ set of chemicals (two or three), then increase the number of compounds stepwise. In this manner, case studies of increasing complexity will help in shaping the methodology and finding where the practical boundaries are.

Selection criteria

The meeting group expects that experts conducting risk assessments of mixtures will often be confronted with incomplete information about the compounds involved. As indicated earlier they support the use of the ‘surrogate chemicals’ approach, directing the assessment at the substances presumed to be the toxicologically major ones, to overcome this difficulty. Which compounds are ignored and what are the consequences? Both the known constituents of toxicologically minor importance and the unknown constituents are typically disregarded. In the case of the former this is based on judgment of the available data; as mentioned above, the best selection criterion is the component risk, as long as interaction with other key components is considered unlikely. With regard to the latter, other criteria are needed to decide whether disregarding them is acceptable. During the working conference four situations were identified in which the unknowns need no further attention. These are situations in which the health or toxicological effects of the mixture as a whole are obvious (*e.g*. tobacco smoke), and in which the total mixture has been tested properly, because in both situations the mixture-as-an-entity approach provides the solution. The latter applies to many ‘single’ chemicals that are ‘technical grade’, *i.e.* contain (unidentified) impurities. A further situation in which the unknowns need no attention occurs when health benefits compensate for the toxicity of the mixture (*e.g.* drinking water with its contaminants). In such a situation the decision is not up to toxicologists, as public health requires balancing the toxicity of contaminants due, among other things, to chemical disinfection, against infectious diseases. It is a political issue.

Finally, it may be decided to ignore the unknown constituents of a mixture when the exposure is low (in terms of either the exposure level (below the threshold of toxicological concern), or the number of people exposed). This is also a political issue.

According to the report, the representative subset of chemicals can be selected on the basis of

- toxicity (type and severity of effect)
- toxicokinetics
- chemical properties
- risk (suggested measure: the quotient of exposure and recommended exposure limit (or lowest exposure at which toxicity has been observed))
- representativeness of the group (if a surrogate replaces several chemicals).
- The workshop participants agreed that the risk (hazard * exposure) is the best criterion. The 'top *n'* thus ideally consists of the *n* most 'risky' chemicals.

The group was unanimous that general rules to select the surrogate chemicals cannot be given and that case-to-case judgment by experts is required. This judgment will have to include the decision as to whether a certain number of unknowns is tolerable in that particular situation. Transparency of the assessment can promote the acceptance of the outcome. This means, among other things, mentioning the percentage of the chemicals identified, and the conservative assumptions applied, if any are deemed necessary to compensate for data gaps. An option worth mentioning in the report is to require a higher percentage of known components when exposure is higher.

In the event that a mixture is very complex, because it predominantly consists of unknowns for example, and has not properly been examined for health or toxicity effects, a participant suggested that so-called pattern recognition approaches be applied. Pattern recognition can be used to identify mixtures with sufficient resemblance, to find one on which toxicity data has been collected. To this purpose Principal Component Analysis is applied to data detailing the chemical characterization of the mixtures, either as 'finger prints' (obtained by e.g. gas chromatography/mass spectrometry) or as detailed identification and quantification of each compound. Pattern recognition can also be used to extrapolate the toxicity of a complex mixture from chemical and toxicological data on similar mixtures, applying multivariate regression techniques such as Projections to Latent Structures. PLS may also be used to identify the major contributors to a mixture's toxicity (the top *n*).

Furthermore, it was concluded that testing mixtures with unknown constituents as an entity is common practice. Many 'single' chemicals, among them numerous pesticides,

are ‘technical grade’ material, *i.e*. contain (unidentified) impurities, and have been tested as such. Consequently they belong to the ‘mixtures’ of which the unknown part can be disregarded without any health risk.

Incompleteness of toxicity data

The meeting participants agreed that conclusions on the nature of the combined action of two chemicals would generally have to be drawn from incomplete data. Consequently, uncertainty as to whether the substances act similarly or can interact will often be the outcome of the assessment. Figure 4 does not reflect this uncertainty properly. It contains a diamond ‘similar action?’ and two diamonds ‘interaction?’, each with the outcomes ‘yes’ and ‘no’. A third, ‘do not know’, needs to be added.

Similar action without interaction can lead to health effects, even if the levels of the individual compounds remain below their toxicological effect levels. There was no doubt about that among the workshop participants. They agreed that the consequence of interaction and dissimilar action without interaction below the effect levels of individual components is not known. At such (low) levels effects due to these types of action have only occasionally been observed. However, the research performed to resolve the combination toxicity at these levels is limited. Therefore this aspect was felt to need further study. Thus, if the effects of the chemicals are characterized by thresholds the following holds true: combinations of dose-additive chemicals, all below their effect levels, may be toxic, whereas whether combinations of response-additive chemicals are toxic is not known, although theoretically they would pose no risk.

If they are characterized by the absence of thresholds, on the other hand, the magnitudes of the effects under dose addition and response addition approximate each other with decreasing dose. There are no clues, however, as to the dose at which the difference becomes negligible. Whether the two addition models result in identical effects at and just below effect levels of the individual compounds thus remains enigmatic.

The consequences of interaction were subdivided into those concerning kinetics and those concerning dynamics. So far, effects caused by kinetic interaction have not been seen at these levels. The same holds true for dynamic interaction, defined as leading to effects greater than predicted by the mathematical model describing independence.

The toxicity of a pair of chemicals can be much more complex than it looks at first sight. Combinations may express several modes of action, e.g. both similar action and dissimilar action. When evaluating the toxicity of a combination, one should therefore consider all endpoints involved, as well as the underlying mechanisms of action. The toxicity picture can have additional levels of complexity. Compounds with dissimilar modes of action may together contribute to one endpoint. For example, two substances with tumour-initiating and tumour-promoting activity respectively, are jointly capable of causing cancer. This illustrates that endpoint and effect measurements determine the biological conclusions about a combination's toxicity. The meeting participants reached consensus on the need for an exact description of the four modes of action in the guidance report. They consider it very important to describe for example the meaning of 'additivity': whether it applies to the level of the individual or the group, whether it is based on a biological mechanism or empirical observations without any mechanistic information. In line with this, the terms 'response addition' and 'effect addition' require clarification, as does the issue of the correlation of effects.

Mathematical base for toxicity assessment of combinations of chemicals

The mathematical concept discussed at the meeting had been developed to find toxicological principles that would lead to the rule appropriate for describing the toxicity of combinations of chemicals. However, during the workshop, its meaning and its possible contribution to the field, particularly to standard setting and risk assessment, remained unclear. According to the external reviewers the concept should be explained and evaluated with the help of extensive existing data sets, in close cooperation with toxicologists, and simulated data sets obtained by computer calculations.

With elaboration for other combination effects, such as antagonism and synergism, it can be explored further as an approach to understanding the concepts underlying mixture models.

Furthermore, it was concluded that various definitions and concepts are often used to describe the combined effects of chemicals. One of the reviewers considered it important to distinguish clearly between evaluation methods based on empirical facts and those based on mechanistic information. The benefit would be a considerable reduction of the discrepancies among findings. In this light, the meeting participants felt that a glossary with the Committee's use of terms should form part of the report.

Prioritization

The Hazard Index (HI) was found useful for setting priorities for risk assessment among combinations of chemicals, whether a mixture or not. It is a measure suitable in cases of similar and dissimilar action without interaction. Modification of the HI by the Weight of Evidence (WOE), that conveys the biological plausibility of interaction being a property of the combination, was found a helpful additional concept. Nevertheless, it was noted that the WOE probably has limited applicability, as lack of data will be the rule rather than the exception. Finally, the others also shared the Committee's opinion that the surrogate approach can be applied to the HI/WOE. Some recommended extending the discussion of the WOE methodology, including the merits of the latest version of the method, developed at the U.S. EPA.

In the HI/WOE both hazard and (potential or actual) exposure are reckoned with. The latter is usually characterized less well than the former. One of the participants said that the accuracy of its assessment depends on the sort of information. In situations of actual exposure an internal measure, of the 'biomonitoring' type, is preferable; when absent an external exposure indicator will have to do.

There was general agreement that the characteristics of the exposed population should be taken into account in the priority setting process, as regards not only the number of people involved, but also the variability in toxicokinetics and toxicodynamics and the background incidence of the health effect studied.