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Position paper of

PAN Germany (Pestizid Aktions-Netzwerk e.V.)

on the combination effects of pesticides

Why Risk Assessment is necessary for Substance Mixtures

Hamburg, February of 2007

Combination effects of pesticides

Why risk assessment is also necessary for substance mixtures

For over 20 years the German non-profit, non-governmental organization PAN Germany has committed itself, both at the national and international level, to promoting sustainable agriculture, which abandons the use of hazardous pesticides. This commitment involves, among other things, drawing attention to health and environmental hazards of pesticides and pointing out possibilities of reducing these hazards. It is commonly thought that pesticides have been well-examined toxicologically and that their use is regulated in numerous regulations. However, in general it is assumed that pesticides are simply used as single substances and that hazards and risks therefore need to be estimated only for the substances individually. Nevertheless, for over 100 years almost all biomedical sciences have studied the simultaneous presence of pollutants and reported that the effects differed from those of the individual substances.

The state of art in assessing combination effects is laid out in various scientific synopses of methodological as well as experimental studies. On this basis, recommendations are made on how to consider the combination effects of pesticides in risk assessment and risk management. However, the pesticide legal framework has not consequently implemented this aspect up to now. Neither are these findings being considered in the establishment of maximum residue limits for food so far, although the new MRL Regulation (396/2005/EC) stated that „it is also important to take into account cumulative and synergistic effects".

Also, the Commissions proposal of a Regulation concerning the placing of plant protection products on the market from July 2006 pointed out: The residues of the plant protection products "shall not have any harmful effects on human health, including vulnerable groups, or animal health, taking into account known cumulative and synergistic effects when the methods to assess such effects are available, or on ground water" (COM (2006)388, Article 4).

The current knowledge on combination effects is summarized in this paper. PAN Germany demands that the authorities for pesticide regulation should consider combination effects regularly and in a methodically well-grounded manner in the risk assessment of pesticides. Problems caused by multiple exposures need to be considered also within the implementation of National Action Plans on the sustainable use of pesticides, e.g. the 'German Reduction Programme Chemical Plant Protection'. This should lead to the introduction of specific protective measures that refer to the precautionary principle.

Background 1

Pesticides and other chemicals do not occur in the human environment as single substances. On the contrary, the multiple exposure is the usual case.

Example food: For example, the report of the European Commission on the monitoring of pesticide residues in products of plant origin shows that in 2004 the upward trend in the presence of several residues in the same sample (multiple residues) was particularly serious. Since 1999 the proportion of samples with multiple residues of pesticides has been rapidly increasing up to 23.4%. The Netherlands, Czech Republic and Germany are in the leading position with 41.6%, 38.6%, and 3.2% of samples containing multiple residues in the EU/EFTA region. The proportion of samples with eight or more pesticides is about 2.3% in Germany.[1] There appears to be a trend of trying to avoid that the individual pesticides exceed the maximum residue limits (MRLs) by using several pesticides, each at a lower dose.

Example environment: For example, between 1990 and 1999 water of the Rhine near Bad Honnef in Germany contained 3 out of 9 pesticides on an average (measured annually) at detectable concentrations. In some analyses even 7 out of 9 substances were detected.[2]

Example workplace: The working environment is commonly understood to be a place where multiple exposures occur. For example, in Germany the new Directive on security in industry ('Betriebssicherheitsverordnung') explicitly takes into consideration the possible consequences of several factors acting simultaneously, such as physical factors (e.g. noise), psychological factors (e.g. stress), and chemical factors (e.g. hazardous chemicals). According to this directive the employers are obliged, within the required hazard assessment, to "consider the hazards connected with the working tools themselves and resulting at the workplace from the interaction between different tools, the substances being used or the working environment".[3]

Example body burden: For example, within a campaign of the World Wildlife Fund parliamentarians of the EU volunteered to have blood samples taken and analysed for 101 selected contaminants. The study included 47 persons from 17 European countries. Out of the 101 substances analysed, 41 were detected on an average and the number ranged up to 54.[4]

Background 2

The fact that several substances are simultaneously present in the environment is not only an unintentional effect but also the consequence of deliberately using substance mixtures. E.g. it is a common practice in agriculture to use several active ingredients that are biologically highly potent on the very same field.

Example Combined formulations: For example, the products on the market are often formulated on the basis of several active ingredients, particularly products used as herbicides. Searching the directory of plant protection substances approved in Germany shows that the most-sold herbicides like diflufenican are marketed together with other herbicides in formulations that contain two to three substances, such as mecoprop, isoproturon, flurtamone, flufenacet, or ioxynil.[5]

Example Tank mixtures: Mixing different pesticide formulations and applying these together is such a common agricultural practice that specialized internet sites provide information on how well the marketed formulations can be mixed and whether they are compatible or not.

Example Treatment series: Within a season agricultural crops are often treated several times and with different pesticides in a series of treatments. Residues can be transferred to surface waters by just one incident of rainfall and contaminate organisms with several substances.

Background 3

While an intentional use of several chemicals is supposed to have a pronounced effect due to their combination, current risk assessment rules out that there is a particular hazard from these mixtures. Authority bodies either doubt that scientific procedures exist for assessing combination effects, or deny that combination effects are relevant to hazard and risk assessment by referring to the low concentrations present compared to limits that are higher. However, cases that are known and well-documented have clearly proven that the latter reference to an apparently lacking toxicological evidence of combination effects is not correct.

Example Pharmaceuticals: For example, in the development of medicinal drugs for humans undesired effects have been studied and hazardous interactions were reported again and again. In just one recent case, e.g., marketing of the product 'Lipobay' was halted as combination effects with other cholesterol-lowering drugs (statins) were proven to cause serious harm.[6]

Example Environment: Chemical contaminations of the environment often occur at relatively low concentrations, especially in 'near-natural' areas. As long as the focus is only on individual substances, in many cases not enough evidence can be supplied to support the view that the environmental contaminants are a hazard. However, the effects of substance mixtures have been studied and there are documented cases of harmful effects on organisms. The toxic effects of oil pollution on organisms in sediments were traced to polycyclic aromatic hydrocarbons (PAHs) that were present in mixtures and appeared to be nontoxic when studied individually at the measured levels.[7]

Example Workplace: The Court decision in Germany has ruled in a case that the recognition of lung cancer as an occupational disease and the claim for damages were justified. This ruling was based on the fact that asbestos fibres and PAHs affected the claimant simultaneously while he was working as a tiler.10 The worker had been exposed to cumulative doses of asbestos (14.6 years of exposure) and benzo[a]pyrene (BaP) (39 years of exposure). For each substance individually the dose was below the maximum exposure limit required for the recognition of an occupational disease (25 years for asbestos and 100 years for BaP). However, the court was convinced that there had been 'at the very least an additive or co-carcinogenic combination effect of the substances, both of which were work-related and are proven human carcinogens'. Therefore the court concluded that the risk had been increased as a consequence of the combination so that it appeared likely that the illness was caused occupationally.

Background 4

Science has been studying and analyzing combination effects for over 100 years. This research has resulted in an immense number of theoretical and experimental papers in nearly all biological and medical sciences. Textbooks have been written and Scientific associations on this topic have been founded. The following sections present the current state of art in the analysis and assessment of combination effects of chemicals.

Fact No. 1

In general, combination effects can be predicted on the basis of two models used in pharmacology and toxicology, i.e. 'concentration addition' and 'independent action'.

For the assessment of combination effects of substances it is agreed that these effects can be described essentially by using two models that are based on simple pharmacological or biostatistical reasoning (see background paper 1). Provided that information is available on the effectiveness of individual substances (for a certain biological effect), these models allow making prognoses for the combination effect when a substance mixture is present. Both models were initially developed about a century ago. They differ primarily on basic assumptions about the sites in an organism that are affected by substances and about the mode of action. The model of 'concentration addition' assumes that substances have similar modes of action. On the other hand, the model of 'independent action' was established on the premise that the modes of action are different. In accordance, the model of 'concentration addition' is generally considered to be a suitable model for predicting combination effects of similar acting substances, while the model of 'independent action' is suited for dissimilar substances. Innumerable experiments in different branches of science have tested both models and proven their prognostic value for the combination effects of substance mixtures.

Fact No. 2

Terms such as 'synergism' or 'antagonism' are only meaningful with regard to an expected effect.

The discussion about combination effects is characterised by the use of terms such as 'synergism', 'additivity' or 'potentiation'. By these terms it is intended to highlight that the combination of substances results in enhanced or reduced effects. Unfortunately, no consistent terminology has been established, therefore it can be difficult to reach a common understanding in this field. Sometimes the same term is connected to opposed notions, which may cause identical findings to be evaluated differently (see background paper 2). Indeed, any term standing alone does not express clearly what is meant. For example, one can only refer to an enhanced effectiveness if one knows what effect is expected to result from a substance combination. Expected values for an effect can be deduced from various pharmacological or

toxicological considerations. Referring to the model of ‘independent action’, e.g., a synergistic effect indicates that the observed effects are greater than would be expected from assuming that substances act independently. At the same time, however, and referring to the model of ‘concentration addition’, the observed effects could be antagonistic. Without referring to an expected effect these terms therefore are not scientifically meaningful.

Fact No. 3

Combination effects of chemicals are usually additive or independent.

A vast abundance of experimental results in almost all biological and medical sciences has shown that combination effects of substances can be described with the above-mentioned models (see background paper 3). In some branches of research, e.g. in ecotoxicology, the model of ‘concentration addition’ has clearly become widely established. The question has occasionally been raised whether an additional model is needed at all or if the model ‘concentration addition’ could perhaps serve as a general basis of evaluating combination effects. However, experimental results have shown meanwhile that under certain conditions the model of ‘independent action’ allowed a better prediction. While it is not possible to determine generally which model is “true”, there are important consequences for the consideration of combination effects in regulations. These findings show that the combined effects can predominantly be described by simple rules and therefore can be assessed prospectively. On the other hand, in literature only sporadic references to synergistic combination effects are found.

Fact No. 4

Both additive and independent action of several substances lead to greater effects than of the individual substances.

The combination effect expected to result from a substance mixture in any case is greater than the effects expected for the individual components when present on their own. This statement remains valid irrespective of substances acting in a similar or dissimilar way. It also applies to substances in mixtures where each component (studied individually in the mixture) differs from the others in effectiveness by one or more orders of magnitude (see background paper 4).

Fact No. 5

Substances present at concentrations below their ‘no-observed effect concentration’ (NOEC) also result in combination effects that are relevant.

In discussions about the relevance of risk assessment of substance mixtures it is often stated that combination effects are not expected to occur when the individual components each are present at such low concentrations that they are unable to cause any observable effects on their own. It is argued that regulations for chemicals aim to achieve this in any case and that therefore it should not be generally assumed that combination effects occur. However, both models on combination effects

(see background 1 above) and evidence from experimental studies show that toxic effects of mixtures can arise also from substance concentrations which cause no statistically significant effect. On the one hand, the model 'concentration addition' assumes explicitly that the concentrations alone, not the effects observable individually, are relevant to a combination effect (an effect occurs irrespective of how many doses a poison is divided among). Thus for any mixture of components acting similarly it makes no sense to assume that there is a concentration threshold for the combination effect. On the other hand, establishing a threshold concentration for 'no observable effect' is methodologically difficult and controversial. In studies on substance mixtures containing components with a dissimilar action combination effects have been observed even at concentrations where the substances on their own showed no statistically significant effects (see background paper 5).

Fact No. 6

It is possible to consider combination effects in chemical risk assessment.

Different approaches have been proposed for taking combination effects into consideration in risk assessment and management of chemical mixtures. For example, recommendations have been submitted for regulating the water quality in fish breeding already in the 1970s and also for establishing quality targets for water in the 1980s that considered the toxicity of mixtures.[9,10] Proposals for considering the effects of mixtures exist also for assessing the risk of multiple residues in food, permissible exposure limits for substance mixtures at the workplace, and the classification of products containing several active ingredients.[11,12] In the US the Environmental Protection Agency (EPA) has recently published a revised framework for risk assessments of cumulative exposures and established a long-term initiative of research on this issue.[13] Research will focus on human health and the risk assessment of exposures specified in locality and duration. In the UK the 'Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment' has recently presented distinct proposals for analyzing and evaluating the risks of mixtures of pesticides and similar substances and the potential combination effects.[14] In all these documents there is absolutely no doubt that one has to take cumulative exposures into consideration.

Conclusions

Mixtures of pesticides are present in the environment on a regular basis due to their 'proper use'. Combination effects caused by exposure to mixtures are considered to be probable and can be assessed in a risk assessment based on state-of-the-art scientific knowledge. However, this is not implemented in the current practice of regulatory risk evaluations. This situation presents a serious risk to human health and the environment.

Various institutions have made proposals on how to consider multiple exposures. All these proposals are based on the identical assumption that simple models, together with the information about individual substances, are suitable for predicting combination effects realistically or at least estimating a 'worst case' scenario. There are simple options, methodologically, for performing this:

1. Establishment of a limit for combination effects, by dividing the limits for individual substances by the number of components in the mixture.
2. Establishment of a limit for the sum of all substances similar to the EU limit for the sum of all pesticide contaminants in drinking-water (Directive 80/778/EEC).
3. Application of additional uncertainty factors referring to combination effects explicitly in the risk assessment of individual substances.

PAN Germany holds the view that it is the duty of regulatory authorities to examine which are the best options within the respective legal framework to achieve the level of protection aimed for. The authorities are called upon to consider the occurrence of pesticide mixtures in risk assessment and should develop concrete guidelines for action. However, synergistic effects are occasionally observed and the above-mentioned options do not provide any protection against these. No simple model exists so far for making predictions in this case.

Therefore one needs to refer to another important option: application of the precautionary principle. Policy needs to proceed from here by, e.g., effectively improving the MRL-setting procedure by the European Food Safety Authority (EFSA), the setting of environmental quality standards in the field of water policy, the pesticide authorisation or the National Action Plans like the 'German Reduction Programme Chemical Plant Protection'.

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Annex

Background papers

Background paper 1

Models for analyzing combination effects

Scientific research in the analysis of combination effects has developed from two different origins historically (Unkelbach & Wolf 1984; Boedeker et al 1990). The following sections present short citations from a comprehensive review on this topic by Grimme et al (1998).

Concentration addition *(LOEWE additivity)*

Basic assumptions:	Identical sites in organism affected by individual substances; similar mechanisms of action of substances
Quantitative model:	$\mathbf{c_1/EC_{x,1} + c_2/EC_{x,2} = 1}$

Independent action *(additivity of actions, BLISS independence, response addition)*

Basic assumptions:	Different sites in organism affected by individual substances; dissimilar mechanisms of action of substances.
Quantitative model:	$\mathbf{E(c1,2) = E(c1) + E(c2) - E(c1)E(c2)}$

Table: Models for analyzing combination effects. c denotes the applied concentrations (of substance 1 and 2, respectively), E observed effects, and EC_x the concentration that causes a certain effect (x). (For further information on terms used, see Greco et al 1995; Grimme et al 1998).

CONCENTRATION ADDITION

The model of concentration addition mainly originates from work of the pharmacologist Loewe (Loewe & Muischnek 1926). This model basically assumes that the components of a mixture have a similar effect. In the formulation of the model considerations started with the simplest mixture imaginable, the combination of a substance with itself (or a so-called 'sham combination'). In its most general form, 'concentration addition' refers to the property that, regarding a parameter for the effects studied one substance apparently acts like the dilution of another. When interpreted more narrowly in view of the mechanism of action, concentration addition is taken to be applicable if the substances have an identical molecular mechanism and display a similar mode of action. In the case of additive concentrations the effect of a substance mixture is constant when a component is wholly or partially replaced by another substance in the proportion leading to the same effect at that concentration. The table above presents a quantitative formulation applied to a mixture of two substances.

INDEPENDENT ACTION

The model of 'independent action' was first developed by Bliss (1939) who used the term 'independent joint action'. Contrary to concentration addition it starts with the assumption that the effects of substances in a mixture are dissimilar. The term 'dissimilar' refers to the primary interaction with various sites in an organism and the induction of a joint action through various sequels of effects. It is postulated that under these conditions the relative effect of a substance (e.g. 50% inhibition relative to a control group treated identically save for the substance studied) remains unaltered in the presence of a second substance. The quantitative formulation for a mixture of two substances is given in the table above. An example illustrates what this means for a mixture of two components that each cause 50% of the organisms in a test population to die: 50% mortality in a population that has been already reduced by 50% results in a further reduction of the population to 25% (viz. 0.5·0.5 = 0.25) or an increase in mortality to 75%.

SUMMATION OF EFFECTS

Regarding combination effects it is often stated that effects of a combination of substances are greater or smaller than the sum of the individual effects. Without further substantiation this means implicitly that the sum of the parameters for the individual effects represent a value reasonably expected for the overall effect of a mixture. Although the comprehensible formulation of sum of individual effects may appear plausible intuitively, a closer inspection reveals disturbing short-comings of this approach. The fact alone incurs suspicion that in a combination of, e.g., ten substances each causing 20% inhibition of a certain parameter, it is calculated that the combined effect should be 200%. From a hypothetic experiment that combines agents which are actually just different dilutions of the very same substance it can also be deduced that summing up of the effects might be expected in special cases.

Background paper 2

Terminology in the analysis of combination effects

The irregular and inconsistent use of terms used for evaluating combination effects has at all times caused confusion and misunderstandings in this field of research. Current terms are presented in the table below.

Table: Common terms for combination effects

Terms for combined toxicity higher than the value expected from individual effects:
Augmentation, enhancement, potentiation, sensitation, superadditivity, supraadditivism, synergism, synergy
Terms for combined toxicity that equals the value expected from individual effects:
Additivity, additivism, independence, indifference, non-interaction, summation, zero-interaction
Terms for combined toxicity lower than the value expected from individual effects:
Antagonism, antergism, depotentiation, desensitation, infraadditivity, negative synergism, non-interaction, potentiation, subadditivity, zero-interaction, no addition

[adapted from Boedeker et al. 1992]

There is much confusion in terminology that makes it difficult to communicate the results. Different authors who re-analyzed the very same data have published conflicting results (Unkelbach & Wolf 1984). A different understanding of the terms synergism, antagonism and potentiation is particularly evident. For example, in literature up to seven different types of antagonisms and synergisms can be found (Fedeli et al 1972; Golden & Mantel 1957). Contrary to general opinion that synergism means a greater than additive effect, some authors consider this to be a special case of additivity (Unkelbach & Wolf 1984). Also no clear distinction between synergism and potentiation is discernible. While it appears to be generally accepted that potentiation refers to a combination effect that is greater than a synergistic effect, both terms are also used synonymously (Le Blanc 1974). Apart from this, and based on pharmacological considerations, it was proposed to regard potentiation as a special case of antagonism (Loewe 1927).

The term ‘interaction’ is also used with numerous different meanings. Unkelbach (1992) examined this term carefully and concluded that it would be better to refrain from using it in future analysis of combination effects. Its different use in pharmacokinetics and -dynamics or in biostatistics makes it seem unlikely that it will be used in a clear and meaningful way. Principal terms applied to expected combination effects are ‘additivity’ and ‘independent action’, besides their connotations. These expressions are also only meaningful when they refer to

pharmacological models that have been stated explicitly. Separating terms from their basic pharmacological context continues to cause misunderstandings. On the one hand, ‘additivity’ has repeatedly been misinterpreted as a combination effect that results exclusively from the addition of the effects of individual substances, while on the other, the term ‘independent action’ has often led to an incorrect notion that these combination effects are not relevant toxicologically or ecotoxicologically (RSU 1987).

Also the naming of models (both qualitative and quantitative) is confusing. For example, the model of independent action is referred to in literature as ‘simple independent action’ (Finney 1971), ‘response addition’ (Anderson & Weber 1975), ‘multiplicative survival model’ (Morse 1978), ‘effect multiplication’ (Berenbaum 1981), ‘response multiplication’ (Christensen & Chen 1985), and even as ‘effect summation’ (Gessner 1988). At least within ecotoxicology this situation has led to the present discourse that only refers to models and deviations from expectations depending on a model (e.g., “more or less than concentration-additive”). This corresponds with the so-called ‘Saariselkä agreement’ (Greco et al 1993). However, in the various branches of human toxicology such a consensus has not been reached so far.

Background paper 3

Experimental findings

Various studies have meanwhile systematically viewed the experimental results of tests on combination effects. Basically a synopsis is confronted with the difficulty that specifications for the models and references applied in the evaluation of results are frequently missing in original papers. As a consequence, results are not comparable or must first be rendered comparable in additional evaluation. Nevertheless, summary evaluations have apparently been based on an intuitive assumption of the (eco)toxicological relevance of combination effects. These evaluations have been incorporated in general assessments of this issue by important institutions, e.g., scientific research (DFG), national authorities (US EPA), EU commissions (EIFAC) and industrial associations (GIFAP, ECETOC). Illustrative examples of these assessments are presented in the overview below. It is characteristic that they unanimously emphasize an additive combination effect of combined active ingredients or pollutants. Even where experimental evidence is inconclusive, the assumption of additivity is considered to be a reliable approximation of the expected combination effects.

To summarize, it is thought to be indisputable in science that when mixed exposures occur combination effects are likely. Further, if mixtures of substances with a similar action are present it is thought to be plausible that a combined effect will be concentration-additive. So the controversies arise from the following questions:

1. What are substances with a “dissimilar” effect?
2. Which model of additivity enables the description of combination effects from dissimilar substances?

The terms ‘similar’ and ‘dissimilar’ effect are used in literature with various degrees of stringency. Thus in pharmacology, strictly speaking, an identical molecular mechanism of action at the same substructure of an acceptor is stated as prerequisite for a similar effect of different substances, and correspondingly in this view dissimilar effects involve different mechanisms of action (Pöch 1993). Other authors require that a similar effect is localized at least in the “same site of primary action”, while a dissimilar effect is distinguished from this by the criterion of differences in a substances’ fundamental structure (different “parent compounds”) (Calamari & Vighi 1992). Finally, the weakest requirement for a similar effect is that there is a similarity in the mode of action, which is not specified further (Könemann 1980; Hermens et al 1984; van Leuwen 1991).

These different ways of understanding and defining ‘similar effect’ have led to a variety of propositions for regulations, e.g. targets for water quality. In the Netherlands formulation of quality objectives for water has considered the findings of Joop Hermens’ group at the University of Utrecht (van der Gaag et al 1991). They have shown that ‘concentration addition’ is a model with good prognostic value for a broad class of substances called non-reactive organic compounds, mixtures of which exhibited only basic toxicity. Regarding an eventual regulation in the EU, however, Calamari and Vighi (1991; 1992) proposed to consider concentration addition only for chlorinated aliphatic hydrocarbons and to divide this class of

compounds into six subgroups that are congeneric (comprising substances that have an identical quantitative structure-activity relation). The authors argued that one can only assume there is a similar effect for substances within a congeneric group and when the effect is known well enough.

There continues to be a controversial debate about the question when it can be assumed that substances have a similar mode of action so that the model of concentration addition can be applied for making suitable prognoses of combined toxicity (US EPA 2000; Borgert et al 2004). Often one fails to see, however, that the combination effect expected to result from multiple exposures is greater in any case than the individual effects, irrespective of whether concentration addition applies or independent action. Occasionally, quantitative prognoses based on either of the two models are even indistinguishable and this has been shown both in theoretical analysis (Drescher & Boedeker 1995) as well as in experimental studies (Backhaus et al 2004).

Overview: Evaluation of combination effects by relevant institutions

DFG (1975):

“The available results of published studies on combination effects of pesticides relate to 86 references in literature. This list includes approximately 90% of the papers published up to the end of 1974. (...) By summarizing the results compiled in this listing it can be observed: 1. Greater than additive effects were found only when higher doses were administered, i.e. when the acute toxicity was increased. These combinations could present a hazard to the user, however, these are identified in toxicological examination of such mixed formulations and categorized in the corresponding toxicity class. 2. All combinations that were tested in repeated doses only showed additive effects at low doses.”

EPA (1986):

“When little or no quantitative information is available on the potential interaction among the components, additive models are recommended for systemic toxicants. Several studies have demonstrated that dose additive models often predict reasonable well the toxicities of mixtures composed of a substantial variety of both similar and dissimilar compounds ... . The problem of multiple toxicant exposure has been addressed by the American Conference of Governmental Industrial Hygienists, the Occupational Safety and Health Administration, the World Health Organization, and the National Research Council. Although the focus and purpose of each group was somewhat different, all groups that recommend an approach elected to adopt some type of dose additive model.“

EIFAC (1987):

“Meanwhile, the concentration-addition model appears to be adequate to describe the joint effect of commonly-occurring constituents of sewage and industrial wastes, and to be used to make the tentative predictions of the joint effect on fish populations of toxicants present at concentrations higher than the EIFAC recommended values.“

GIFAP (1988):

“Studies on acute toxicity of combinations can help in the evaluation of combination effects of pesticides. Experience has shown that the great majority of such products display additive toxicity in combination.“

ECETOC (2001):

„When large numbers of substances are present in mixtures at low concentrations relative to their individual acute toxicities, additivity of acute toxic effects is closely followed. This holds true even when the substances are not related chemically, or exhibit different modes of action when acting as acute toxicants alone.”

Background paper 4

Both the additive effects and independent action of several substances are greater than the effects of individual substances

The statement that the effects of several substances are usually greater than those of the individual substances is illustrated in the following diagram (figure 1). The s-shaped curve describes the relationship between the concentration of the herbicide dinitramine and its biological effect on algae. The curve represents a quantitative model that summarizes the experimental observations and underlies toxicological parameters in hazard assessment of the substance dinitramine, e.g. the EC50 value (“effective concentration” or concentration that affects 50% of a test population after a specified time). Asterisks indicate the toxicity to algae observed experimentally when dinitramine is present in the same testing system with 13 different aromatic nitro compounds. To cause the same effect by exposure to the single substance as by multiple exposure, the concentration needs to shift by about one order of magnitude towards the lower values on the concentration scale. A shift on the vertical scale for the effect is more drastic: e.g. a concentration of 10-8 mol/L dinitramine (or 3.2 parts per billion) has no measurable effect, while an exposure to the mixture results in almost 100% inhibition of reproduction. For each of the other 13 components present in the mixture the diagram would be identical (adapted from Altenburger et al 2005).

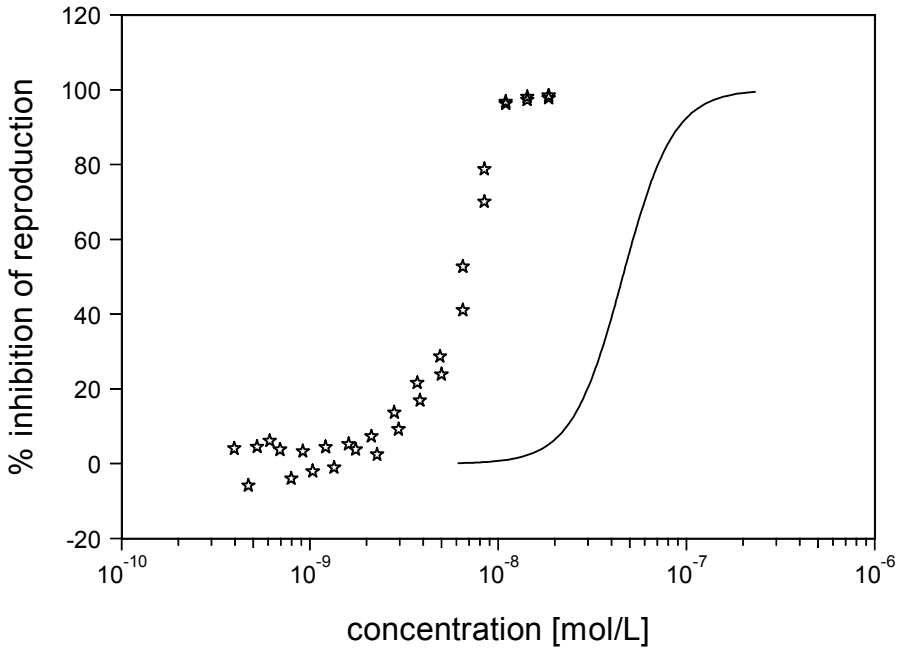

Figure 1: Experimental results for the phytotoxicity of the herbicide dinitramine in the simultaneous presence of 13 nitrobenzene derivatives (indicated by asterisks). For the single substance dinitramine the effect is plotted as a function of concentration (continuous line) (adapted from Altenburger et al 2005).

Background paper 5

Substances present at concentrations below their 'no-observable effect concentration' (NOEC) also result in combination effects that are relevant

Combination effects at concentrations below the NOEC of individual substances with a similar effect:

In an experiment a mixture composed of 16 phenol derivatives was studied. The bars in figure 2 represent the effect that components of the mixture each could have on their own (1% inhibition of vital functions of a certain bacteria species). The effects of the individual substances are regarded as not statistically significant for the experiment. They result from a quantitative relationship between dose and effect simulated in a model. Effects usually become observable (and are thus rated as significant) between 5% and 10%, depending on the substance. The corresponding concentration is called the 'no-observable (adverse) effect concentration' (NOEC). Based on the two different models used for analyzing combination effects (see background paper 1) the expected toxicity of the mixture can be predicted and is represented in figure 1 by bars with a hatching. The combination effect that is actually observed is represented by a black bar (adapted from Grimme et al 1998). It is found firstly that the combination of the mixture is clearly visible and also statistically significant, and secondly that the combination effect can be well predicted on the basis of the model 'concentration addition'.

Combination effects at concentrations below the NOEC of individual substances with a dissimilar effect:

An experiment studied a mixture containing 16 herbicides with different mechanisms of action. Figure 3 depicts the results (in the same way as in figure 2) (adapted from Faust et al 2003). The combination effect of the mixture is clearly visible and significant as above. In this case of a mixture of substances with a dissimilar effect the quantitative prediction of the combination effect is more precise when based on the model 'independent action'.

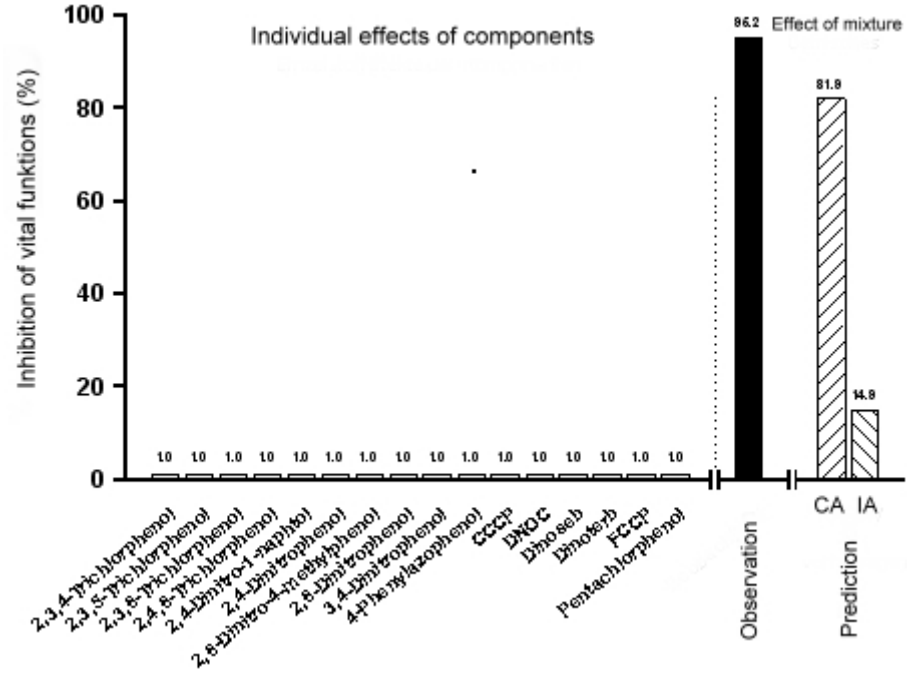

Figure 2: Combination effects at concentrations below the NOEC of individual substances with a similar effect: the prediction is based either on concentration addition (CA) or on independent action (IA) (adapted from Grimme et al 1998).

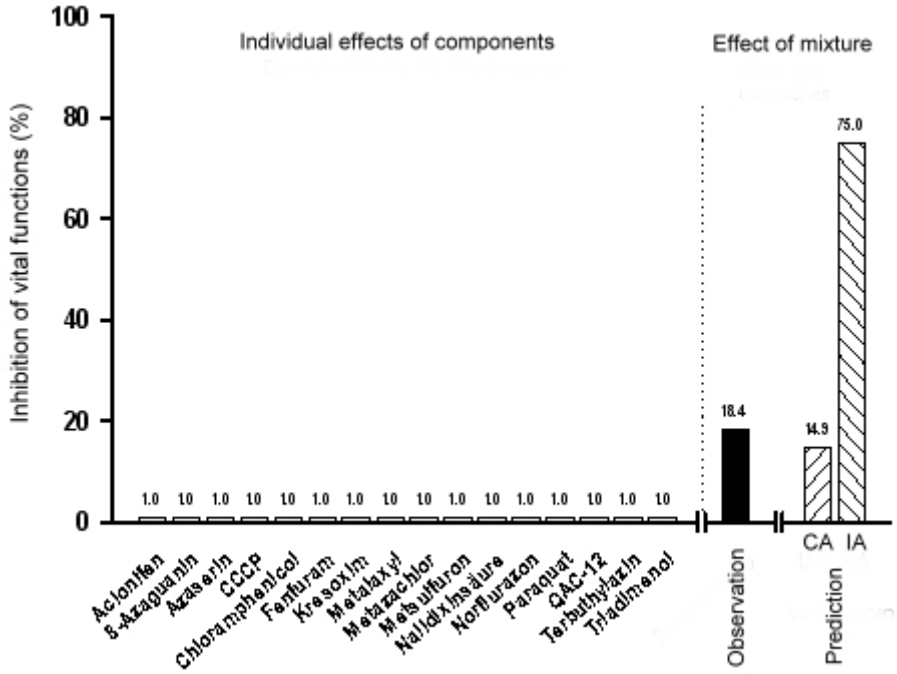

Figure 3: Combination effects at concentrations below the NOEC of individual substances with a dissimilar effect: the prediction is based either on concentration addition (CA) or on independent action (IA) (adapted from Faust et al 2003).

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