

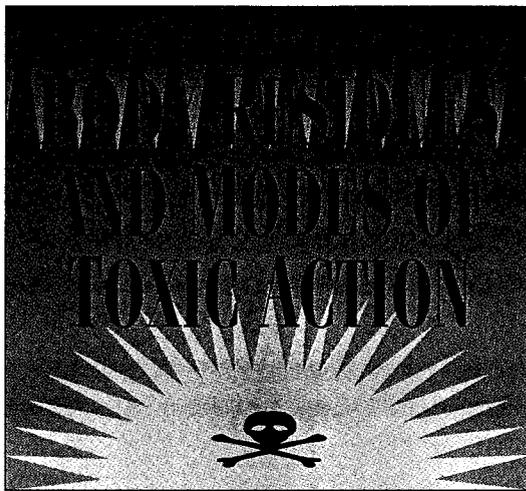
Enhancing Ecotoxicological Modeling and Assessment

In this review we discuss and advocate an evolving approach for the assessment of ecological risk from chemicals. Although the examples are for aquatic systems, the principles are applicable to all parts of the ecosystem. The "critical body residue" (CBR) method is feasible as a result of marked improvements in our ability to quantify three related sets of environmental processes, as illustrated in Figure 1 and discussed in more detail later.

The first improvement is in our ability to model and predict for aquatic systems the fate of chemicals that originate from various sources, including direct industrial and municipal discharges, tributaries, runoff, and atmospheric deposition, with realistic treatment of chemical exchange with sediments. These models yield statements of concentrations as ranges or probability distributions at various times in the various aquatic ecosystem media.

The second improvement is in the ability to use these data to estimate the accumulation of chemical residues in organisms and in assemblies of organisms in a food chain or web. The outcome is the concentration (in milligrams per kilogram or in millimoles per kilogram) of chemicals in organisms or even in specific tissues within organisms.

The third improvement is in the ability to relate these body or tissue residues to various acute and chronic effects as determined in toxicity tests and bioassays. Not only can the effects be estimated for a single chemical, but in many cases it should be possible to treat several



chemicals acting in concert. This latter feature is important because, in many real-world situations, toxic effects result from mixtures rather than from single substances.

The link between CBR and adverse biological responses—whether laboratory-based toxicity endpoints or field-based ecological effects—is currently the most poorly understood aspect. However, shifting from comparison between ambient water concentrations and water concentrations known to cause toxic effects (e.g., LC_{50} s) to comparison between organism concentrations and CBRs has several advantages, including:

- bioavailability is explicitly considered;
- accumulation kinetics are considered, which reduces the confounding effect of organism exposure duration when interpreting results;
- uptake from food (as distinct from water) is explicitly considered;
- toxic potencies are expressed in a less ambiguous manner, facilitating identification and investigation of different modes of toxic action;
- effects of metabolism on accumulation are considered;
- mixture toxicity may be more readily assessed; and
- experimental verification can be readily sought in the lab and the field.

The CBR approach is neither new nor radical; rather, it represents a more complete exploitation of existing information via fundamental toxicological principles. Mancini (1), Connolly (2), Menzel (3), Bartell et al. (4), and others modeling environmental fate, bioaccumulation, and toxicity have recognized the need for residue-effect relationships and advocated a body-residue-based approach in environmental toxicity and risk assessment. They recognize that using models to estimate ecosystem concentrations and comparing these concentrations with LC_{50} s can be complicated and misleading.

In this article we discuss and justify these assertions, emphasizing the third stage, body-residue-based toxicity interpretation and assessment. We will briefly review the current status of models of environmental fate, bioaccumulation, and toxicity, then review information about critical body residues associated with acute and chronic toxicity

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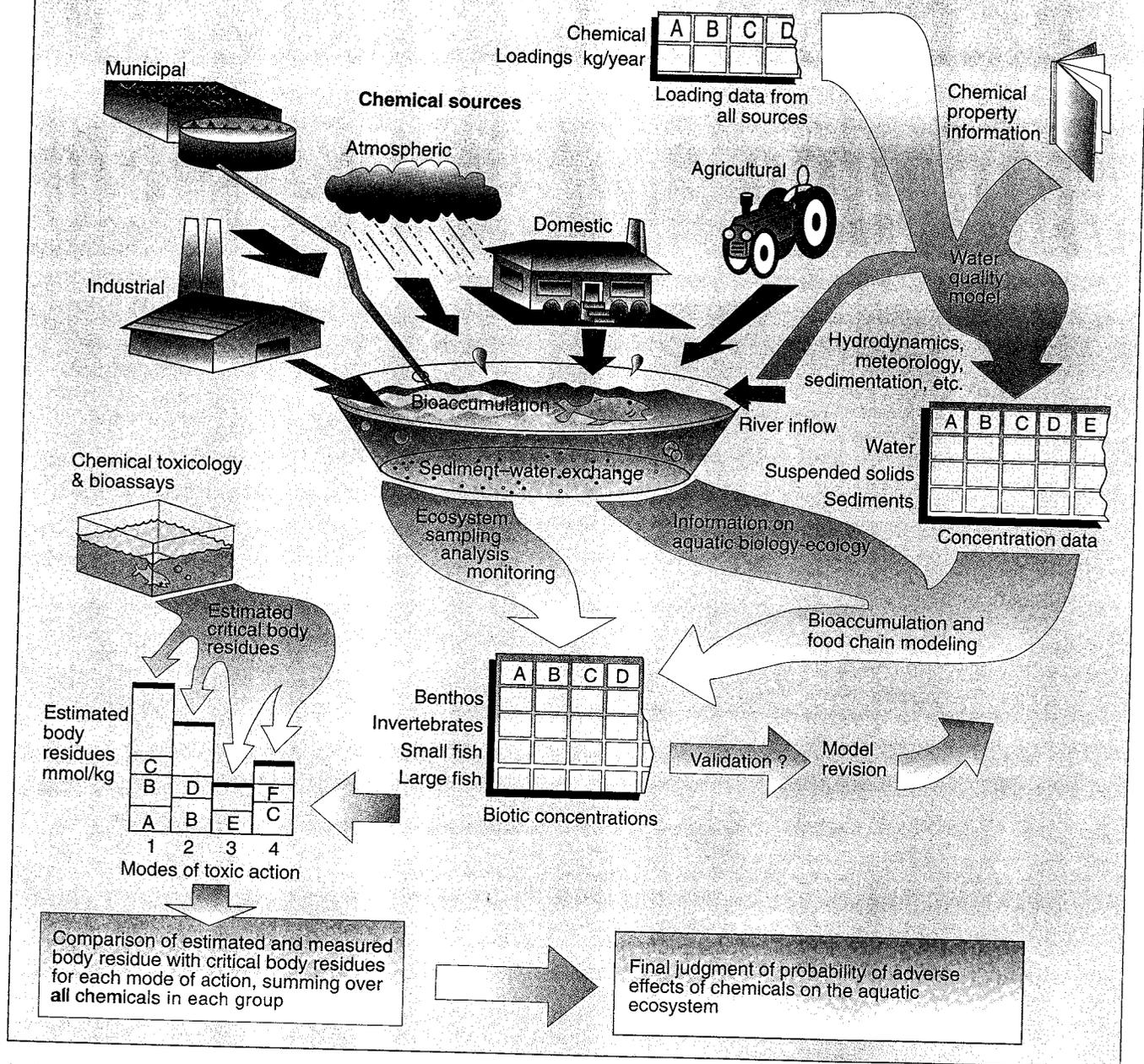
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FIGURE 1

Integrated scheme for the use of critical body residues and modes of toxic action in environmental risk assessment



(primarily for organic chemicals). Within the framework of ecological risk assessment, we will discuss the implications and advantages of this approach for modeling and interpreting the toxicity of single chemicals and mixtures, as well as for developing regulations.

Environmental fate models

Several factors have combined in recent years to improve the reliability of environmental fate models of chemicals to the point that they are essential in any meaningful assessment. Much modeling work has been focused on examining water

quality in aquatic ecosystems. The condition and persistence of chemicals in aquatic systems can now be established. Techniques have improved for handling the systems of equations that describe the basic mass balance in the necessary compartments, and reliable parameter values for physical-chemical properties, organic chemical reactivity, and metal speciation are now available. The advent of low-cost, rapid computing and an increasingly computer-literate society has demystified computing. User-friendly programs now make it possible to present findings in attractive,

readily assimilable outputs.

EPA, through its Center for Exposure Assessment Modeling in Athens, Georgia, supports a number of models including the widely employed Exposure Analysis Modeling System (EXAMS) and Water Quality Analysis Simulation Program (WASP) water quality models. Using these and other related models, scientists have simulated the fate of a chemical in a specified environment to the extent that there is a near-complete quantitative understanding of fate. The key capability is that of relating concentrations throughout the system to past and

present (and hence future) discharge rates. Notable in spearheading these successful applications have been the Manhattan College group of O'Connor, Thomann, Di-Toro, Connolly, and their colleagues, and the EPA Group at Athens, Georgia. Although most models employ conventional concentration-based equations, it is possible to use the fugacity approach. This approach may simplify interpretation, and it yields a particularly elegant set of equations expressing the various rates of transport and transformation as they contribute to the mass balance statement (5).

Bioaccumulation

Remarkable progress has been made since pioneers such as Neely, Hamelink, and their colleagues (6, 7) first elucidated the basic bioconcentration phenomena of chemical uptake from water. Bioaccumulation is a manifestation of lipid-water partitioning modified by species-specific factors such as feeding, metabolism, growth dilution, digestion and egestion efficiency, as well as the bioavailability of the chemical in the water. It is now possible to write simple first-order models that adequately describe the relationship between quantities of chemical in the body of an aquatic organism and the concentrations in the surrounding aquatic environment (8).

Furthermore, it is possible to assemble equations for systems of organisms that comprise a food web or chain and, by estimating food preferences, show how contaminant levels will change with trophic level. Notable in this context are the studies by Thomann (9, 10) and Clark et al. (11). Multi-compartment pharmacokinetic models bring an even higher level of sophistication to the determination of chemical fate in organism tissues, as shown by Tarr et al. (12) and Nicholls et al. (13). However, an important shortcoming is that such models are rarely applied to the small aquatic organisms typically used in routine aquatic bioassay work. This is due both to limitations in physiological information and to technical problems with chemical analysis of very small organ-tissue samples. There is now a well-developed capability of calculating concentrations, or body residues, in organisms from loading data. Validity can be tested by well-designed monitoring programs. The recent

texts by Gobas and McCorquodale (14), Suter (15), and Bartell et al. (16) review many aspects of this issue.

Toxicity

The final and most difficult task in any assessment is to relate body residues to levels known, or suspected, to be associated with adverse biological responses. Paracelsus stated in 1564 that "What is there that is not poison? All things

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are poison and nothing is without poison. Solely the dose determines that a thing is not a poison" (17). In other words the magnitude of the biological response produced by a toxicant is a function of the amount of toxicant to which the organism is exposed (18). This cause-effect, dose-response principle [often termed concentration-response in ecotoxicology (19)] involves three assumptions:

- Amounts of chemical in the body and at site(s) of toxic action are proportional to the concentration and nature of the exposure(s).
- Biological responses can occur when chemical(s) in the organism are present at site(s) of toxic action where effects are initiated.

• Above an effect threshold the magnitude of biological response elicited is proportional to the amount of chemical at the site(s) of toxic action.

Because it is difficult to measure the amount of toxicant at target site(s) within organism(s), a surrogate measurement, such as concentration in the exposure medium, is normally used. Franks and Lieb (20) state the situation clearly: "One must be very careful, when comparing potencies, not to get confused between observed values and the potency at the site itself." For example, the 96-h LC₅₀ water concentration is simply a surrogate for the amount of toxicant in the organism at the site(s) of toxic action producing the observed mortality.

Perhaps most convincing, as a general example of the importance of understanding the surrogate dose-target dose relationship, are the plots of log bioconcentration factor (BCF) and log LC₅₀ versus log K_{ow} as illustrated in Figure 2 (21-24). Although this example was developed with bioconcentration and acute toxicity data for narcotic organics, chronic toxicity data for small freshwater fish (21-23) and for modifications in marine mussel energetics (25) exhibit a similar relationship.

These plots usually have slopes of approximately +1 and -1, respectively. The BCF plot is obtained by setting the concentration in water, C_w, measuring the organism concentration, C_f, as a response, then deducing BCF as C_f/C_w. In principle it would be possible, but inconvenient, to decide on C_f, then explore what values of C_w will achieve the desired C_f. The plot would then logically be of C_w/C_f as the dependent variable. As illustrated in Figure 2, this is effectively the BCF graph turned upside down, and takes the form of the toxicity plot. The LC₅₀ test can thus be regarded as a bioconcentration experiment in which, rather than analyze for CF, the researcher uses the organism response to CF. The condition of the organism replaces the gas chromatograph as the detector.

These slopes indicate that, to a first approximation, narcotic toxicity results from a near-constant body residue. Although this is a well-recognized relationship elucidated in the classic work of Ferguson (26) and McGowan (27), we believe that the basic toxicological

principles embodied in this relationship, and their usefulness in ecotoxicology problems and environmental risk assessment, have not been fully appreciated or exploited.

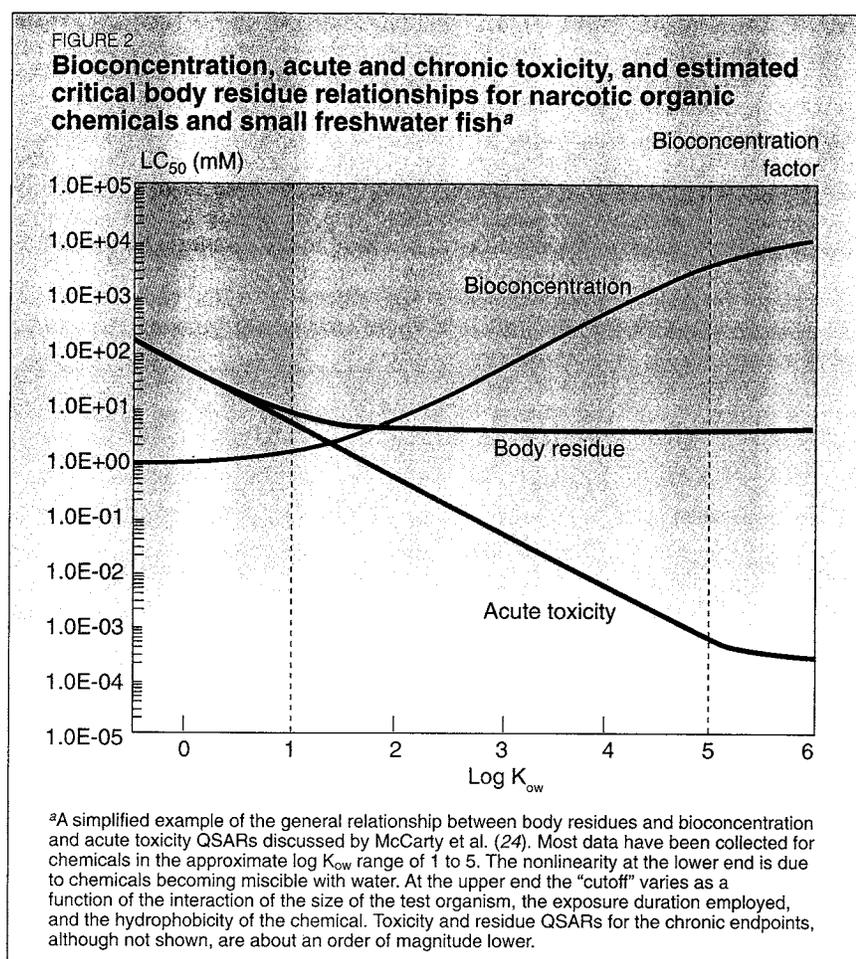
These relationships also suggest that, in many toxicological circumstances, living organisms are much more similar than it would appear from a superficial examination of toxicity test results. For example, Hodson (28) reported that good correlations existed between rat or mouse oral LD_{50} s and fish intraperitoneal LD_{50} s for some organic chemicals. Kaiser and Palabrica (29) reported good correlations between toxicity of organics to fish or invertebrates and *Photobacterium phosphoreum* toxicity. The growth-based EC_{50} s for the algae *Selenastrum* (30) and for the terrestrial plant lettuce, as well as the EC_{50} for photosynthesis inhibition of the algae *Chlorella* and *Chlamydomonas* (31), also exhibit $\log K_{ow}$ -based QSARs with slopes near unity for some groups of hydrophobic organics.

This suggests that residues producing narcotic effect endpoints in various organisms may also be approximately constant. Although the CBR associated with narcosis may be somewhat different for different species, much of this is because of disparity in body character and composition rather than differences in target site concentrations. Toxicity differences are not likely to be as dramatic as suggested by the concentration data derived from exposure-based bioassays.

Limitations

It should not be construed that the exposure concentration methodology, which continues to be widely used, is not valid or useful. When the intermediary relationships are understood, an exposure dose can be an effective surrogate dose. In some cases biological effects are best explained by exposure concentrations, for example, where deaths of organisms result from exposure to strong acid, alkali, or irritant or when exposure to high concentrations of certain metals produces copious mucus secretion and suffocation.

Situations in which essentially irreversible damage or injury is caused by the presence of the chemical in the organism may not be readily amenable to CBR interpretation if the chemical residue cannot act as its own marker or descriptor of exposure. Thus, investigations of



many carcinogens and mutagens, especially ones with short half-lives where organisms are briefly or intermittently exposed, may be problematic. Some chemicals are rapidly metabolized and, in some cases, an intermediate metabolite is the toxic agent.

Nevertheless, knowledge about the amount and time course of a chemical that has entered the body of an organism is always critically important for toxicological evaluation—whether the information be explicit, as discussed above, or implicit, as advocated by Sprague in his recommendation for "threshold" or "incipient" bioassay endpoints (32).

Risk assessment

Toxicity bioassay data are used extensively in the emerging field of environmental risk assessment. Three basic categories of factors—exposure, toxicokinetics, and toxicodynamics (or what might be called the three *P*'s of toxicology: exPosure, Partitioning, and Potency)—interact to determine the responses in bioassays. Figure 3 shows that the relationships for toxicity and risk are equivalent; the dif-

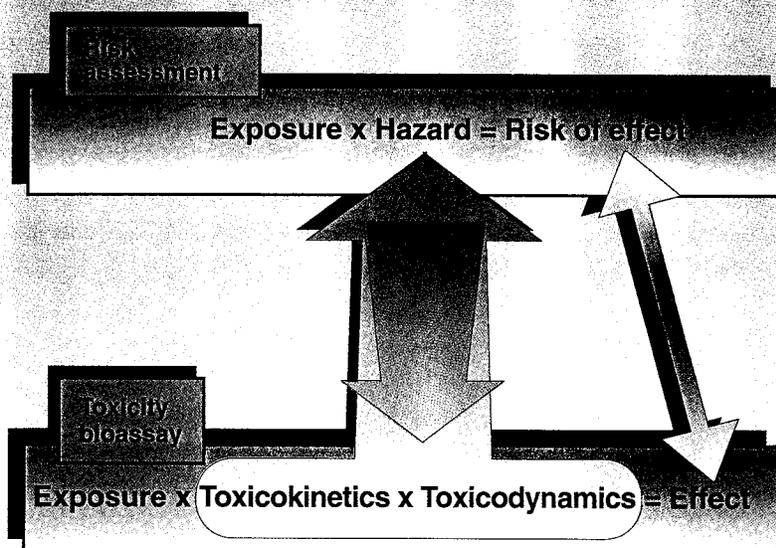
ference lies in the application.

Basic toxicological principles are used to interpret bioassays; in risk assessment, those principles are used to take bioassay information and interpolate or extrapolate to new circumstances and situations. Although explicit modeling is important to bioassay interpretation, it is the essence of risk assessment. Thus, any improvements in understanding basic toxicology gained from greater knowledge of bioassay results will also improve risk assessment (3).

Modes of toxic action

When applying the CBR approach, it is important to recognize the existence of various modes of toxic action. In addition to the general, nonspecific mode of toxic action known as narcosis there are other more specific modes of action. As noted by Drummond and Russon (33), more than one biochemical mechanism may be associated with a whole-organism response mode. Investigators at EPA-Duluth carried out pioneering comparative investigations of various modes of toxic action in environmental toxicology using fish (34-39).

FIGURE 3
Risk assessment and toxicity bioassays^a



^a The relationship between the commonly employed expressions describing toxicity and risk assessment illustrating the fundamental similarities.

Researchers have identified seven major categories for organic chemicals and have recently reviewed acutely toxic body residue estimates for large (600–1000 g) rainbow trout in six of these categories (40). We have added the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin category because it is considered to have a different mode of action. Available aquatic acute and chronic residue data for these eight groups are presented in Table 1 and summarized graphically in Figure 4. Both estimated (bioconcentration times toxicity estimates) and measured residue data are used, and the data have been converted to molar concentrations.

Based on the data for small fish (Figure 4), as well as the CBR estimates for larger fish by McKim and Schmieder (40), different modes of toxic action generally appear to be associated with differing ranges of body residues. Although less data for chronic toxicity exist, a similar type of residue-toxicity relationship is apparent. Overall, this information indicates that whole-body residues are reasonable first approximations of the amount of chemical present at the toxic action site(s). For acutely toxic exposures, data confirm this conclusion for both continuous and intermittent exposure regimes (46).

As noted earlier, using whole-body residues as surrogates for target tissue residues in the organism has shortcomings, many of which

are shared with the external exposure approach. These include metabolic breakdown or activation (e.g., polycyclic aromatic hydrocarbons); internal distribution; lipid types and content; temperature; and general biological factors such as species, sex, life stage, and season. As with exposure-based interpretation, a number of these potential difficulties can be minimized by experimental design, and interpretation of field residue and effect data can be substantially improved.

Most of the modifying factors noted above primarily affect *toxicokinetics*, the time course of accumulation of the chemical, rather than *toxicodynamics*, the time course of the adverse biological response by organisms to the accumulated chemical. The influence of toxicokinetics on the overall toxic response elicited in a situation is often poorly understood quantitatively but is critical to a thorough understanding of the situation.

As can be seen in Figure 2, the 6-orders-of-magnitude difference in exposure-based bioassay results for narcotic organics is caused largely by differences in partitioning behavior (i.e., toxicokinetics) rather than by differences after the chemicals have reached the site(s) of toxic action in the body where the adverse effects are initiated (i.e., toxicodynamics). It is hoped that improvements in investigative techniques and quantitative determination of the influence of these

modifying factors will reduce the observed variability within some of the modes of action, further clarifying intermodal differences.

We believe that the combination of chemical fate and bioaccumulation modeling, and ultimate comparison of deduced whole-body residues with CBRs for chemical groups and each mode of action, as depicted in Figure 1, can be a powerful interpretive and regulatory tool.

Metals, organometals, metalloids

Although the primary focus of this paper is organic chemicals, the same basic toxicological principles apply to metalloids, organometals, and metals. For example, tin, arsenic, mercury, and lead can be found in the environment in organic (i.e., alkylated) forms originating from either natural or anthropogenic sources. These compounds exhibit many of the characteristics of organic chemicals. Again there is organism- and species-specific variability in accumulation as well as in response.

Tas et al. (70) reported that the lethal residue for tributyl and triphenyltins in fish was approximately 0.02 mmol/kg. They also noted that the residue level and mode of action (neurotoxicity) were similar to those of the pyrethroid insecticides. A lethal tri-*n*-butyl tin residue of 0.002 mmol/kg can be estimated from for 96h-LC₅₀ and bioconcentration factor obtained in bioassays with rainbow trout (71). Page and Widdows (72) noted that tissue levels greater than 0.001 mmol/kg wet weight (converted from dry weight assuming 85% water content) of alkyltins are associated with chronic effects in marine mussels. Moore et al. (73) reported that chronic effects occurred in marine polychaetes exposed to tributyltin at residues of 0.003 mmol/kg wet weight, and that substantial mortality occurred at residues of about 0.009 mmol/kg wet weight. Overt toxicity of mercury to various fish species occurs at body residue levels in the range of 0.05–0.15 mmol/kg wet weight (74).

Other metals also appear to exhibit residue-effect relationships. Connolly (2) estimated that the 96-h acute zinc toxicity in trout occurred at about 5500 mg/kg (84 mmol/kg). Peterson et al. (75) reported that aluminum residues of about 0.3 mmol/kg were associated with 30-day LC₅₀ in salmon alevins. McGeachy and Dixon (76, 77) reported that acute and chronic toxicities in rainbow trout exposed to ar-

senic were associated with residues of 0.11–0.12 and 0.05–0.08 mmol/kg, respectively.

Enserink et al. (78) provided bio-concentration and toxicity data for daphnids exposed to arsenic, cadmium, chromium, copper, mercury, nickel, lead, and zinc. Body residues calculated by us indicate 21-day LC₅₀ estimates were associated with 1.2, 2.1, 1.1, 1.1, 0.46, 3.8, 24, and 41 mmol/kg (converted from dry weight assuming 80% water content), respectively. Borgmann et al. (79) reported that the body residue of cadmium associated with 6-week survival-based EC₅₀ estimates for the amphipod *Hyalella azteca* varied over a narrow range, from 0.068 to 0.17 mmol/kg (converted from dry weight assuming 80% water content).

Residue-based interpretation of metals toxicity is problematic. There are exposure-dose-dependent mechanisms of toxic action noted earlier, active or facilitated transport into the organism, preferential accumulation in certain organs, especially liver and kidney, and metallothionein complexation. Some metals are essential micronutrients that can be actively regulated by organisms. However, for environmental assessment purposes, further investigation of body residues and modes of toxic action appears to be warranted for organometals, metalloids, and metals.

Residues for chronic toxicity

Knowledge of the relationship between acute and chronic data is rather qualitative. Chronic toxicity is often assumed a priori to be caused by a different mode or mechanism of toxicity. This is not necessarily the case, and assuming that it violates the null hypothesis assumption of similarity until proven otherwise. Much work has been focused on the ratio of acute bioassay data to chronic bioassay data, often termed the acute to chronic ratio (A/C). For a variety of organisms and chemicals A/C is typically of the order of about 10, averaging 12 for organics (43). This is supported by quantitative structure–activity relationships (QSARs) for some chemical groups for which acute and chronic regressions are about an order of magnitude apart (21, 23, 80). Although these observations are based on exposure-based data, acute and chronic residues can also be estimated, and we interpret these relationships as follows.

In typical aquatic bioassays the

TABLE 1
Summary of modes of toxic action and associated critical body residue estimates in fish^a

Chemical and effect	Estimated residue (mmol/kg)	Reference
Narcosis		
Acute (summary)	2 to 8	24
Chronic (summary)	0.2 to 0.8	24
Acute (octanol, MS222)	1.68 or 6.32 ^b	40
Polar narcosis		
Acute (summary)	0.6 to 1.9	41
Acute (2,3,4,5-tetrachloroaniline)	0.7 to 1.8	42
Chronic (summary)	0.2 to 0.7 (chronic/acute = 0.1–0.3)	43, 22, 44
Chronic (2, 4, 5-trichlorophenol)	0.2	45
Acute (aniline, phenol, 2-chloroaniline, 2, 4-dimethylphenol)	0.68 or 1.76	40
Respiratory uncoupler		
Acute (pentachlorophenol)	0.3	46, 47, 48
Acute (2, 4-dinitrophenol)	0.0015 or 0.2	47
Chronic (pentachlorophenol, 2, 4-dinitrophenol)	0.09 to 0.00015 (chronic/acute = 0.1–0.3)	43, 22, 44
Chronic (pentachlorophenol)	0.094	49
Chronic (pentachlorophenol)	0.08	45
Acute (pentachlorophenol, 2, 4-dinitrophenol)	0.11 or 0.20	40
AChE inhibitor		
Acute (malathion and carbaryl, chlorpyrifos)	0.5 and 2.7	47
Acute (chlorpyrifos)	2.2	50
Acute (aminocarb)	0.05 and 2	51, 52
Acute (parathion in blood)	0.13 to 0.2	53

actual dose is an unknown amount of chemical at the site(s) of toxic action in the organism. Thus, a surrogate dose measurement, such as the exposure concentration, is employed in lieu of the actual dose. Each degree of biological response is considered to be associated with a different dose. There may or may not be an actual threshold dose, depending on the mode and mechanism of toxic action, but an effective threshold is present. When responses are plotted against the respective exposure doses at a given exposure duration, a normal distribution is often obtained or, if plotted as a cumulative response, a sigmoidal curve (see Figure 5). The actual nature of the distribution—normal (probit), logit, Weibull, or other—can affect extrapolation into the tails (81, 82), but this does not affect the essence of our argument.

The result of considering the time course is a three-dimensional surface formed from exposure times, exposure concentrations, and response. Hong et al. (83) prepared a growth curve polynomial model that fitted such a response surface to bioassay data. Mayer et al. (84) developed a probit-based method that employed acute toxicity data to predict chronic toxicity. Mackay et al. (61) also prepared a response surface model, incorporating a constant lethal body residue for the mode of toxic action, log K_{ow} for the chemical, and a Weibull distribution factor. Unlike the previous two, this model employs body residues explicitly. After initial calibration with experimental data to obtain the appropriate Weibull shape factor, only the log K_{ow} of the chemical and the acute CBR for the mode of toxic action in question are required

Chemical and effect	Estimated residue (mmol/kg)	Reference
Chronic (chlorpyrifos)	0.003	50
Acute (malathion, carbaryl)	0.16 or 0.38	40
Membrane irritant		
Acute (benzaldehyde)	0.16	(Estimated with BCF = 1.6, acute fathead LC ₅₀ = 0.1 mmol/L)
Acute (benzaldehyde)	2.1 or 13.2	40
Acute (acrolein)	0.0014 or 0.94	40
CNS convulsant ^a		
Acute (fenvalerate, permethrin, cypermethrin)	0.002 to 0.017	54
Acute (fenvalerate, permethrin, cypermethrin)	0.000048 to 0.0013	55
Acute (endrin in blood)	0.0007	56
Acute (endrin)	0.0018 to 0.0026	57
Acute (endrin)	0.005	58
Chronic (fenvalerate, permethrin)	0.0005 and 0.015	59
Respiratory blockers		
Acute (rotenone)	0.0006 to 0.003	60
Acute (rotenone)	0.008	61
Acute (rotenone)	0.0009 or 0.0028	40
Dioxin (TCDD)-like		
Lethal (TCDD)	0.000003 to 0.00004	62, 63, 64, 65, 66
Growth/survival (TCDD)	0.0000003 to 0.000008	67, 65
Early life stages, lethal (TCDD)	0.00000015 to 0.0000014	68, 66, 69
Early life stages, NOAEL (TCDD)	0.0000001 to 0.0000002	66

^a The rainbow trout used by McKim and Schmieder (40) weighed 600–1000 g; the other data presented are largely for small fish, sometimes early life stages, that typically weighed less than 1 g. Most estimates have been converted from mass-based data.

^b The two values represent residues estimated by two different methods.

^c Includes three subgroups characterized by styrycholine; fenvalerate and cypermethrin; endosulfan and endrin (38).

to predict various acute or chronic toxicity endpoints. When necessary, the effects of modifying factors, such as metabolic breakdown of the toxicant, can be addressed by the model.

The essence of this approach is illustrated in Figure 5. When the influence of modifying factors, especially metabolism, is minimal, the body residue is equal to the exposure toxicity concentration times the bioconcentration factor. It is assumed that all organisms reach the same body residue at the same time; we have not considered the statistical variability and uncertainty in the data. Such simplifying assumptions facilitate discussion but may be approximately correct only in some situations.

A key finding by Mayer and co-workers was that chronic endpoints employed in early life stage tests

with fish were similar to 0.01% acute lethality estimates obtained from probit-based extrapolation of the acute toxicity dose-response curve for the same species and chemical (84, 85). Thus, a critical body residue for chronic effects can be calculated by the same method used with acute data. In Figure 5 this produces a chronic CBR estimate of 1 mmol/kg. Once the relationship between exposure-based and residue-based estimates of dose is established, this simple procedure can be used to estimate CBRs associated with any exposure-based response endpoint on the curve, whether it be LC_{0.01}, LC₀₁, LC₁₀, or another response level of interest.

In summary, toxicity test data yield information on the biological response (usually expressed as a percentage of the population responding) as a function of concen-

tration and duration. Cumulative distribution curves can be deduced and responses at low exposures estimated from appropriate statistical models. A key finding is that exposures yielding chronic endpoints appear to be similar to those yielding 0.01% mortality at incipient, acutely lethal concentrations (84, 85). Application of basic toxicological relationships allows the estimation of acute and chronic CBRs from exposure-based toxicity data.

Body residues and mixtures

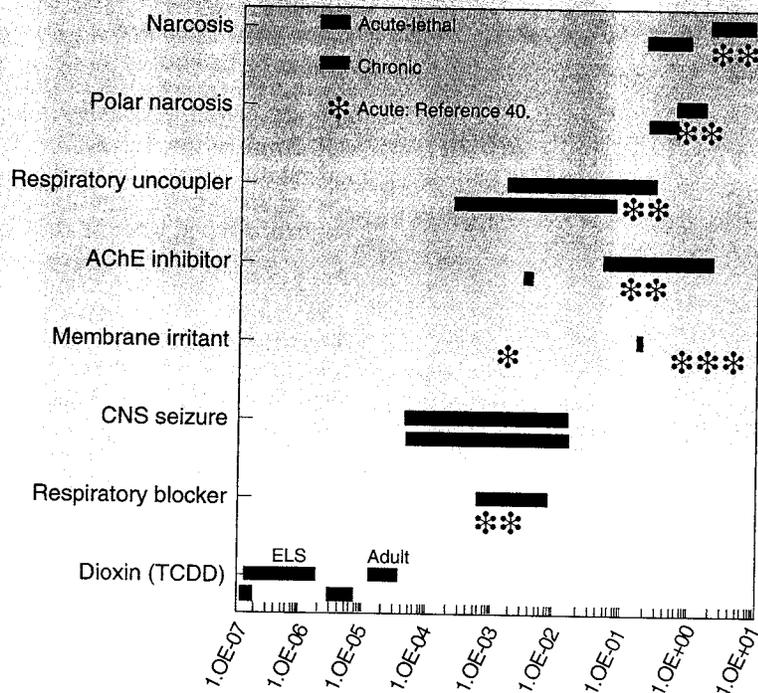
Hermens and co-workers conducted extensive investigations into the toxicity of mixtures in aquatic systems. Although independence, potentiation, or antagonism may be present, they reported that for a variety of mixture studies with equitoxic contributions from 3 to 50 organic chemicals, the mixture toxicity was generally additive (86–88). Indeed, it appears that the larger the number of chemicals in the mixture the better the approximation of concentration addition. Components present as low as 0.0025 of the LC₅₀ contributed to the mixture toxicity. This occurred even when chemicals known to have dissimilar modes of toxic action were present in the mixture and for both acute and chronic endpoints.

A likely explanation for this phenomenon is that when chemicals that act by specific modes of toxic action (i.e., non-narcotic) are present in a mixture below their threshold for specific toxic action (i.e., below 0.3 to 0.02 of their threshold LC₅₀), they do not express the specific toxic action. Instead, they merely contribute to the narcotic activity of the mixture. In these circumstances, it is simple addition of the narcotic toxicity of the components of the mixture, rather than any interaction between specific modes of toxic action, that is producing the observed biological response. Approximate additivity is likely common in real-world situations with many organic chemicals, especially when considered on a residue basis rather than an exposure basis.

We speculate that, as a practical general approach to mixture toxicity, it may be possible to establish guidelines that would allow classification of mixture toxicity into those situations expected to be approximately additive and those that are not. This may be extended to estimating when a single mode of specific toxicity was expected to dominate the mixture. A knowledge base of CBRs would have to be devel-

FIGURE 4

Estimated body residue levels (mM) associated with acute and chronic toxicity endpoints for fish exposed to eight categories of organic chemicals^a



^aA summary of the data presented in Table 1. Measured and estimated residue data from the literature are graphed according to mode of toxic action and acute or chronic biological response endpoint.

Implications

We believe that the CBR approach could contribute to integrated models of environmental fate and toxicity. Current environmental fate and bioaccumulation models can predict chemical residues in organisms, but the toxicological significance is uncertain. If residue-effect relationships can be better defined, the ability to interpret existing laboratory and field data, as well as predict situations of impact in advance, will be substantially improved. The following areas are worthy of note.

Modifications to standard bioassay protocols. For initial investigation a single enhanced acute bioassay protocol may be more effective, both scientifically and economically, than the current trio of standard aquatic bioassays: acute, chronic, and bioconcentration (92). Some of the chronic toxicity test results needed for regulatory purposes could be obtained or refined from enhanced acute tests (61, 84). Simultaneously examining exposure levels and body residue would improve understanding of factors such as bioavailability and metabolic degradation. Furthermore, increased quantitative understanding of bioassay data, which will result from residue-based approaches, will allow advances to be made beyond the exposure-medium-based, no-observed-effect-level Safety Factor approach currently employed in estimating acceptable levels and developing environmental contaminant regulations.

Incorporating CBR into standard bioassays should substantially improve the toxicological understanding of results and, hence, any regulations based on those results.

An improved method of addressing mixture toxicity. To a first approximation, neutral hydrophobic narcotic chemicals are equipotent on a body residue basis. Thus, the toxicity of a mixture of narcotics can be explained by a proportional contribution to a critical body residue associated with an adverse effect endpoint. This approach allows the contribution to the total residue through time for each component to be approximated by simple kinetics models (93) and facilitates investigation based on hypothesis testing.

A residue-based approach to understanding and predicting the toxicity of mixtures of chemicals with different modes of toxic action appears promising. The scheme outlined earlier provides a working ap-

oped so that typical residue values and ranges for acute and chronic endpoints could be established for various test organisms and modes of toxic action. It is unlikely that every chemical has the same mode of toxic action in every organism, so this variability would have to be considered. Despite obvious impediments, this approach offers many advantages over current methods for examining mixture toxicity.

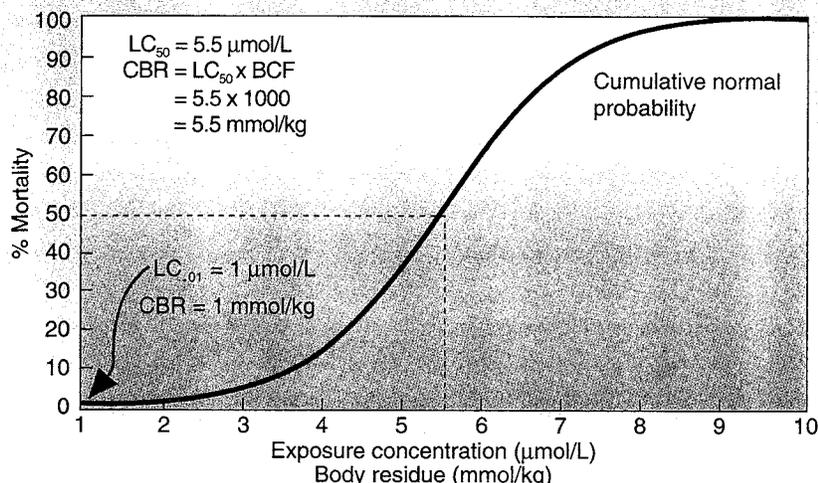
In particular, a scheme of this type could serve as a framework for much-needed research that provides basic toxicological data as well as information directly usable for regulatory purposes (see Figure 1). For just this reason a residue-based scheme is the logical extension of the various mixture toxicity classification plans that have been proposed in the past (81). Van Leeuwen et al. (89) and Verharr et al. (90) have recently made such attempts implicitly considering body residues. We believe explicit consideration of biological responses associated with accumulated body residues would assist in the broad acceptance of any such schemes.

A critical problem with exposure-based mixture toxicity assessment is that deviations from additivity

may be caused by metabolic breakdown of one or more of the components of the mixture. De Wolf et al. (42) and de Bruijn et al. (91) recently confirmed that the apparent reduced toxicity observed in many exposure-based bioassays with organic chemicals and fish resulted from metabolic activity reducing body residues. It is likely that metabolic degradation of component chemicals, which might be relatively minor at the acutely lethal level in a single chemical test, could be more influential at lower exposure levels. This could contribute to the substantial variability observed in chronic single-chemical and mixture bioassays. Inducible metabolic degradation pathways could further confound the situation.

However, without residue information it is not possible to distinguish between situations in which chemicals contribute differentially to the toxicity of the mixture and situations in which a chemical simply modifies the toxicokinetics of accumulation of other chemicals in the mixture. Clearly, residue-based interpretation may provide the means to investigate and resolve these major impediments to mixture toxicity interpretation.

FIGURE 5

Relationship between acute and chronic toxicity and body residues^a

^a A CBR associated with an acute or chronic toxicity endpoint is estimated in the same way as the product of the exposure-based water concentration and the bioconcentration factor. In this example the observation of Mayer et al. (84, 85), that the 0.01% mortality point on the acute toxicity tolerance is similar to the chronic exposure-based concentration estimated by typical bioassay methods, is exploited to obtain a chronic value to use in the calculation.

proach to regulation of some mixtures of chemicals, while also providing a framework for further investigation. Mixture toxicity is rarely addressed in environmental regulations; the CBR-based approach is attractive because it both highlights the problem and provides a mechanism to examine it.

Integrated fate, accumulation, and effects modeling. The primary interest of the field ecologist, the laboratory toxicologist, the government regulator, and the public is not the avoidance of adverse responses just in populations of certain organisms, but the avoidance of adverse effects in the community and ecosystem. Debate continues about the assessment of ecosystem health; positions range from the "top-down" population and community level supporters to the "bottom-up" biochemical advocates (94).

In exposure-based assessment of aquatic systems, effects are referenced to the concentration in water or sediment. Although feasible at the organism level and above, it becomes increasingly difficult to interpret exposure-based data at levels of organization below the whole organism, this being the realm of physiological-biochemical toxicology and toxicokinetics. To achieve some reconciliation a common factor must provide a link between laboratory test data and ecological effects observed in the field. A residue-based "middle-out" ap-

proach—whole-body residues in average organisms—should provide a common dose surrogate for the bottom-up and top-down supporters. A residue-based approach should provide a good overall strategy for those who wish to understand entire ecosystem structures, from biochemistry to ecology.

Summary

We view residue-effects relationships as integral in an overall scheme of environmental risk assessment involving fate modeling, bioaccumulation modeling (including bioconcentration, food chain accumulation, and metabolism), and community dynamics, as discussed by Bartell et al. (4). Residue-effects relationships will allow the substantial progress environmental toxicology has made in the past few decades to continue without losing touch with either the large body of exposure-based information or the field-based observations of adverse responses and residue monitoring data. The opportunity to correlate and validate the observations of laboratory toxicologists and field ecologists via residue levels in organisms and population effects (or lack of them), is particularly attractive.

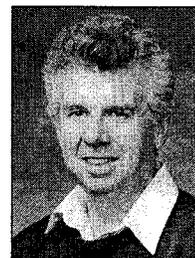
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