Methods for Assessing the Effects of Mixtures of Chemicals
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Toward Modelling the Ecological Effects of Chemicals Acting Jointly

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ABSTRACT

A physical basis is sought for modelling the ecological effects of chemicals acting jointly. Neutral hydrophobic chemicals comprise the class of chemicals considered. It is assumed that these chemicals cross biological membranes passively, driven by gradients in chemical potential, and are distributed among chemical phases (types of tissue) of an organism's body by equilibrium partitioning. On this basis a model is developed to account for exchange of chemicals between the external aqueous medium and an organism's body. The bioconcentration factor is expressed in terms of the model as a weighted sum of concentrations in the different body phases. Based on the model, the relative times required for different chemicals to reach a steady state in the body relative to external concentrations is found to be in direct proportion to the ratio of their octanol–water partition coefficients. Experiments using chemicals with partition coefficients that are widely different will be very difficult to conduct or complex to interpret. Misleading results will be obtained from experiments conducted without due consideration of the influence of the partition coefficients on chemical exchange. Discussion of future directions of research toward applying models of joint toxicity in ecosystem models is given.

1 INTRODUCTION

Defining the toxicity of chemical mixtures is a major problem at both the theoretical and practical level. Sufficient research has not been conducted to establish a widely applicable, rational, and workable approach for evaluating or predicting the joint action of toxicants in the aquatic environment. Recent reviews (Alabaster and Lloyd, 1980; Calamari and Alabaster, 1980; EIFAC, 1980) have summarized the available research on the combined effects of mixtures of toxicants on aquatic organisms and approaches used in evaluating these effects. In this paper we present an initial attempt at drawing together a consistent basis for predicting ecological effects of mixtures of toxicants in aquatic systems. Our

approach considers both controlled experiments and natural populations in an attempt to approach a rationalization of the two in a way that will permit use of common toxicological measures in models of natural populations. We also present a mathematical model of the uptake and distribution of neutral organic chemicals and proceed to consider certain aspects of toxicity measures as implied by this model.

2 TERMINOLOGY USED IN TOXICOLOGICAL EXPERIMENTS WITH MIXTURES OF CHEMICALS

Different forms of joint action have been discussed by Sprague (1970), Muska and Weber (1977), and Calamari and Alabaster (1980). The concepts of similarity and interaction, proposed by Bliss (1939) for two substances and later developed further by Plackett and Hewlett (1948, 1952, 1967) and Hewlett and Plackett (1959, 1964), are central to current approaches to analyses of joint action. The types of joint action are defined as similar or dissimilar depending on whether the sites of primary action within the organism are the same or different, and as interactive or non-interactive depending on whether one toxicant influences the biological action of another. Models for interactive joint toxicity are complex and require information beyond toxicity of the individual components. Most investigators studying effects of toxicant mixtures on measures such as survival and growth of aquatic organisms consider only special cases of non-interactive joint action.

Development of predictive methodology to describe the joint action of multiple toxicants has been approached in two distinct ways (Marubini and Boranoni, 1970). One approach has been to describe responses using statistical models. The other approach has been to postulate physical mechanisms at receptor sites, to derive theoretical response curves, and finally to relate experimental and theoretical results. Our approach—as far as it goes—fits neither approach exactly, but utilizes aspects of both. Processes leading to effects are considered to be uptake, distribution in the organism, and action at critical sites. We consider these steps via elementary assumptions leading to simple models of the processes. To represent the joint action of chemicals, models of their separate action are first needed. Accordingly, we consider as a fundamental model an expression relating uptake and distribution of a single chemical to properties of the chemical and to characteristics of the organism.

3 EXCHANGE OF CHEMICALS BETWEEN ORGANISM AND ENVIRONMENT

Uptake of neutral organic chemicals by organisms occurs by passive movement of the chemicals across the membranes that separate organism from environment. In developing the concepts of uptake and distribution of chemicals by aquatic

organisms, we use the property of chemicals to partition between two phases, such as organic solvent and water, and its standard measure, the octanol–water partition coefficient, K_{ow}. Body fat is an organic phase and blood is a water phase. Accordingly, we represent organisms as consisting of these two components plus all other tissues lumped into a remainder category.

The rate expression for the exchange of chemicals between organism and aqueous medium is based on simple assumptions. The rate of increase in the organism is directly related to area of the exchange surface and inversely related to body volume. Thus, the overall exchange rate coefficient is kS/V. Taking uptake as the positive direction, the exchange rate (proportional to concentration gradient between the aqueous medium and the blood) is $kS/V(C_E - C_B)$. We follow Yalkowsky *et al.* (1973) who, citing confirming independent results, noted that biological membranes are non-directional for neutral non-polar molecules, and therefore assumed uptake and excretion rates to be identical.

Exchange between aquatic organisms and the environment is through the blood which comprises only a portion, P_B, of the total body. The rate of change of the whole body is the fraction, P_B, times the rate of change in the blood. Including metabolic loss rate, the expression for the whole body is (see glossary for explanation of symbols):

$$\frac{dC_T}{dt} = P_B\left[\frac{kS}{V}(C_E - C_B) - k_m C_B\right] \tag{1}$$

The expression is more readily applied, however, when the terms are referenced to whole body concentration, C_T. Use of a distribution fraction, $D_B = P_B C_B / C_T$ (ratio of C in blood to that in whole body, Equation (7)) permits this. Introducing k_e, an organism-specific exchange constant, to replace kS/V gives:

$$\frac{dC_T}{dt} = P_B k_e C_E - D_B(k_e + k_m) C_T \tag{2}$$

On integration, the time-dependent concentration for whole body is

$$C_{Tt} = \frac{P_B k_e C_E}{D_B(k_e + k_m)}[1 - e^{-D_B(k_e + k_m)t}] + C_{T0}\, e^{-D_B(k_e + k_m)t} \tag{3}$$

4 CHEMICAL DISTRIBUTION WITHIN ORGANISMS: AN EQUILIBRIUM ASSUMPTION

We assume that the distribution of chemicals among the body components of aquatic organisms is rapid in comparison with exchange between the blood and the external aqueous medium. On this basis we calculate the equilibrium distribution of the chemical within an organism. Concentration in the whole organism can be expressed as a weighted sum of concentrations in the component parts, the weights being the mass-referenced fractions of the whole body that the

components comprise:

$$C_T = P_B C_B + P_F C_F + P_N C_N \tag{4}$$

Equilibrium constants for partitioning between blood and the two other components (the fat and the remainder) are:

$$K_N = C_N / C_B \quad \text{and} \quad K_F = C_F / C_B \tag{5}$$

Substituting for C_F and C_N in Equation (4) from Equation (5) gives:

$$C_T = P_B C_B + K_F P_F C_B + K_N P_N C_B \tag{6}$$

from which the distribution fraction, $D_B = P_B C_B / C_T$ is:

$$D_B = (1 + K_F P_F / P_B + K_N P_N / P_B)^{-1} \tag{7}$$

5 ORGANISM–ENVIRONMENT DISTRIBUTION: THE BIOCONCENTRATION FACTOR

Bioconcentration factors (BCFs) are widely discussed in the literature (Mackay, 1982; Neely, 1979; Spacie and Hamelink, 1983; Veith *et al.*, 1979). BCFs can be defined with reference to any source, such as food, aqueous environment, or sediments. Here, in terms of the preceding model, we refer BCF to concentration in the aqueous environment: the ratio of concentration in the organism (whole body basis) to concentration in the aqueous environment. It is unambiguous only if the environmental concentration is constant and the organism's body burden has reached a steady state with the chemical in the environment. With these assumptions, Equation (2) can be solved for C_T / C_E:

$$\text{BCF} = C_T / C_E = \frac{P_B k_e}{D_B (k_e + k_m)} \tag{8}$$

Although BCF is often treated solely as a property of the chemical, it is quite sensitive to properties of individual organisms as well. The exchange coefficient, k_e, is a function of the ratio of exchange surface area to body volume, and the distribution fraction, D_B, is a function of the relative sizes of the blood, fat, and other body components. A fat organism is expected to exhibit a high BCF for a given hydrophobic compound, relative to an organism with low fat content. Differences in BCF values among compounds depend primarily on the degree of hydrophobicity. The degree of hydrophobicity influences both exchange rates and distribution within organisms.

6 UPTAKE AND DISTRIBUTION OF MIXTURES OF CHEMICALS

Generally, uptake of mixtures of toxicants can be represented as a set of independent uptake processes by using a separate describing equation like

Equation (1) for each toxicant, which will distribute internally as described by Equations (4)–(7). Application of these equations to separate chemicals will generate different behaviours based upon differences in their partition coefficients.

Mixtures of chemicals cross biological membranes at rates that vary with both K_F and solubility. The uptake rate coefficient, k (Equation (1)), is a function of the partition coefficient (Yalkowsky and Flynn, 1973; Yalkowsky *et al.*, 1973). These authors give an expression for flux from which the rate coefficient expression can be extracted:

$$k = (R_m/K_F + R_{aq})^{-1} \tag{9}$$

The partition coefficient to fat, K_F, accounts for partitioning to the fatty components of biological membranes. The octanol–water partition coefficient, however, approximates K_F and can be used instead. For increasingly large values of K_F, k approaches R_{aq}^{-1}as a limit, i.e. the resistance to transport shifts to the aqueous phase. The uptake flux generally is reduced at higher values of K_F, however, because solubility decreases for larger compounds (Flynn and Yalkowsky, 1972).

7 INFLUENCE OF HYDROPHOBICITY ON THE JOINT ACTION OF CHEMICAL MIXTURES

Except in the rare instance of direct interaction among chemicals, the distribution of chemical mixtures within organisms can be treated as a set of independent processes. Assuming the applicability of Equations (4)–(7), the distribution of the set is controlled primarily by the distribution of hydrophobicities of the chemicals. Hydrophobicity differences can be extreme, K_{ow} ranging through nearly seven orders of magnitude. Because of this range of hydrophobicity, partitioning into organisms' component phases differs widely among chemicals as does the time required to approach a steady state with the chemical sources. If blood or other body fluids is the component in which toxic action occurs, chemicals with higher partition coefficients will reach action levels more slowly than those with lower partition coefficients—other factors being equal. Therefore, in environmental or experimental exposures of organisms to mixtures of chemicals, in interpreting response data it is necessary to consider the relative time required to reach action levels. Expressing a body burden concentration as a fraction, f, of the steady state with the aqueous medium, the time required to reach this fraction of steady state from a body burden concentration of zero can be calculated:

$$t_f = \frac{\ln (1-f)}{D_B k_e} \tag{10}$$

This result is obtained assuming that the rate of metabolism of the chemical is

negligible. Values of t_f can vary as a result of differences in any of the parameters of the exchange rate coefficient or distribution coefficient (body volume, K_F, P_F, etc.). The relative time required to achieve the fraction, f, can be obtained using the time ratio, $T_{ij} = t_{fi}/t_{fj}$:

$$T_{ij} = \frac{D_{Bj} k_{ej}}{D_{Bi} k_{ei}} \tag{11}$$

The indices i and j identify the two conditions being compared, e.g. times to a given fraction of steady state for two chemicals by a single organism, or times to a fraction of steady state for a single chemical in two organisms differing in fat content. For one chemical and two organisms the measure reduces to a ratio of proportions representing the two body compositions:

$$T_{ij} \approx \frac{P_{Bj} P_{Fi}}{P_{Bi} P_{Fj}} \tag{12}$$

For a single organism exposed to two chemicals simultaneously, the time ratio reduces simply to approximately the ratio of the two partition coefficients:

$$T_{ij} \approx K_{owi}/K_{owj} \tag{13}$$

Considering the large potential range of values of K_{ow}, one can appreciate the potential importance of transport and distribution of mixtures of chemicals on their observed joint action. Variation in the exchange rate coefficient with variation in K_F, counter-variation resulting from decreasing solubility with increasing molecular weight, and resulting variation in body concentration and times required to achieve it, all complicate analysis of short-term experiments with mixtures of chemicals. In the analysis of toxicological results variance among responses of test individuals is used to estimate such quantities as the concentration that produces the median effect. These quantities cannot be applied directly in models of environmental situations where concentrations differ considerably and unpredictably, and where the susceptibility of exposed populations varies continuously in response to the effects of the toxicant.

8 PROBLEMS IN THE APPLICATION OF TOXICOLOGICAL MEASURES IN ENVIRONMENTAL MODELS

Concern with the relationship between chemical toxicity as measured in bioassays and its expression in natural environments has led to various modelling approaches (e.g. Mancini, 1983). Several steps seem necessary if prediction of effects in the natural environment is to be successful. The most obvious relationship that needs to be clarified is that between the toxicity of a chemical to a species as measured and as expressed in the natural environment. Another implied relationship is that between the susceptibility of species that have been measured and those that have not. That such an interspecies relationship is assumed to exist

is implied by the practice of testing using a few species available in the laboratory for the purpose of setting limits and standards for all. A more fundamental difficulty than differences between field and laboratory populations exists for the application of toxicological measures to predictions for natural populations. The nature of bioassays is that they represent the state of a population at the instant of the assay. They do not relate to the way that the population susceptibility is distributed immediately after the effects of a toxin have been exercised. They are static measures, whereas predictions of the effects of toxicants in natural situations would require knowledge of the tolerance distribution through time under stress of the toxicant. There is, thus, the need for an assumption or a method to permit static-to-dynamic translation of the toxicological measures.

Two extreme approaches can be identified. One is to find an algorithm to alter the tolerance distribution as more susceptible individuals are lost, so that the distribution continually evolves toward higher resistance. The problem of accounting for genetic recombination and subsequent reintroduction of susceptibility into the population by natality, however, may be insurmountable. The other approach is the simplest. It is to assume that no evolution of the tolerance distribution occurs as a result of mortality of susceptible individuals or from other causes. For the joint action of chemicals where different mechanisms are involved, there is the question of correlation of the distributions of tolerance to the different chemicals. If the individuals that are susceptible to one chemical die in response to that chemical, are they the same individuals that would have succumbed to another, and further, are they simply the weaker individuals that are more likely to succumb to any cause whatever, including predation? It is simplest to assume that natural populations respond in the same way as do laboratory populations. We suggest that differences in the variance of susceptibility between laboratory and field populations are due largely to factors that cause variance in blood concentration. Thus, we interpret similarity to mean that, after removing variation associated with both field and laboratory populations, the remaining tolerance distributions for laboratory and field populations will be similar.

A rough idea of the magnitude of the effects on the variance of susceptibility and the temporal characteristics of uptake introduced by variation in body size and fat content can be obtained by use of a hypothetical example. Consider two individuals exposed to a given concentration of a toxicant whose value for K_{ow} is 10^4. We assume that the toxicant is associated primarily with the body fat and that there is equal affinity of the toxicant for blood and water. If the fat content of one individual (i) is 0.03 and the other (j) is 0.04, then, by Equation (12), the ratio of times to equilibrium, T_{ij}, is about 0.75. So if the individual with 3% fat responded at 75 hours, the one with 4% would respond at 100 hours. If organisms ranged in size between, say, 300 and 500 g, the ratio of times to response (Equation (11)) due only to body size would be $T_{ij} = 0.84$. Combining both fat and size considerations, the extreme ratio of times to response would be $T_{ij} = 0.63$. Of course one

cannot say, from these calculations for the hypothetical situation, what part—if any—of the observed variance in susceptibility results from variation in fat content and body size. The hypothetical situation is not extreme, however, and the possibility that these factors underlie a significant portion of the variance appears to be worthy of further consideration.

9 REPRESENTATION OF THE JOINT ACTION OF TOXICANTS IN DYNAMIC POPULATION MODELS UNDER THE HYPOTHESIS OF NO EVOLUTION OF THE TOLERANCE DISTRIBUTION

Dynamic population models usually take the form, $dy/dt = y(G - L)$. Toxicants acting jointly alter either the gain or loss rate (G or L). We consider, briefly, a possible means of incorporating into equations for population rates of change, loss rates due to two simple models of joint action of toxicants. In simple similar action or concentration addition (Anderson and d'Appolonia, 1978) it is implied that the two chemicals act identically except for relative potencies. The expected effect in a bioassay experiment from several chemicals acting jointly through simple similar action can be represented generally as

$$E = g^*\left(\sum_i p_i C_i\right) \tag{14}$$

Ferguson (1939) indicated that the potencies, p_i, are closely related to thermodynamic activity coefficients. If the potencies were identical to thermodynamic activity coefficients, two chemicals acting through simple similar action would produce the same effect at the same activity (product of activity coefficient and concentration). Perfect concordance is not achieved by use of activities, but relative variance among activities required to produce a given effect is lower than among concentrations (Filov, 1979).

Death rate is obtained through invocation of the static-to-dynamic assumption:

$$L_c = g\left(\sum_i p_i C_i\right) \tag{15}$$

The concentration–response functions, g^* and g, while unspecified here, would take into account individual tolerances for the chemicals, C_i (for example, see Plackett and Hewlett, 1952).

In independent action, response (response addition) occurs when a chemical exceeds the organism's tolerance for that chemical. Chemicals acting independently do not contribute jointly toward exceeding a single tolerance. As noted, tolerances to two or more chemicals can be correlated, i.e. one tolerance level is a function of the concentration of other chemicals. This type of action is termed interactive joint toxicity. Here, inferences about joint responses cannot be drawn from the responses to chemicals separately. Without a theoretical basis for the

derivation of the joint probability distributions, the problem of representing interactive joint toxicity is unfeasible. Experiments with all possible mixtures of chemicals would have to be conducted for complete information. The need for a theoretical approach is apparent.

In either independent or interactive joint toxicity, conceivably a common response could result from groups of chemicals, each group acting by simple similar action. These classes of joint action can be represented as

$$L_r = \sum_i h_i \left(\sum_i p_{ij} C_{ij} \right) \tag{16}$$

Where dependencies exist, the potencies, p_{ij}, are altered from the values obtained in single chemical experiments.

Equations (14)–(16) are given merely to indicate a direction for thinking about representing mortality rates by joint action of chemicals in dynamic population or ecosystem models. Development of explicit representations probably will be most fruitful if some of the existing general theory is used as a basis for particular theory. We reiterate the fundamental difficulty caused by toxicological test measures relating only to the static situation of the laboratory population at the time of the test.

10 SUMMARY AND CONCLUSIONS

In this paper, we have dealt with problems of predicting the uptake, distribution, and effects of neutral, hydrophobic chemicals acting jointly. To aid the discussion we proposed a model for uptake and distribution based on the following assumptions:

(1) Neutral, hydrophobic chemicals exchange between organisms and the external aqueous environment via passive transport across biological membranes.
(2) Both organism to environment transport and organism compartment to organism compartment transport of chemicals are driven only by gradients in chemical potential.
(3) Distribution of chemicals among the body compartments (phases) of an aquatic organism is rapid in comparison with uptake.

We believe the first two assumptions to be valid. The third assumption, however, is not well supported. If assumption 3 is invalid, our relationships dealing with times to fraction of steady-state body burden and the ratios of these times will not apply. Other concepts are not affected.

There are relevant implications of a model based upon these assumptions. Equal chemical potential is expected between all phases within the organism, if metabolism is negligible, and between the organism and the environment when the organism has reached steady state. Times to any fraction of steady state are

strong functions of the hydrophobicity of the chemical. Design of experiments with multiple chemicals should take into account the considerable differences in time for individuals to reach stable body burdens for each of the chemicals. Individual differences in body size, and particularly body fat, can contribute further to variation in the times to stable body burdens. Variation among potencies of different chemicals is expected to be reduced by expressing dose as thermodynamic activity.

The application of toxicological measures in population or ecosystem models is hindered by the difficulty of interpreting these measures. The response of the tolerance distribution under chronic exposure conditions is needed if these measures are to be properly applied. The greatest obstacle to the development of a predictive theory appears to be the lack of well-developed theory to account for dependencies in the joint tolerance distributions of different chemicals.

We do not expect that all the theoretical and hypothetical aspects put forth in this paper will be found to be appropriate descriptions of the processes, even for the purpose of discussion in this paper. We advance these ideas as an initial attempt to develop a coherent basis to predict the expected effects of chemicals acting jointly on aquatic organisms. This is a task of large magnitude, with concepts at widely different levels in the hierarchy bounded by biochemical processes at one extreme and interactions of groups of populations in the ecosystem at the other. It is our view that the ultimate purpose will best be served if we organize our inquiries from the perspective of the ecosystem, but study and describe component processes at fundamental levels. We have attempted to address joint toxicity from this perspective, in the hope of asking the right, or at least useful, questions, as well as framing the answers in terms that could be useful for ultimate system predictive modelling.

GLOSSARY OF TERMS USED IN TEXT

BCF	bioconcentration factor
C_B	chemical concentration in the blood
C_E	chemical concentration in the aqueous environment
C_T	chemical concentration in the whole body
f	body burden as fraction of steady-state body burden
g	concentration–response function for mortality rate, simple similar joint action
g^*	concentration–response function for effect, simple similar joint action
G	population growth rate coefficient
h	concentration–response function for mortality rate from interactive joint action
k	chemical exchange rate coefficient, $V\,\mathrm{cm}^{-2}\,t^{-1}$
k_e	organism-specific chemical exchange rate coefficient, t^{-1}

K_F	partition coefficient of chemical in fat referenced to that in blood
k_m	metabolism rate coefficient, t^{-1}
K_N	partition coefficient of chemical in the non-fat non-blood component referenced to that in blood
K_{ow}	the octanol–water partition coefficient
L_c	mortality rate from concentration addition (simple similar action)
L_r	mortality rate, independent of interactive joint action
p	potency coefficient
P_B	fraction of body composed of blood
P_F	fraction of body composed of fat
P_N	fraction of body composed of non-fat, non-blood
R_{aq}	resistance to chemical transport in the aqueous phase
R_m	resistance to chemical transport in the membrane
S	surface area of an organism active in chemical exchange
T_{ij}	ratio of times to response under conditions i and j
V	volume of an organism's body

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