8 Mixed and Multiple Agents

Can any mortal mixture of earth's mould breathe such divine enchanting ravishment?

John Milton

Risk assessments often deal with single agents such as a new chemical, an exotic species, or a harvesting method. However, organisms and ecosystems are always exposed to mixtures of chemicals and to diverse and multiple hazardous agents. Often, things that are treated as single agents for regulatory purposes such as crude oil, polychlorinated biphenyls (PCBs), and pesticide formulations are actually mixtures. This chapter begins with a discussion of methods for addressing chemical mixtures and then presents an expansion of those methods to address combined risks from multiple activities and agents at a site.

This chapter is limited to assessment of mixed and multiple agents per se. An alternative approach to multiple agents is to measure or observe their effects in the field and then infer their causes, which may be mixed or multiple (Foran and Ferenc 1999). Such bioassessments, which are limited to existing and ongoing conditions, are presented in Chapter 4. Also, some discussions of multiple agents treat background environmental conditions such as temperature and light as part of the set of agents to be assessed. Here, the multiple agents to be assessed are limited to those that may be subject to regulation, remediation, or management, and environmental conditions are treated as cofactors.

8.1 CHEMICAL MIXTURES

Methods for estimating risks from chemical mixtures can be divided into those that use test results for whole mixtures and those that use test results for component chemicals. The choice depends primarily on three considerations:

Availability of effects data: Methods that employ whole mixtures require that a mixture be available for testing. Methods that are based on components require that the components have been tested in a consistent and appropriate manner or that the resources be available to perform the needed tests.

Complexity of the mixture: In general, the models that estimate effects of mixtures from toxicity data for components are feasible only for simple mixtures. If a mixture contains many chemicals, not only is it unlikely that suitable toxicity data will be available for each, but it is also difficult to defend assumptions about their combined effects.

Availability of exposure data: Whole-mixture methods require that the exposures be defined in terms of dilution in an ambient medium of a mixture with a sufficiently consistent composition. Processes such as differential partitioning or degradation of constituents may render test data for mixtures irrelevant. Component-based methods require a characterization of the chemical composition of the mixture to which organisms are exposed, derived by analysis of contaminated media or by modeling of the transport

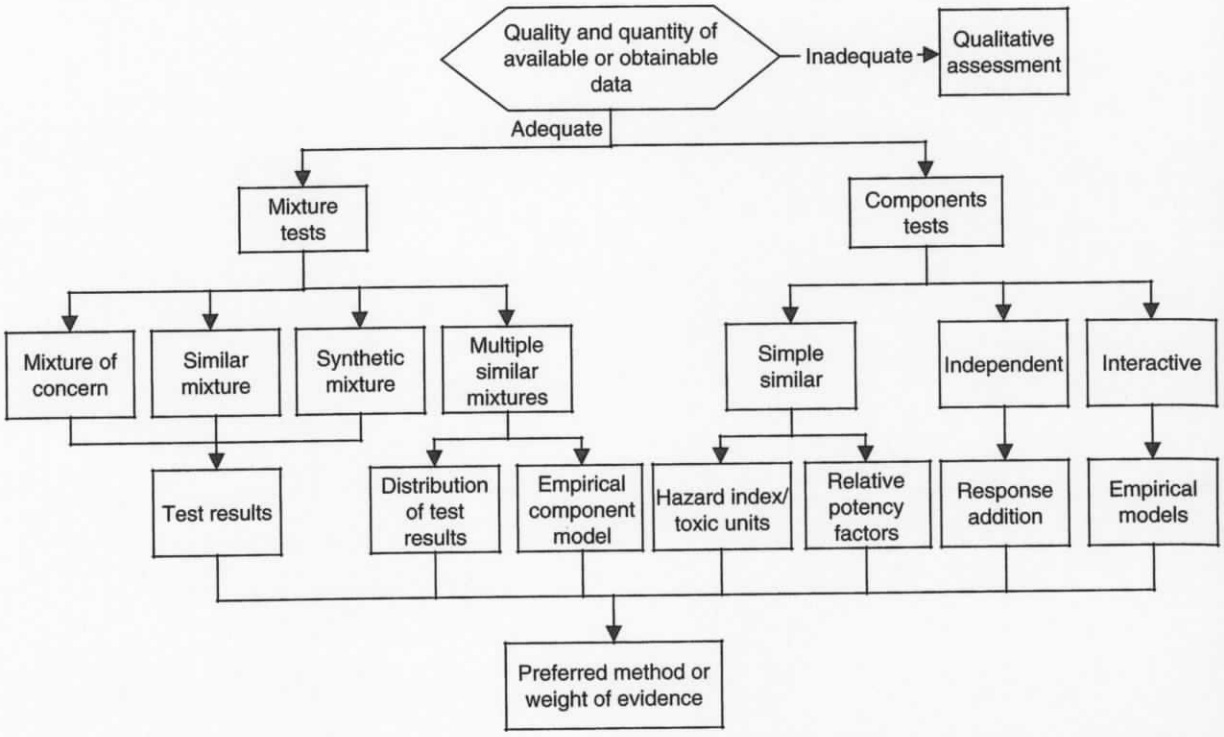

FIGURE 8.1 The different methods of assessment of chemical mixtures. (Highly modified from Risk Assessment Forum, Supplementary guidance for conducting health risk assessment of chemical mixtures, EPA/630/R-00/002, US Environmental Protection Agency,Washington, DC, 2000.)

and fate of a release that has been chemically characterized (Part III). Modeling is complicated by the effects of the mixture on the transport and fate of the constituents.

The options for assessment of mixtures are diagramed in Figure 8.1. With the availability of exposure information and effects data concerning mixtures or their constituents, the quality of those data, and the possibility of performing new tests to provide necessary data, one may proceed to an assessment based on whole mixtures, constituents, or a qualitative assessment. If only a qualitative assessment is possible, it should explain why mixture risks cannot be quantified and also that the combined effects of a mixture are likely to be greater than those of the constituents.

This section focuses primarily on estimation of effects of mixtures, because that is the most difficult problem. However, estimating the transport and fate of mixtures presents its own problems. For example, the antibacterial properties of one chemical may slow the biodegradation of others in a mixture. Similarly, the fate of benzene dissolved in an aqueous effluent is quite different from its fate in petroleum, a nonaqueous liquid. It is important to assure that the effects analysis and exposure analysis produce consistent descriptions of a mixture at the point of exposure.

This section is consistent with the guidelines from the US Environmental Protection Agency (Risk Assessment Forum 2000). It is not inconsistent with guidance and practices in Europe and elsewhere. The basic concepts and models are the same, but terminology differs, and different approaches are emphasized, even among agencies in a nation.

8.1.1 Methods Based on Whole Mixtures

Some whole mixtures are materials such as pesticide formulations that have a consistent composition and may be tested, assessed, and regulated like an individual chemical. In such cases, assessors are primarily concerned with the fate of the mixture in the environment. If the

constituents have highly diverse properties, the toxicity of the original mixture can quickly become irrelevant. Over time, such a mixture in the environment will be transformed from the original mixture, to a different but still similar mixture, and then to a mixture that is so dissimilar that it must be tested as a new mixture or assessed in terms of its constituents. An important example is the current situation with PCBs. Because all of the isomers in a commercial PCB mixture such as the Aroclors are relatively similar, their fates are similar; so it has been possible to relate mixtures in the environment back to the commercial mixture. However, in the years since PCBs were banned, the differences in partitioning and degradation rates among isomers have resulted in changed mixtures in the environment. There is now a lively debate between those who believe that toxicity should be estimated for the individual isomers and then combined to estimate mixture risks and those who believe that a concentration that is reported as Aroclor 1242 is still sufficiently similar to the tested commercial mixture. The debate continues because it is not clear whether our lack of knowledge about the toxicity and interactions of the constituent isomers or the changes in the mixtures are larger sources of uncertainty.

Other mixtures are undesigned and variable. They include combinations of wastes, pesticides, fertilizers, and other chemicals brought together at a location by various processes (e.g., the mixture of chemicals in the sediments of an urban estuary) or complex effluents from various industrial, commercial, and domestic processes. These mixtures differ from the materials discussed above in that their compositions are often unknown or poorly known and are highly variable over space or time. However, these mixtures may still be collected and tested, and, in fact, tests have been developed for that purpose (Box 8.1). These tests differ from conventional toxicity tests in that they are designed to be relatively inexpensive, so that they can be performed in large numbers, yet sensitive enough to be protective. For example, the standard toxicity tests for aqueous effluents and contaminated freshwaters in the United

BOX 8.1
Mixtures Risks to Chesapeake Bay Striped Bass

In situ toxicity tests with larval striped bass have shown that the waters of some spawning areas in Chesapeake Bay tributaries are acutely lethal and that these effects are associated with mixtures of metals (Hall et al. 1985). Logan and Wilson (1995) developed a predictive risk characterization method and used it to explain and extend the results of the tests. They defined risk under the assumption of simple similar effects as

$$P(\Sigma \mathrm{TU}_i > 1)$$

or, since the data were log-normally distributed, as

$$P[\log(\Sigma \mathrm{TU}_i) > 0]$$

Risks were estimated for six test sites. The means and variances of concentrations of five metals were taken from measurements at the sites. The toxic concentrations were LC_{50}s with an estimated variance of 0.018 for log-transformed values from the pooled variance in Barnthouse and Suter (1986). At the least and most metal-contaminated sites, the sum of toxic units was 0.17 and 3.27, respectively, and the risks were 0.00 and 0.99, respectively. The three sites with risks greater than 0.2 had greater than 85% mortality of striped bass larvae. However, for the sites with risks less than 0.2, mortalities ranged from 60% to 83%. The mortality at low risks suggests that either the caging or contaminants other than metals affected many larvae.

States are 7 d tests of fathead minnow (*Pimephales promelas*) larvae and the life cycle of the cladoceran *Ceriodaphnia dubia*. These tests may be performed periodically to determine the temporal variance in toxicity of effluents or ambient waters, whereas the conventional chronic tests would be prohibitively expensive and might not detect important short-term variance in toxicity. Similar soil or sediment tests can be used to determine the distribution of mixture toxicity across space (Figure 20.2).

In some cases, the tested mixture is the mixture of chemicals accumulated by organisms. The advantage of this approach is that the tested mixture is the mixture to which organisms in the field are internally exposed. It integrates spatial and temporal variance in exposure concentrations, bioavailability, and differential uptake and retention. This approach can be particularly useful in establishing causation (Chapter 4). For example, exposure to hydrocarbons extracted from blue mussels with reduced feeding rates collected from contaminated sites was shown to cause reduced feeding in clean mussels (Donkin et al. 2003). Similarly, Tillitt et al. (1989) tested chlorinated hydrocarbon mixtures extracted from eggs collected from colonies of cormorants and terns that had experienced reproductive impairments.

If adequate data are not available for the toxicity, biological oxygen demand, or other property of a mixture, test or measurement results for a similar mixture may be substituted. In such cases, the assessors must determine whether the mixtures are sufficiently similar, which

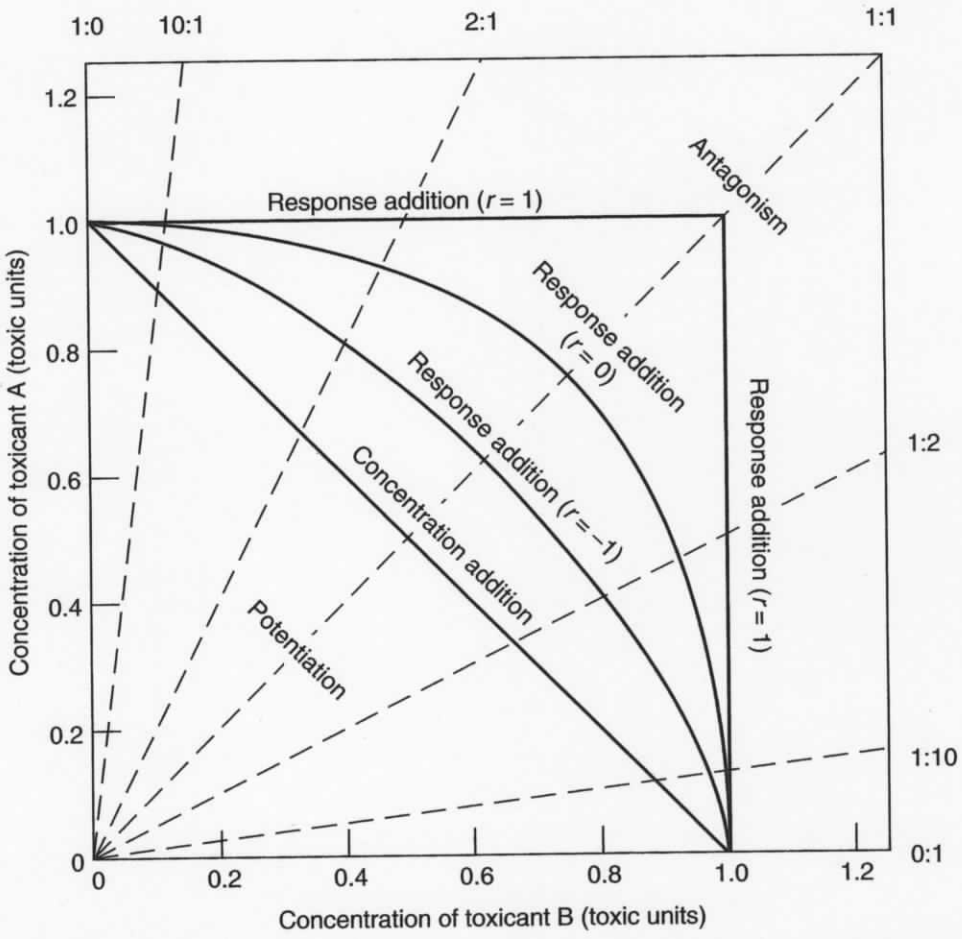

FIGURE 8.2 Isobologram (isobole diagram) for a quantal response to a mixture of two toxic substances. Dashed lines represent constant ratios of concentrations of the two chemicals. Solid lines are isoboles, lines of equal response, representing the set of concentrations of A and B at which the endpoint response (e.g., LC_{50}) occurs.

depends on the use of the data. For screening purposes, to determine whether a hazard requires more assessment, a roughly similar mixture may be adequate. For example, we may assume that all heavy crude oils or all polycyclic aromatic hydrocarbon (PAH) mixtures are similar, perhaps with a safety factor. However, in more definitive assessments in which the risks are near the margin of acceptability, the mixtures should be highly similar. The criteria for determining similarity include the proportion of components that are common to the mixtures, their proportions in the mixture, the presence of components in one mixture with a different mode of action (MoA), and the presence of components in one mixture from a different chemical class.

In some cases, data are available for multiple similar mixtures. For example, we may know the degradation rates of several heavy crude oils, the fathead minnow LC_{50}s for effluents from multiple chrome-plating operations, or the *C. dubia* chronic value for wastewater from a municipal treatment plant at multiple times. In such cases, the distribution of those properties could be used to estimate the same property for an untested heavy crude oil, chrome-plating effluent, or municipal effluent. If chemical composition data are available for the similar mixtures, a model may be developed using multiple regression to relate concentrations of the constituents to the toxicity or fate-related property. That empirical constituent model could then be used to estimate the properties of the mixture of concern, if it has been analyzed. However, that approach depends on the assumption that the concentrations of the constituents are independent.

Finally, tests may be performed with synthetic mixtures. That is, if the composition of a future effluent or other unavailable mixture can be estimated, the mixture can be approximated by mixing reagent chemicals in an appropriate medium. This mixture and, if appropriate, dilutions of the mixture may then be tested. Because stability of the mixture over time is not an issue, these tests may be performed with longer durations and more life stages than the short-term tests used with effluents and contaminated ambient media. This approach also provides the opportunity to systematically study the effects of variation in composition of the mixture or characteristics of the medium such as pH or hardness.

The results of mixture tests may be modeled as exposure–response relationships like individual chemicals. That is, if the mixture is tested at full strength and in dilutions, responses such as proportional mortality p may be modeled as

$$p = f(s) \tag{8.1}$$

where s is strength from 0 to 1 and f is the probit, logit, Weibull, or some other fitted function. In the field, dilutions may occur naturally due to dilution of an effluent plume, leachate, aerial deposition, or spill. In such cases, the contaminated media may be collected and tested on a transect or grid and tested. The results may be modeled as in Equation 8.1, with dilution defined by concentration of an index chemical or some other measure of contamination (e.g., total PAHs) relative to concentration in the source or maximally contaminated sample.

In some cases, only the full-strength mixture is tested. In such cases, one can only describe the differences in response from the control (e.g., clean water) or reference (e.g., upstream water). Statistical significance tests are commonly applied to these differences, but the usual warnings about the differences between statistical and biological or regulatory significance apply (Section 5.3).

8.1.2 Methods Based on Tests of Components

In many instances it is not possible to test the mixture of concern or, because the composition of a mixture is not stable, it is not feasible to assess risks based on tests of the mixture. In such cases, the toxicity and other properties of the mixture must be estimated from the properties

TABLE 8.1
Categories of Interaction of Pairs of Chemicals

	Similar Action	Dissimilar Action
Noninteractive	Simple similar (concentration or dose addition)	Independent (response addition)
Interactive	Complex similar	Dependent

Source: Adapted from Plackett, R.L. and Hewlett, P.S., *J. Royal Stat. Soc.*, B14, 141–163, 1952; Hewlett, P.S. and Plackett, R.L., *The Interpretation of Quantal Responses in Biology*, Edward Arnold, London, 1979. With permission.

of the individual chemicals. The occurrence of chemicals in mixtures can affect toxicity in two ways. First, the presence of one chemical may influence the kinetics of uptake, transport, metabolism, and excretion of other chemicals. Examples include the thickening of the mucous coating of fish gills by zinc and damage to kidney nephridia by cadmium–metalothionine complexes. A common form of kinetic interaction is the induction or inhibition of metabolic enzymes. Second, by jointly inducing effects, chemicals in a mixture can cause types or levels of effects that are different from those of any of the chemicals acting alone.

The combined effects of toxic chemicals can be categorized based on whether they interact and whether they have similar joint action (Table 8.1). Similar action can refer simply to having the same mode of action (e.g., reproductive failure) or more strictly to having the same mechanism of action in the same organ (e.g., inhibition of the shell gland) (Chapter 7). Chemicals may be assumed to have similar action if they are fitted well by the same structure–activity relationship (Section 26.1). Interactions include effects of one chemical on either the kinetics of the other or on dynamics, including modification of the site of action or combining to produce an effect that neither could produce alone. Both chemicals with similar action and dissimilar action may interact. In either case, the interactions may be potentiating (also termed synergistic or enhancing) or inhibiting (also termed antagonistic or attenuating). Models of simple similar action are commonly referred to as concentration- or dose-additive, and models of independent action are commonly referred to as response-additive.

Figure 8.2 is an isobologram, a diagram of the possible combinations of two chemicals, normalized as proportions of the concentrations producing a particular effect such as an LC_{50}. Isoboles are the lines of equal effect in that space.

Care should be taken when extrapolating knowledge of the nature of chemical interactions between taxa, life stages, or exposure patterns. In particular, most of the studies of chemical interactions use acute lethality as the test endpoint. Chemicals that have similar action for that brief exposure and simple response may act dissimilarly in chronic exposures where more mechanisms such as developmental or behavioral toxicity may come into play.

8.1.2.1 Simple Similar Action and Concentration Addition

If chemicals have the same mechanism of action and do not interfere with, or potentiate, each other, their joint action is simple similar and their combined effects may be estimated by concentration addition or dose addition. (In the remainder of this section, read "concentration" as "concentration or dose.") Simple similarity can be assumed if the mechanism of action of the chemicals and their uptake, transport, and elimination (i.e., toxicokinetics) are known to be the same based on mechanistic toxicology. For example, organophosphate insecticides, which inhibit cholinesterase, have simple similar combined toxicity. Simple similarity can be determined empirically by testing mixtures of the chemicals. That is, if different proportional

mixtures of the chemicals are tested and the results fall on the concentration addition line of Figure 8.2, the interaction is simple similar addition. This mode of interaction is suggested by the observation that chemicals have parallel exposure–response curves. Finally, concentration addition models are commonly used as a default for mixtures containing a large number of chemicals with various mechanisms of action (Risk Assessment Forum 2000). This is based on the observation that the toxicities of many mixtures of chemicals are reasonably approximated by a concentration addition model (Alabaster and Lloyd 1982).

Concentration addition models are all based on the assumption that the chemicals in the mixtures are functionally the same, but differ in their potencies by a constant factor t. For two chemicals occurring at C_1 and C_2, and a response R such as proportional mortality, this model is

$$R_1 = f(C_1) \tag{8.2}$$

$$R_2 = g(C_2) = f(tC_2) \tag{8.3}$$

where f and g are concentration–response functions. Equation 8.3 shows how the factor converts the concentration of one chemical to an effective concentration of the other. As a result, the response to binary mixtures of simple similar chemicals (R_m) can be represented as

$$R_m = f(C_1 + tC_2) \tag{8.4}$$

For example, quantal responses (e.g., death or deformity) may be modeled by a log-probit function and for a set of n concentration-additive chemicals, the function would be

$$P_m = a + b\log(C_1 + t_2C_2 + \ldots t_nC_n) \tag{8.5}$$

where P_m is the probit of the response to the mixture, and a and b are fitted variables.

For a particular level of effect such as an LC_{50}, concentration addition models reduce to

$$\Sigma(C_i/LC_{50i}) = 1 \tag{8.6}$$

For example, if a mixture contains three similar chemicals, each at a third of its LC_{50} for a species, that mixture is expected to kill half of the exposed organisms. The concentration (or dose) divided by its effective concentration (e.g., C_i/LC_{50i}) is known to ecotoxicologists as a toxic unit (TU) following Sprague (1970). However, it is known more generally in risk assessment as the hazard quotient (HQ) (Chapter 23). Sums of HQs are called hazard indexes (HI):

$$\Sigma TU = \Sigma HQ = HI \tag{8.7}$$

If the HI is greater than 1, we would expect the prescribed effect to occur. This HI approach depends on having toxicity data for all chemicals in the mixture for an appropriate species and response (e.g., *Daphnia magna* LC_{50} values for each).

Minor constituents of a mixture should not be ignored; if chemicals act by the same mechanism, even those at very low concentrations contribute to effects (Deneer et al. 2005). A solution to the lack of data for some chemicals in a mixture is to use a quantitative structure–activity relationship (QSAR) model to estimate missing values (Section 26.1). If chemicals are additive, they have the same mechanism of action and therefore their toxicity should be predicted by the same QSAR. For example, QSARs using the octanol–water partitioning coefficient (Kow) as the independent variable have been used to estimate the acute lethality of PAHs to amphipods for a TU model of PAH mixtures in sediments

(Swartz et al. 1995). The approach has been extended to the estimation of sediment quality guidelines for PAH mixtures (DiToro and McGrath 2000).

If at least one chemical in a mixture of similar chemicals has well-characterized toxicity, the effects of the mixture can be estimated if their relative potencies (the t values in Equation 8.3 through Equation 8.5) can be estimated. The best-developed example is the dioxin-like chemicals. The toxicity of 2,3,7,8-TCDD is relatively well characterized for a number of vertebrates. Other halogenated dibenzodioxins and dibenzofurans, and some PCBs, have the same mechanism of action, which involves binding to the Ah receptor, but less potency. On the basis of data for toxicity, enzyme induction, and receptor binding, and with expert judgment, toxicity equivalency factors (TEFs) have been developed for the compounds that are known to be dioxin-like for mammals, birds, and fish (van den Berg et al. 1998). The TEF terminology is limited to the dioxin-like compounds, but equivalent factors termed relative potency factors (RPFs) may be developed for other chemical classes such as PAHs (Schwarz et al. 1995; Safe 1998; Risk Assessment Forum 2000), organophosphate pesticides (EPA 2003e), and chlorinated phenols (Kovacs et al. 1993). The general formula is

$$C_m = \Sigma RPF_i * C_i \tag{8.8}$$

where C_m is the mixture concentration expressed as concentration of the index chemical C_i. This normalized concentration can then be used in concentration–response models for the index chemical to estimate effects of the mixture.

The utility of concentration addition models is illustrated by a classic study of the acute lethality of 27 industrial chemicals to fathead minnows (Broderius and Kahl 1985). The hypothesis that these chemicals all acted by narcosis was affirmed by testing them individually and finding that the slopes of log-probit models fitted to test results of each chemical were approximately equal. That is, the concentration–response curves were parallel, and the relative potencies were indicated by the intercepts (Figure 8.3a). Hence, a single concentration–response function could be created by using RPFs to normalize all of the chemicals to the toxicity of 1-octanol (Figure 8.3b). The common narcotic mechanism of action was also suggested by common symptomology: after an initial excitatory phase, the fish darkened and became passive and if death occurred it was within hours. Finally, LC_{50} values from eight binary tests of these chemicals all fell along the concentration addition line of an isobologram (Figure 8.4).

A test of the use of literature values and assumed additivity to predict effluent toxicity is found in Bervoets et al. (1996). They found that the ΣTU for the four most toxic constituents based on 24 and 48 h LC_{50}s were predictive of 24 and 48 h LC_{50}s in tests of the effluent. However, the ΣTU based on chronic no-observed-effect concentrations (NOECs) overestimated the toxicity of the effluent as indicated by the NOEC. The latter result is not surprising given that the NOEC does not correspond to a level of effect and therefore does not provide a consistent TU for addition.

An approach similar to RPFs is used in life cycle assessments (Section 2.3). Ecotoxicity factors and other components of life cycle assessments are commonly normalized to some well-characterized chemical or agent (Guinee 2003). The primary purpose, however, is to create a common unit for comparison rather than to estimate the effects of a mixture.

8.1.2.2 Independent Action and Response Addition

Chemicals have independent action when they are toxicologically dissimilar and exposure to one chemical does not influence the effects of another. Hence, an organism will die of the

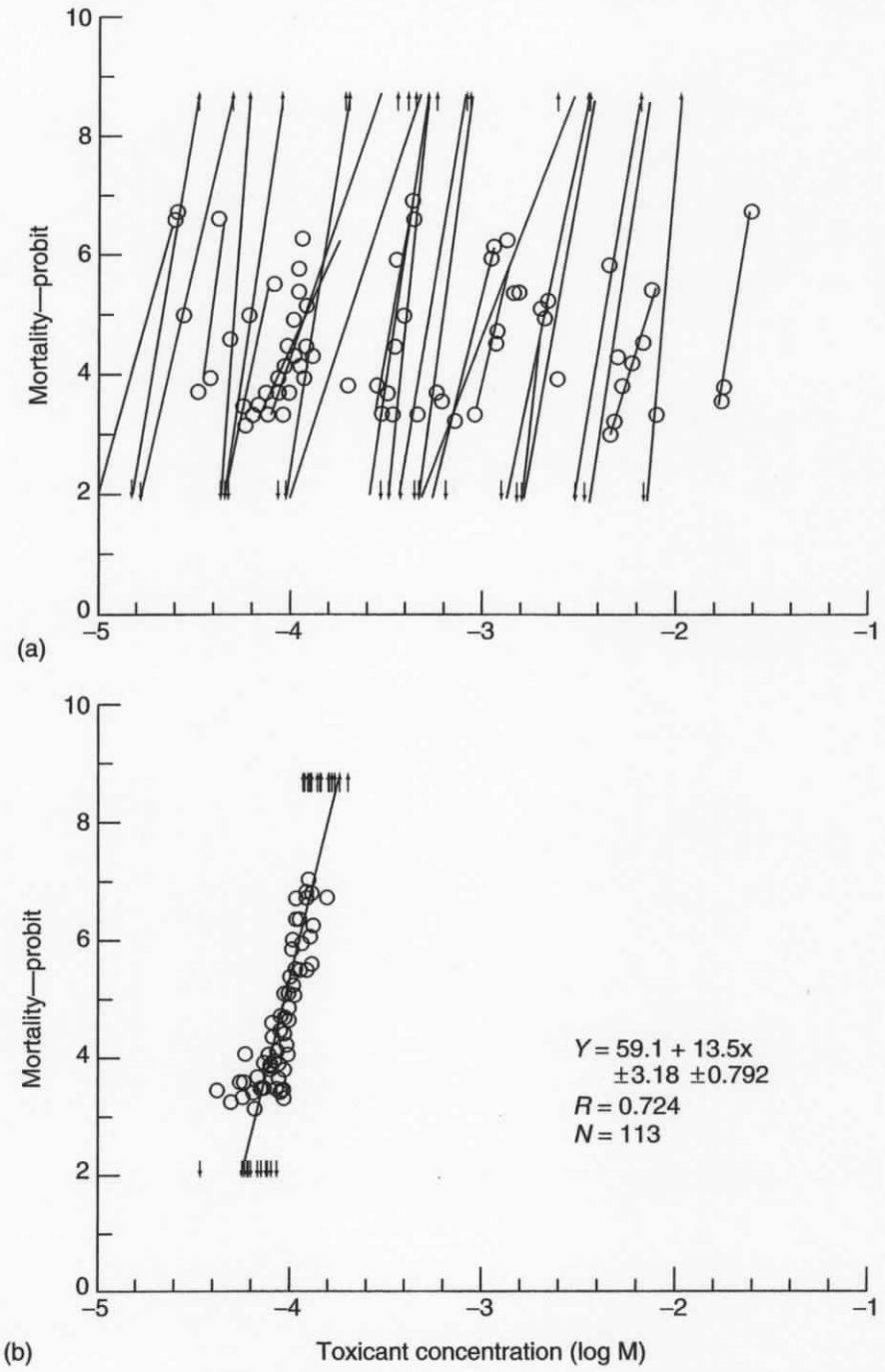

FIGURE 8.3 (a) Percent mortality in probits as a function of log chemical concentrations for 27 chemicals. Up and down arrows represent 100% and 0% mortality, respectively. (b) Plot of the data in part (a) normalized to the toxicity of 1-octanol. (From Broderius, S. and Kahl, M., *Aquat. Toxicol.*, 6, 307–322, 1985. With permission.)

effects of the chemical to which it is most sensitive relative to the concentration. Independent action is represented by response addition models, which sum the effects of each component. Response addition models are applied to mixtures of carcinogens in the United States and elsewhere, because it is assumed that at low concentrations each chemical in a mixture has a

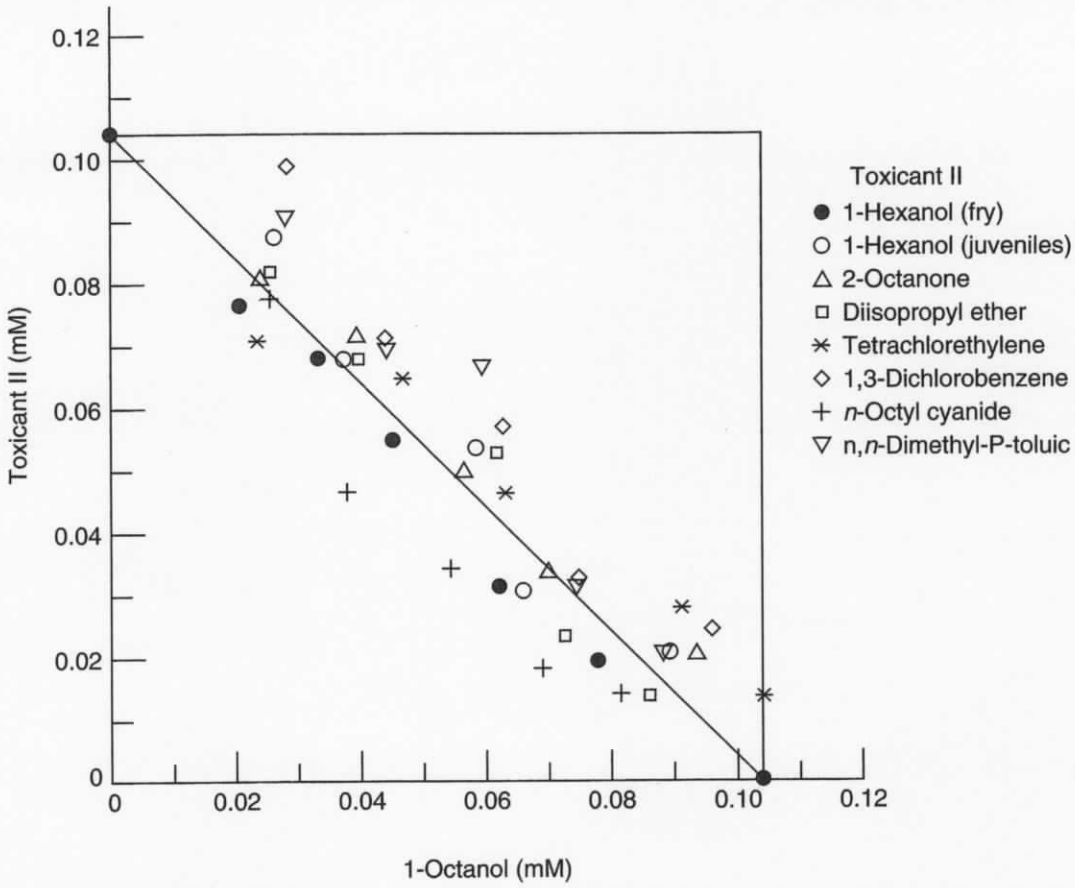

FIGURE 8.4 An isobologram of the tests results of mixtures of organic chemicals and octanol. The diagonal line indicates equal toxic potency on a molar basis. (From Broderius, S. and Kahl, M., *Aquat. Toxicol.*, 6, 307, 1985. With permission.)

small probability of causing a particular tumor type in a particular organ; other chemicals are unlikely to contribute but may cause another type of tumor in another location. A response addition model for two chemicals is

$$P(C_1 + C_2) = P(C_1) + P(C_2) - P(C_1) * P(C_2) \tag{8.9}$$

where $P(C_i)$ is the probability (or, equivalently, the rate or incidence) of a quantal response given concentration C_i. This is simply the probability of an effect that has two independent potential causes. The subtracted term accounts for the practical impossibility of two independent causes of the same effect (if one chemical kills you, the other cannot). The general formula for response addition is

$$P_m = 1 - (1 - P_1) * (1 - P_2) \cdots (1 - P_n) \tag{8.10}$$

To determine whether test results for a pair of chemicals are consistent with independent action, an appropriate exposure–response function is fitted to concentration–response data for each chemical, and the goodness of fit of mixture test data is determined for

$$P(C_1 + C_2) = F_1(C_1) + F_2(C_2) - F_1(C_1) * F_2(C_2) \tag{8.11}$$

where F_x is the fitted functions. Note that a different function may be fitted to each chemical (e.g., probit, logit, Weibull). If the response levels are small, the formula may be simplified to

$$P(C_1 + C_2) = F_1(C_1) + F_2(C_2) \tag{8.12}$$

If, as is usually the case in ecological risk assessment, we are interested in the frequency of an effect in a population rather than the probability of effects on an individual, the formula is the same (P becomes proportion rather than probability), but correlations of sensitivities to the chemicals must be considered. If the probabilities are perfectly negatively correlated (i.e., organisms that are most tolerant of one chemical are least tolerant of the other so that $r = -1$, Figure 8.2), the probability for the mixture is the sum of the independent probabilities, up to a maximum of 1. The other extreme of response addition is perfect correlation of sensitivity to the chemicals ($r = 1$), so organisms respond to the most toxic chemical and the other chemicals do not contribute to the frequency of response. If there is no correlation ($r = 0$), Equation 8.10 is unchanged.

Response addition models are seldom applied to populations because of the difficulty of determining the correlations of sensitivities and the inability to calculate higher-order associations from pairwise associations (Risk Assessment Forum 2000). One might be tempted to use a default assumption of $r = 0$, but there are no good biological reasons to expect that. Hence, if independent action is assumed or demonstrated, perfect positive and negative correlations could be used as bounding assumptions.

8.1.2.3 Interactive Action

Chemicals may interact in a variety of ways to increase or decrease toxic effects. Most of the described cases are antagonistic. For example, one chemical may inhibit the uptake of another, promote the metabolism of another by promoting enzyme expression, or compete for a binding site. However, synergism may occur. For example, injury caused by one chemical may decrease the rate of metabolism or excretion of another, thereby increasing its effective dose. No standard methods are available for modeling these interactive combined actions, and available test data indicating interactions are for pairwise tests that have no clear relevance to typical multichemical exposures (Risk Assessment Forum 2000; Hertzberg and MacDonald 2002). If the mixture cannot be tested, the best solution is to employ toxicokinetic and toxicodynamic models that mechanistically represent the interactions (Section 22.9) (Haddad and Krishnan 1998; Krishnan et al. 2002; Liao et al. 2002). However, suitable models and interaction data are seldom available for nonhuman species and chemicals of concern. Information from the literature may be used to adjust effects estimates ad hoc and generate a qualitative index of combined toxicity such as the mixture toxicity index (Konemann 1981a) or the interaction-based HI (Risk Assessment Forum 2000; Hertzberg and MacDonald 2002). However, if interactions are believed to be important, it is generally better to perform tests of the mixture (Chapter 24).

8.1.2.4 Multiple Chemicals and Multiple Species

These discussions of combined toxic effects have been framed in terms of conventional exposure–response relationships for individual organisms of a species. However, species sensitivity distributions (SSDs), the equivalent multispecies functions, may be treated in the same way (Traas et al. 2002; deZwart and Posthuma 2005) (Chapter 26). That is, if the SSDs for a set of chemicals are parallel or for some other reason are thought to have a common mechanism of action, concentration addition models may be employed. For example, a TU

approach might be used as in Equation 8.6 and Equation 8.7, where the common effect might be the concentration causing some effect (e.g., LC_{50}) in half of the species, the median hazardous concentration for 50% of species (HC_{50}). Similarly, if the chemicals in a mixture are believed to act independently, the response addition models could be applied (Equation 8.10 and Equation 8.11). In place of the probability of effect on an organism [$P(C_i)$], one would use the potentially affected fraction (PAF_i).

8.1.3 Integration of Complex Chemical Mixtures

In some cases, more than one method is available for estimating the toxicity or other property of a mixture. The US EPA mixtures guidelines say: "all possible assessment paths should be performed" (Risk Assessment Forum 2000). If multiple estimates are obtained, the results of one method may be chosen or the evidence from all methods may be weighed. One method may be chosen when it is clearly superior to the others, and the uncertainty in its results is low. For example, testing the mixture of concern is generally preferable to using models based on assumed MoAs of the component chemicals. This generalization may be applied if the tests are of high quality and use species, life stages, and responses that are relevant to the assessment endpoint. However, the quality of tests may be marginal, tests of the components may be more relevant, mechanisms of action may be known, or for various other reasons, testing may not be superior or have low uncertainty. In such cases, judgment must be used to weigh the results in terms of their similarity and relative quality to derive a weight-of-evidence estimate of the response to the mixture. Even when only one method is used to generate the risk estimate, the results of other methods may be used to determine the assessor's knowledge of the nature of the toxic effects and confidence in the estimates.

One particular integration problem is the question of how to estimate the effects of combinations of heterogeneous mixtures of chemicals. Typically, when using a component-based approach, one begins by categorizing chemicals into classes with the same mechanism (or at least the same mode) of action and applying a concentration addition model to each class. The problem is that the combined effects of the classes are unknown. The conceptually conservative approach is to simply report the class results and the fact that their combined effects are unknown. If the effects of each group are sufficiently heterogeneous, the results may be acceptable. For example, if one class of chemicals causes tumors and another causes reduced fecundity at the estimated exposure levels, it is sufficient to report the estimated levels of each effect. However, in most cases, the decision maker will require an estimate of the combined effect. One alternative is to use concentration addition across all classes. Although it is unlikely that chemicals with different MoAs will be truly additive, concentration addition is the default assumption of some regulatory programs such as the risk assessment guidance for Superfund (Office of Emergency and Remedial Response 1991), and it provides good estimates of effects for many complex mixtures (Alabaster and Lloyd 1982). Another alternative is to use a response addition model to combine the effects of the chemical classes. This approach has been recommended in the Netherlands (Traas et al. 2002; deZwart and Posthuma 2005) and the EPA (2003b,e). In a study of toxicity to algae of 10 chemicals commonly co-occurring in sediments, use of a concentration addition model for the non-specifically acting components followed by response addition to include the others resulted in a better model than concentration addition for all components (Altenburger et al. 2004). This approach is based on the assumption that chemical classes with different MoAs will act independently. However, for nonspecific effects such as death or decrements in growth, toxic effects are unlikely to be fully independent. It seems advisable to apply both methods and report those results, along with the results for the individual classes. That is, to report (1) the concentration addition results for heavy metals, baseline narcotics, ionizing narcotics,

cholinesterase inhibitors, etc.; (2) the results for the whole mixture from using concentration addition for all chemicals; and (3) results using concentration addition within classes and response addition to combine the classes.

These approaches require toxicity data for all components of the mixture. If this requirement is not met, one may simply identify a well-characterized representative chemical from each class and assume that the entire class is composed of that chemical (Suter et al. 1984; MADEP 2002).

8.2 MULTIPLE AND DIVERSE AGENTS

While chemical mixtures are a common risk assessment issue, they are relatively simple compared to the typical real-world situation of an ecosystem or region exposed to various anthropogenic agents associated with a set of activities. The activities may be coincidentally associated, as in the sewage treatment plant added to a watershed that already contains other sewage treatment plants, farms, industries, etc. In other cases, they are parts of a program that must be assessed (Table 8.2).

The logic for assessing the cumulative risks of multiple activities generating multiple agents is presented in Figure 8.5. It can be applied to a program or to a new activity that may significantly interact with existing activities. It is based on a series of logical steps that consider first the spatial and temporal relationship of the activities and agents and then the mechanistic relationships among the interacting agents (Suter 1999a). The process diagramed represents cumulative risk characterization. It presupposes that the activities have been characterized, endpoints selected, and the location characterized in the problem formulation, and that the analysis phase has defined the transport, fate, exposure, and exposure–response relationships for each agent. The characterization must be performed for each assessment endpoint (receptor), because the extent of overlap and the mechanisms of action will differ.

The logic of this process is based on attempting to combine the risks of each activity with the minimum of additional analysis. That is, first determine whether the multiple agents are effectively the same. If they are not, are the risks independent? If they are not independent, can they be added or otherwise combined? If effects are not additive, can exposures be added and the risks recalculated? Finally, if exposures are not additive, one must estimate the

TABLE 8.2
Examples of Programs that Involve Multiple Activities and Agents

Program	Example Activities	Example Agents
Pulp mill	Cutting native forest; planting plantation trees; cutting plantation trees; road construction; aqueous emissions; atmospheric emissions	Saws, construction equipment; herbicides, alien monoculture; silt; channel modification; polyphenolic chemicals; nitrogen oxides
Military training	Infantry maneuvers; tank maneuvers; artillery practice	Smokes and obscurants; noise; live ammunition firing; digging; tracked vehicle traffic
Cattle farming	Pasturage; hay production; feed production; feed lots	Trampling; nonnative vegetation; silt, pesticides, nutrients in runoff; manure
Coal-fired electrical generation	Coal mining; coal processing; coal combustion; condenser cooling; ash disposal	Acid drainage; fines in runoff; sulfur and nitrogen oxides; entrainment of aquatic organisms; chlorinated blowdown; metal leachate

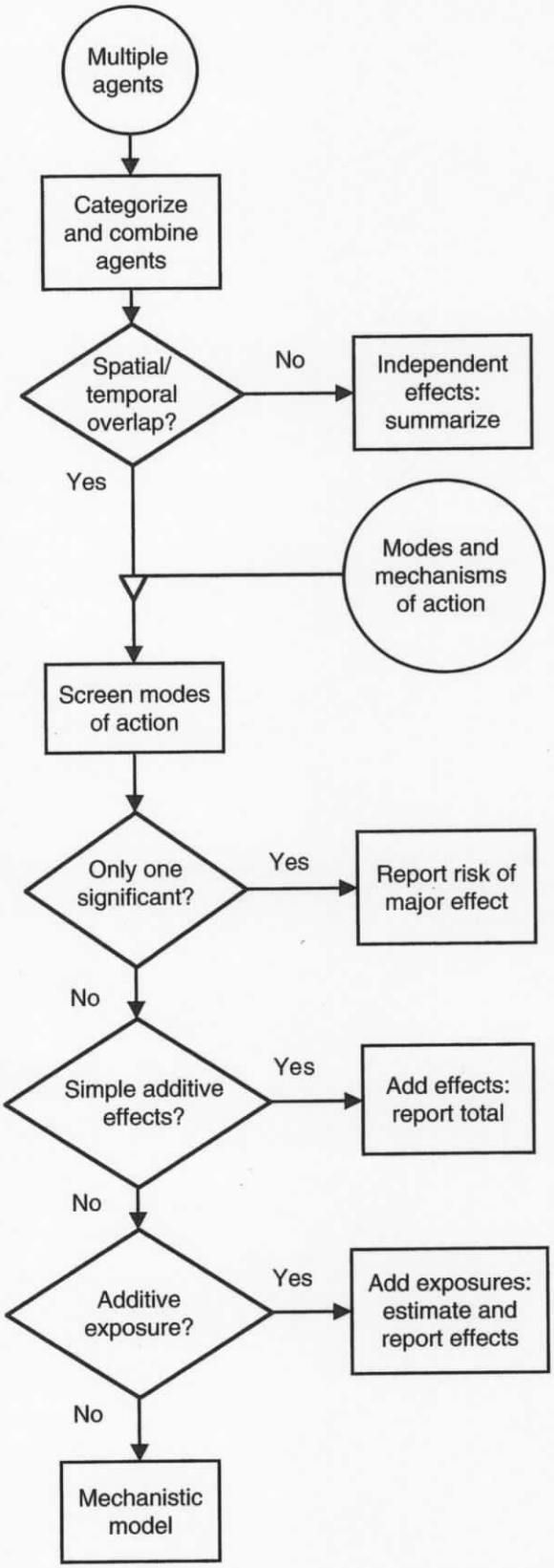

FIGURE 8.5 A diagram of a logical procedure for estimating the combined risks of multiple activities. (From Suter, G.W. II, *Hum. Ecol. Risk Assess.*, 5, 375, 1999. With permission.)

combined risks using a mechanistic model. This strategy depends on the spatial and temporal relationships among the activities as well as the MoAs of the agents.

This process was developed for cases of multiple activities. However, it can also be applied to a single activity that generates multiple agents by skipping the first two steps. There would be no need to combine agents or determine spatial and temporal overlap for a single activity.

8.2.1 Categorize and Combine Agents

When characterizing the combined risks of multiple activities, it will often be the case that some of the activities have hazardous agents in common. For example, dairy and beef farms within a watershed will all have cattle that generate manure, trample streambeds, etc. Similarly, tank maneuvers, training in refueling or rearming tanks, and mechanized artillery training all involve driving tracked vehicles. These sets of agents may be combined with respect to crushing organisms and disturbing the soil.

8.2.2 Determine Spatial and Temporal Overlap

If there is no spatial and temporal overlap of the activities or their consequences, the risks of the individual activities need not be integrated. The spatial and temporal extent of the program should have been defined in the programmatic problem formulation. However, this estimate of the temporal and spatial extent is completed for planning purposes and may have been shown to be inaccurate by subsequent assessment activities. The spatial and temporal extent of each activity should have been defined in the problem formulation in terms of a core area (the area in which the activity occurs) and influence area (the additional area to which the influences of the activity extend). At this step in the process, the core and influence areas for all activities must be overlain geographically and on a time line to determine whether the activities are spatially or temporally independent. Independence may be determined by asking the following questions:

- Are the activities performed in the same or overlapping areas?
- If not, do the agents generated directly or indirectly by an activity extend to a significant extent into areas where other activities occur?
- If not, is there significant interaction between the endpoint populations or ecosystems in the areas?

If the answer to these questions is no, the activities are spatially independent. If they are not spatially independent, temporal independence must be considered by asking the following questions:

- Are the activities performed at the same time?
- If not, do subsequent activities occur before the system has effectively recovered from prior activities?

If the answer to these questions is no, the activities are temporally independent. If the agents or activities are either spatially or temporally independent, the programmatic risk characterization consists of a summary of the risks from all of the component activities.

8.2.3 Define Effects and Mode of Action

If risks to a particular endpoint are a product of effects from multiple exposures, those effects must be combined in a common model that estimates the combined risks. This requires analysis and understanding of the MoAs and potentially the mechanisms of action underlying

the effects and their relationship to the assessment endpoint (Chapter 7). Note that the distinction between endpoint effects and MoA is a matter of perspective. For example, if the effect is reduced abundance, the MoA might be mortality, emigration, or reduced reproduction. At a lower level of organization, the effect might be acutely lethal toxicity and the MoA might be narcosis, uncoupling of oxidative phosphorylation, asphyxiation, cholinesterase inhibition, etc.

The recommended strategy in these cases is to define the effects at as high and appropriate a level as possible given the definition of the endpoint and the available evidence (i.e., exposure–response models). For example, if an endpoint is survival of individuals of an endangered species, mortality is the highest-level relevant effect. One could simply add the number of individuals killed by each MoA, or estimate the number killed assuming independent action. In this way, risks from different MoAs can be estimated by a single model by treating them as one effect.

Even when multiple effects are involved, aggregation of MoAs to generate higher-level effects is an important first step. For example, if the endpoint is population abundance of an animal species, aggregation of the various mechanisms of action for lethality (the MoA) is appropriate. If other MoAs such as reduced fecundity or increased emigration were potentially significant, the resulting mortality would then be used along with those effects in a demographic model to estimate the population-level endpoint. See, for example, the combination of toxic effects on survival and fecundity of fish with the effects of harvesting on survival in Barnthouse et al. (1990).

Once the MoAs have been properly aggregated into common effects, the appropriate combined risk model may be identified and employed. For example, if tank maneuvers kill some tortoises, cause others to emigrate, and reduce fecundity of the remainder, a simple projection matrix or other demographic model could be used to estimate population reductions.

8.2.4 Screen Effects

If only one effect is significant to the decision, it is not necessary to consider the combined effects. This simplification may occur for two reasons. First, if the magnitude of one effect is much greater than the others, the others may be ignored. In general, if the increment in risk obtained by including the minor effects is less than the uncertainty concerning the magnitude of the effect, the minor effects are negligible and can be screened out. For example, in the case of tank maneuvers and terrestrial herbivores, the effects of loss of food may be negligible relative to the direct effects of crushing for a rare species like the desert tortoise, which is not limited by food. However, for a species that is limited by food and is able to avoid being crushed, the direct effects may be negligible.

Second, if the magnitude of one or more individual effects is clearly so large as to be unacceptable, the risks from the combined effects need not be estimated. For example, if planned tank maneuvers are expected to kill half of the population of desert tortoises, there is no need to consider the additional risks from loss of food.

The decision to dismiss insignificant effects must be made on the basis of screening assessments. These are assessments that are performed using simple assumptions or models to determine whether it is appropriate to continue a line of assessment (Chapter 31). Simple screening methods and models are not available for most nonchemical hazards, but they will not be needed in most cases because the risk models are not highly complex and the number of effects to be screened is not large in most cases. Therefore, the screening assessments may be performed by characterizing the risks for each effect independently and then comparing the results to criteria for absolute or relative significance. These criteria must include the potential significance of indirect effects as well as direct effects.

8.2.5 Simple Additive Effects

The effects of activities on an endpoint are additive if they have the same mechanism of action and if they are independent. To return to the tortoise and tanks example, driving tracked vehicles, driving wheeled vehicles, and firing live artillery rounds all have acute lethality as an effect. Those effects may be simply added (i.e., the number killed is the sum of the numbers killed by each agent). If the probabilities are not small, it will be necessary to consider that you cannot cause the same effect twice. By analogy to Equation 8.9, for two activities a and b, the number of tortoises killed by both is

$$N_k = p_a N + p_b N - (p_a N * p_b N) \tag{8.13}$$

where N_k is the number killed; p_a, the probability of being killed by activity a; N, the number of individuals in the exposed population; and p_b, the probability of being killed by activity b. The probabilities themselves would be functions of properties of the activities such as the number of tanks, the distance traveled, the season, and the time of day. The extension to more than two chemicals and to nonquantile responses should be obvious.

8.2.6 Additive Exposures

Although effects are not additive, exposures may still be additive, as in the concentration addition models discussed above (Section 8.1.2). If the mechanism of action is the same so that the same or parallel exposure–response models are applicable, exposure levels or normalized exposure levels may be added.

Habitat losses can be considered to be exposure-additive. For example, if several activities eliminated habitat or made it unavailable, the losses may be added and the effects on a population or community may be estimated. A more complex case would be activities that each reduced the quality of habitat areas. By analogy with the RPFs for chemicals, one could devise factors that were proportional to the loss in habitat quality. The model for total reduction in habitat would be

$$UHE = \Sigma RHQ_i * a_i \tag{8.13}$$

where UHE stands for undisturbed habitat equivalents; RHQ, for relative habitat quality with activity i; and a_i, for the area affected by activity i.

For example, if tank training is being expanded into a new 1000 ha area involving both construction of paved roads on 1 ha and designation of 600 ha for off-road tank operation, and if studies in the old training areas found that tank traffic reduced tortoise abundance by half, then the tortoise habitat equivalents will be

$$UHE = 0(1\text{ ha}) + 0.5(600\text{ ha}) + 1(399\text{ ha})$$
$$UHE = 699\text{ ha}$$

If undisturbed habitat in the new area supports 0.7 tortoises/ha, the 1000 ha area after conversion to tank training is estimated to support $0.7(699) = 489$ tortoises, a 30% reduction. The information for deriving RHQ values could be derived ad hoc, as in this example, or from the literature on habitat quality for the species of concern, including the Habitat Evaluation Procedure reports (FWS 1980).

Another common mechanism for which exposure addition might be assumed is agents causing reductions in energy resources. That is, if less food is available for heterotrophs or

light for plants, if a contaminant causes energy costs for metabolism or repair, if animals must travel further between water, food, or other resources, or if plants must replace tissues lost from herbivory or harvesting, such energy costs might be summed. This example is hypothetical and would require some creativity because there is no common exposure unit like habitat area or chemical concentration. While common units are not necessary for exposure addition models, the functions are likely to be heterogeneous and would therefore need to be converted so that they are approximately parallel. Simulations using bioenergetic models might be more appropriate.

8.2.7 Mechanistic Models of Combined Effects

If the mechanisms by which multiple agents act are heterogeneous and particularly if they are interactive, a model must be selected that incorporates them all. This requires a consideration of the mechanisms underlying the effects, and selection of an appropriate mechanistic level for the model. In general, selection of higher-level mechanisms results in simpler models that are more easily implemented. However, interactions among the exposure and response processes often require a deeper level of mechanistic description. For example, combined risks to fish populations from contaminants and harvesting can be modeled using relatively simple demographic models by assuming that the contaminants reduce only survivorship, and fecundity and harvesting affect only survivorship (Chapter 27). However, if one wishes to estimate the influence of compensatory processes such as reduced mutual interference among survivors and increased food supply, one must incorporate mechanisms that affect individual fish (Rose et al. 1993 and Chapter 27). In an ecosystem-level example, the combined effects on phytoplankton and zooplankton production of exposure to a mixture of metals (As, Cd, Cu) and nutrient (N, P) enrichment were estimated (Moore and Bartell 2000). Concentration addition was used to estimate direct reductions in plankton production due to the metals; then the CASM model was used to estimate the combined effects of direct metal toxicity, direct nutrient promotion of primary production, and food-web propagation of effects on plankton taxa. In many cases, the extent to which these more deeply mechanistic models can be applied is limited by both scientific knowledge and by lack of needed site-specific information. Nevertheless, simple threshold models or empirical models that may have provided the best estimate of risks from an individual agent are likely to be inappropriate for the estimation of combined risks.

8.2.8 Integration of Complex Sets of Agents and Activities

For cases involving multiple activities generating multiple agents and exposing multiple receptors, integration becomes more complicated because the decision structure is more complicated (Harwell and Gentile 2000). In order to determine risks to an endpoint like wood storks in the everglades, one must consider the effects of agriculture, urban sprawl, tourism, and climate change. However, these activities are not under the control of one agency and they are not legally or politically controllable to the same degree. Hence, risk characterizations must be tailored to particular decision makers and stakeholders by treating different activities and agents either as cofactors or as subject to control, and by adjusting the spatial and temporal scales to match constraints on the decision maker. Such tailoring not only makes the risk assessment more useful but can also reduce the uncertainty in the results by limiting the sources of variance and uncertainty to those that are relevant.