Methods for Assessing the Effects of Mixtures of Chemicals
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Methods for Assessing the Effects on Non-human Biota of Mixtures of Chemicals as Applied to Specific Taxonomic Representatives of Individual or Groups of Species

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ABSTRACT

The testing of the toxicity of individual and mixtures of chemicals to non-human biota, especially aquatic organisms, is discussed. The relationships of a variety of taxonomic groups of species are used to choose representative surrogate species for preliminary screening of chemicals and chemical mixtures. The ranges of toxicity values for many chemicals and for a range of aquatic and terrestrial species are summarized. The practical aspects of synergism occurring with chemical mixtures are examined and needs for further testing for making firm conclusions are outlined. Test organisms used for determining toxicity of single and multiple chemicals need not be different. Recommendations for species to use for chronic toxicity tests are provided, which include all of the stages of the life-cycle of the organism chosen.

Factors such as different modes of action of chemicals, abiotic conditions of the environment, and effects on ecosystems as well as on individuals are considered. The importance of the variables in test methods in laboratory and field tests is discussed. Conclusions are given.

1 INTRODUCTION

The procedure for testing the toxicity of mixtures of chemicals to specific taxa resolves itself into five major questions:

(1) Are taxonomic groups useful as indicators of differences in toxicity of individual chemicals?

(2) Assuming the magnitude of a complete testing programme on even a small proportion of the species on earth, what is the practical answer to selection of taxa for testing individual chemicals?
(3) Are mixtures more or less toxic than the individual chemical components (i.e. synergistic, additive, antagonistic)?
(4) Are more or different species of organisms necessary for testing toxicity of mixtures compared with the individual chemical components?
(5) What other aspects must be considered in assessing the toxicity of chemicals when using the data for comparative purposes?

These questions are addressed in the following sections.

2 RELATIONSHIP OF REPRESENTATIVE TAXONOMIC GROUPS OR SPECIES OF ORGANISMS TO TOXICITY OF VARIOUS KINDS OF INDIVIDUAL OR STRUCTURALLY RELATED CHEMICALS

Taxonomic relationships of organisms are based on morphological, reproductive, biochemical, and other characteristics; some are of major importance, some minor. The greater the taxonomic difference (phylogenic as contrasted with generic or specific relationships), the greater the differences in these characteristics. Large differences in taxonomic characteristics of organisms are reflected in their different reactions to various chemicals, their mode of toxicological action, speed of penetration to toxic sites, reaction at the toxic site, speed of metabolism and excretion, habitat, and mode or probability of exposure to chemicals (i.e. terrestrial versus aquatic).

The toxicity of chemicals is defined by their ability to interfere with any of the specific necessary life functions of the organism. Based on this relationship differences in toxicity should increase with greater taxonomic differences (species < genus < family < order, etc.).

Species of organisms are included under many different large taxonomic groups (kingdoms and phyla being the largest) containing millions of species of animals and plants (see Table 1).

It is obvious that plants and animals, terrestrial and aquatic animals, birds and mammals are different in their toxic response to chemicals. How different is one species of fish from another, one species of insect from another, etc.? It is not physically possible to test all species such as rare and endangered species, species not manageable for determining comparative toxicity with benchmark species, or species not available. Nor can all toxicity testing facilities be exhausted on a few chemicals at the expense of testing all the rest. Therefore, it is necessary to attempt to select surrogate species to represent all or a greater majority of species of organisms.

We need to know the range of toxic sensitivity between closely and distantly related species to given chemicals that are structurally closely or distantly related.

Table 1 Summary of plant and animal species numbers

Taxonomic groupings	Approximate numbers of species
Plant kingdom	
Algae, macrophytes (aquatic and terrestrial)	350 000
Animal kingdom	1 200 000
Vertebrates	37 790
Fishes	17 000
Birds	8 600
Reptiles	6 000
Mammals	4 300
Amphibians	1 500
Invertebrates	1 150 000

The magnitude of the species selection problem facing ecotoxicologists is evident from this generalized summary of known numbers of plant and animal species for the world.

Species of animals which have been most widely tested in the United States include five mammals, five birds, seven fish, two terrestrial insects, nine aquatic arthropods and one mollusc.

Kenaga (1978) tabulated the maximum–minimum range in acute toxicity values for 75 pesticides for eight of the above organisms (see Table 2). Tests for aquatic organisms showed the greatest range. This was because the dosage of toxicant in the organism is dependent on a combination of the water concentration and the bioconcentration factor of the chemical from water to the organisms. Thus the dosages of chemicals received from identical concentrations in water are much higher for those chemicals having high bioconcentration factors. The amount of toxicant obtained by the terrestrial organisms was based on what they fed on or were exposed to and was not usually more than a bioconcentration factor of ten. Under the conditions of these tests, honeybees had the smallest range (2837-fold) and *Daphnia* the largest range (150 100 000-fold) of toxicant values for the 75 chemicals. Daphnids were the most sensitive, generally (see Table 2).

In a study of the toxicity of chemicals to the eight organisms, it was shown that from a given value on a given species the following correlations were highest. Also, new values were predictable by use of regression equations from known values (Kenaga, 1978):

—Rainbow trout LC_{50} from salt-water fish LC_{50}.
—Rat LD_{50} from mallard LD_{50}.
—Rat LD_{50} from bobwhite LC_{50}.
—Shrimp LC_{50} from salt-water fish LC_{50}.

Table 2 Maximum variation in acute toxicity between 75 pesticides

Organism Test method	Organism exposed by[a]	Difference between maximum and minimum value
Honeybee μg/bee	Forced cuticle contact dosage	2827
Rat LD_{50}, mg/kg	Forced oral dosage	>10000
Mallard LD_{50}, mg/kg	Forced oral dosage	>16700
Bobwhite LC_{50}, ppm	Optional oral (food concentration)	16700
Rainbow trout LC_{50}, ppm	Forced contact[b] (water concentration)	>117000
Salt-water fishes LC_{50}, ppm	Forced contact (water concentration)	>500000
Daphnia magna LC_{50}, ppm	Forced contact (water concentration)	150500000
Shrimp LC_{50}, ppm	Forced contact (water concentration)	33000

[a] Measured dosage or intake.

[b] Exposure based on water concentration of chemical and bioconcentration factor.
From Kenaga (1978).

Predictions of toxicity were best if the chemicals used were similar in structure (e.g. chlorinated hydrocarbon insecticides, or phosphorus-containing insecticides, etc.).

Based on the above correlations it appears that the greatest spectrum of toxicity information can be gathered from a few species of the greatest phylogenic differences, and poorest toxicity correlation with each other. Three animal organisms, the rat, a fish, and an aquatic invertebrate such as a daphnid, represent the greatest diversity of sensitivity to chemicals and their toxic reactions.

In other comparisons drawn by Kenaga (1978), correlation coefficients between two species of fish or two species of birds, etc., were the highest indicating a close relationship of toxicity to similar species, genera or families, than to similar orders or phyla of organisms.

Birge and Black (1982) tested a number of aquatic vertebrate organisms for toxicity (LC_{50}) using ten metals and ten organic chemicals. The sensitivity of species of organisms to a given chemical from most sensitive to most tolerant ranged from about ten-fold to 4600-fold (see Table 3).

An example of range of toxicity of one chemical to terrestrial insects is shown by Kenaga *et al.* (1965) who studied the effects of the insecticide chlorpyrifos on

Table 3 Difference in sensitivity of aquatic vertebrates to various metals and organic chemicals

Compound	Number of species tested	LC_{50} (mg/l)		Variation in sensitivity
		Most sensitive species	Most tolerant species	
Silver	8	*Rana pipiens* (0.007)	*Ambystoma opacum* (0.25)	35
Aluminium	7	*Gastrophryne carolinensis* (0.06)	*Bufo fowleri* (277)	4600
Arsenic	8	*Gastrophryne carolinensis* (0.04)	*Bufo fowleri* (78.7)	2000
Cadmium	8	*Gastrophryne carolinensis* (0.04)	*Bufo fowleri* (2.40)	60
Chromium	8	*Gastrophryne carolinensis* (0.03)	*Ambystoma opacum* (2.31)	77
Mercury	8	*Gastrophryne carolinensis* (0.001)	Largemouth bass (0.14)	140
Lanthanum	7	Rainbow trout (0.02)	Goldfish (89.9)	4500
Nickel	8	Rainbow trout (0.05)	*Bufo fowleri* (11.0)	220
Lead	8	*Gastrophryne carolinensis* (0.03)	*Bufo fowleri* (2.16)	72
Zinc	8	*Gastrophryne carolinensis* (0.02)	*Bufo fowleri* (87.3)	4400
Atrazine	6	Catfish (0.22)	*Bufo americanus* (>48)	>130
Carbon tetrachloride	8	*Rana catesbeiana* (0.90)	*Xenoplus laevis* (9.46)	10
Chloroform	8	*Hyla crucifer* (0.27)	*Xenoplus laevis* (>70)	>250
Methylene chloride	9	Rainbow trout (13.16)	*Rana pipiens* (>50)	>4
Phenol	12	*Rana pipiens* (0.04)	Fathead minnow (25.0)	625
Polychlorinated biphenyls				
Aroclor 1254	7	Rainbow trout (0.0003)	*Bufo fowleri* (0.0037)	13
Aroclor 1242	7	Rainbow trout (0.001)	*Bufo fowleri* (0.012)	12
Aroclor 1016	7	Rainbow trout (0.001)	*Bufo fowleri* (0.028)	28
Capacitor 21	7	Rainbow trout (0.002)	*Bufo fowleri* (0.028)	14

From Birge and Black (1982).

20 species of insects by use of a measured topical application to their cuticles. The range in toxicity for these species was a 36-fold difference in LD_{95} values in terms of mg/kg body weight.

What we conclude from a summary of the preceding data (see Table 4) is that the greater the difference between chemical structure, and between the systematic taxonomy of the species tested, the greater the difference in range of toxicity. These differences can be from one to seven orders of magnitude. Terrestrial organisms show a smaller range of toxicity than aquatic organisms.

'A review of data bases for chemicals for which a large number of (aquatic animal) species have been tested, shows clearly that while certain species may frequently be very sensitive or very resistant on the average, there are also frequent exceptions for these species. This is easily seen in Table 5 which is a compilation of data obtained from Ambient Water Quality Criteria documents (USEPA, 1978). *Daphnia magna* is often among the most sensitive of the species tested. However, it is the most resistant species tested for endrin and is among the more resistant for others. Even for closely related species (e.g. such as among trout species or among cladoceran species), the relative sensitivity reverses from one chemical to another. For example, in the revised chromium document there is at least a 40-fold difference in sensitivity among the cladoceran species tested; *D. magna* being very sensitive. To pentachlorophenol *D. magna* is one of the more resistant species and *Ceriodaphnia reticulata* is most sensitive. This is just the reverse of their sensitivity to hexavalent chromium.

'The point is that we have ample data to prove that a species can only represent itself consistently and not a group.

'However, species can be viewed as indicators of sensitivity in quite a different way. We can see that species sensitivity (LC_{50} or LD_{50}) distributes itself in a rather consistent way for most chemicals (Table 5). The distribution resembles a log normal one. We can then take the approach of sampling this distribution in order to predict the range about the mean. This is in reality our objective. Thus, each species we test is not representing any other species but is one estimate of general species sensitivity. With several such estimates, the overall range of sensitivity for all species can be determined. Our problem is to know how many species and what type of species to test to adequately represent the whole range.

'First of all, we should not confuse ecological habits or habitat with sensitivity. One must distinguish here between probability of exposure and sensitivity. There is no reason to expect a relationship between toxicity of man-made chemicals and ecological niche or trophic level. For naturally occurring chemicals such as heavy metals, one might expect a relationship to have developed because evolution of resistance can occur. But the ecological groupings should only enter into species sensitivity indirectly (e.g. if we want to estimate the range of sensitivity only for ecologically important species or species that potentially may be exposed). Thus, our objective is to sample the distribution of species sensitivity (LC_{50} or LD_{50}) in a statistically valid way. Consequently, the selection of species should not be based on such characteristics as trophic levels or benthic habits. But, as stated above, if a chemical is expected to occur only in sediment and not in water, then the population of species of concern would not include birds of prey, trees, or top swimming fish, because they would not be exposed.

'For sampling the sensitivity of the species of concern (as determined by LC_{50} or LD_{50} data) it would be toxicologically more valid to subdivide them into groups based on those characteristics that could logically be expected to change sensitivity

(e.g. type of circulatory system (open or closed), type of excretory system, suite of enzymes present in body, type of respiratory organ, etc.). Then a group of test species would be more likely to represent the full range of sensitivity.

'The difference between testing individual surrogate species and estimating sensitivity by using a cluster of species is subtle but critical. Examination of our data base tells us clearly that it is invalid to say one species can represent any other for determining sensitivity to a variety of chemicals. That is reason to cease pursuing this approach. It is also evident from our data base that the distribution of species sensitivity might be similar for chemicals. If it is, we can devise ways to estimate the range of sensitivity, but that is not using the surrogate species approach as it is commonly viewed.

'In checking the data in Table 5 it can easily be seen that for most chemicals the range of sensitivity of five commonly tested species [rainbow trout, fathead minnow, bluegill, *D. magna* and *Gammarus* spp. (includes 2 species)] is a large part of the range of all species tested. This suggests that tests on a small number of species may be all that is needed and this range (based on a cluster of species) can validly be a surrogate for the range of all species. Surrogate used in this way is an accurate use of the term that can be defended with objective data. Selection of test species as we have been doing is neither valid nor objective. *D. magna* by itself is not a surrogate for any other species.' (Mount, 1982).

Table 4 Summary of ranges of maximum differences between highest and lowest toxicity values for various numbers of species and chemicals

Number of chemicals and number of species tested	Maximum difference in ranges between lowest and highest toxicity values	Reference
Repeated tests on one test method, using one terrestrial organism and one chemical at a time for 52 chemicals	1.4- to 25-fold	Kenaga (1978)
One chemical at a time for 20 chemicals on 6–12 aquatic species	10- to 5000-fold	Birge and Black (1982)
One chemical on 20 species of terrestrial insects	36-fold	Kenaga *et al.* (1965)
Twenty-eight chemicals (closely related) on one species of fish	100- to 1000-fold	Kenaga (1978)
Seventy-five chemicals on 4–7 aquatic species of vertebrates and invertebrates	>32 000- to 150 000 000-fold	Kenaga (1978)
Fifty-one chemicals on 4 species of birds	10- to 100-fold	Kenaga (1978)
Seventy-five chemicals on 3 terrestrial mammal and bird species	10 000- to >16 667-fold	Kenaga (1978)
Seventy-five chemicals on 4 aquatic and 4 terrestrial species	2827- to 15 500 000-fold	Kenaga (1978)

Table 5 A ranking of aquatic species sensitivity[a] to chemicals according to acute toxicity data from USEPA criteria documents

Chemical	No. species tested	Sensitivity ranking					
		Daphnia	Rainbow trout	Fathead minnow	Bluegill	*Gammarus* (two species)	
Aldrin	21	–	2	14	3	21	
Arsenic	12	4	5	7	12	2	
Cadmium	27	3	1	14	20	5	
Chlordane	14	11	4	6	–	5	
Chromium(VI)	12	2	10	8	12	1	
Chromium(III)	18	7	–	3	11	2	
Copper	45	3	8	23	39	4	36
Cyanide	15	–	1	7	8	10	
DDT	42	9	22	34	18	5	11
Dieldrin	20	–	2	11	9	18	
Endosulphan	10	10	1	2	6	7	
Endrin	28	28	9	4	14	21	
Heptachlor	18	14	6	16	9	10	12
Lead	9	2	4	5	6	1	
Lindane	22	21	4	13	11	3	10
Mercury	14	1	7	5	–	2	
Nickel	23	1	16	7	11	15	
Selenium	13	2	6	3	12	–	–
Silver	10	1	7	3	8	2	
Toxaphene	29	16	14	15	9	23	27
Zinc	29	1	5	14	19	20	
No. of times species ranked:							
Most sensitive		4	4	0	0	2	
Among 5 most sensitive		10	10	7	1	10	
Among 5 least sensitive		5	2	2	6	4	

[a] The lower the number the greater the sensitivity.
From Mount (1982).

Another important aspect to consider is whether the chemical is toxic to various species at levels which might be expected to occur in the environment from the use of the chemical.

Recent studies of organic contaminants in water have shown their concentrations rarely exceed 1 mg/l except for spills and planned dosages. Sheldon and Hites (1978) identified 100 chemical contaminants in Delaware River water, but reported none at concentrations higher than 15 μg/l. In a study by the EPA on priority pollutants in waste water effluents, concentrations reported were generally below 10 μg/l, with a few in the 10–100 μg/l range. In view of these observations, it seems appropriate to examine further the relative toxicities of

chemicals to aquatic plants and animals for those compounds that are toxic to plants below 1 mg/l.

The same concentration (1 mg/l) seems to be an excellent guide for upper concentrations in soil and air as well (Kenaga, 1982a).

For a number of years acute toxicity screening tests against a variety of organisms have been carried out at The Dow Chemical Company. Included were a daphnid, four species of fish, an alga, and five species of aquatic vascular plants. In addition, a summary of toxicity data from five species of terrestrial plants (Kenaga, 1981), four species of bacteria, and four species of fungi (Kenaga and Chambers, 1980) was made available (see Table 6).

The number of chemicals tested against the four groups of aquatic organisms listed in Table 6 varied from 27 781 to 49 032 and thus represented a much larger number and more varied sample of chemical structures than is ordinarily available for comparison. At concentrations below 2 mg/l, a greater percentage of chemicals showed toxicity toward fish or daphnids than toward aquatic plants or alga, confirming the generally held view that aquatic animals are more sensitive to chemical toxicants than aquatic plants. None of the 22 781 compounds tested were toxic to aquatic plants below 0.1 mg/l. *Chlorella*, an alga, was generally less sensitive than aquatic vascular plants despite the fact that exposures were for seven days compared with one day for the other organisms. There were only three

Table 6 Comparison of the range of lethal concentrations of chemicals to various plant and animal organisms

Organism	No. of chemicals tested	% of chemicals causing 100 % mortality (ppm range)	
		0.01–0.09	0.1–0.99
Daphnia magna	33 909	0.6	2.4
Composite of 4 fish species[a]	35 305	0.14	1.3
Composite of 5 aquatic plant species[a]	27 781	0	0.1
Alga (*Chlorella*)[a]	49 082	0.006	0.02
Corn[b]	37 517	0.008	0.09
Wild oats[b]	114 897	0.0017	0.045
Cotton[b]	29 938	0.0067	0.31
Soybean[b]	13 199	0.022	0.79
Radish[b]	72 649	0	0.017
Composite of 5 terrestrial plant species[b]	131 596	0.006	0.17
Composite of 8 species of bacteria and fungi[c]	13 409	0	0.2

[a] Kenaga and Moolenaar (1979).

[b] Kenaga (1981), soil pre-emergence seed germination.

[c] Kenaga and Chambers (1980).

chemicals out of 49 082 tested on *Chlorella* that were toxic at concentrations below 0.2 mg/l (Kenaga and Moolenaar, 1979).

Tests on seeds of terrestrial plants and on microorganisms also reveal that their sensitivity for a great many chemicals is lower than that of fish and daphnids and that only relatively few chemicals were toxic below 1 mg/l (see Table 6).

The most useful predictive indicator organisms and methods for acute toxicity among the organisms tested would appear to be the rat LD_{50}, one species of fish LC_{50} and one species of aquatic arthropod LC_{50}.

Our knowledge for testing surrogate species for chronic toxicity is limited to much fewer species than for acute toxicity simply because there is a great deal more time, energy, and 'know-how' needed to raise species through their entire life-cycle as needed for chronic reproductive tests. For a discussion of choice of and extrapolation of representative species of aquatic organisms, see Kenaga (1979, 1982b) and Maki (1979). For chronic toxicity tests, organisms with short life-cycles, such as *Daphnia magna*, are desirable (Maki, 1979).

3 TESTING MIXTURES OF CHEMICALS FOR JOINT ACTION, PARTICULARLY SYNERGISM

The first question one would ask about the subject of testing mixtures of chemicals is, What is the difference in toxicity between testing organisms exposed to chemical mixtures and to single chemicals?

The obvious answer needed is whether the mixture is synergistic (potentiated), merely additive, or antagonistic in relationship to the toxicity of the individual chemicals.

Synergism is variously defined. In general it is agreed that the effect of the mixture is more than additive compared with the effect of the two (or more) ingredients separately. This can be taken as the biological effect (usually mortality) of concentrations of:

(1) Compound A separately *plus* compound B separately = less than the effect of the same concentrations of compounds A and B together.
(2) Either compound A *or* compound B = less than the effect of one-half the concentrations of each of compounds A and B together.

Unfortunately there is very little of this type of synergism data published in the open literature, since it requires that the test concentrations of each ingredient must be carefully chosen and that the test must be conducted for the specific purpose of determining synergism. The chances of demonstrating synergism are slim, as discussed later.

Mixture of chemicals can occur in many ways including:

(a) Technical chemicals containing impurities.
(b) Manufactured formulation mixtures for commercial use.

(c) Effluents from manufacturing locations producing various chemicals, or from municipal effluents handling many chemicals (i.e. those dumped into toilets or washed from the street into sewers).
(d) Effect of manufactured chemicals as mixed in the presence of natural chemicals in the environment (i.e. in the buffered system of water in rivers, lakes, etc.).

In the first two cases the chemical mixtures are usually tested as mixtures in the laboratory using rats and perhaps other organisms, as needed for commercial use. If synergism is shown under these conditions, unusually high toxicity is often detected.

One outstanding example of commercially valuable synergism is the use of pyrethrins and piperonyl butoxide or sesamex (3,4-methylenedioxyphenol derivatives) in about a 1 : 10 ratio for insect control. Ratios of 1 : 1 or less are not synergistic. These mixtures are not synergistic in rats and remain relatively low in toxicity.

A study of pyrethrin and pyrethroid insecticides by Soderlund (1983) showed a wide difference in the rapidity of hydrolysis of the *trans*-isomer compared to the *cis*-isomer in various insect species and rats.

A study by Fukuto (1983) of technical malathion, which constitutes a mixture of chemicals, showed that it contained at least 14 impurities which varied in content from a trace to 1.1 %. Four of the minor impurities added separately to purified malathion each decreased the acute oral LD_{50} to rats 1.8- to 10-fold. The most active impurities were aliphatic phosphates. Malathion held in storage for six months at 40°C resulted in notable *increased* impurities and toxicity to rats but not increased toxicity to the house fly. The ratio of malathion to impurities was about 100 : 1. A similar test with the phosphate insecticide acephate and its impurities resulted in *decreased* toxicity to the rat and no appreciable change in toxicity to the house fly.

Phosphate and carbamate insecticides are known to be acetylcholinesterase (AChE) inhibitors *in vitro*. They are also AChE inhibitors *in vivo* for insects and mammals to varying degrees. However, specific thiophosphate and phosphate insecticides such as malathion and acephate which contains carboxy esters as well as phosphate esters are much less toxic to mammals. Mammals are able to detoxify malathion by de-esterification due to the presence of carboxyesterases which de-esterify at a faster rate than conversion to maloxon, the phosphate AChE inhibitor derived from malathion, not present in insects. These basic differences in toxicity are related to the enzyme systems of these widely separated taxonomic groups of insects and mammals. Any chemical mixtures containing an ingredient which would inhibit decarboxylation would cause malathion to be much more toxic to mammals.

Some synergistic activity can be related to such a simple cause as the inducement of better penetration of the primary chemical to the site of toxic

action, either by increased rapidity of penetration (e.g. dimethyl sulphoxide), or by increased stability (antioxidant, etc.) of the secondary chemical.

Acute vapour toxicity tests on rats by McCollister *et al.* (1956) showed individual fumigant ingredients of fumigant mixtures containing ethylene dichloride, carbon tetrachloride, and ethylene dibromide; carbon tetrachloride and ethylene dibromide possess similar toxic effects and their joint action caused slight potentiation. However, from the practical viewpoint none of the mixtures gave a toxic response greater in magnitude than that exhibited by any one of the individual compounds, i.e. the effect of the mixtures was less than additive.

There are many examples of mixtures of chemicals which have been tested for synergism. Most studies show the toxicity of the basic chemical and of the mixture, but often omit the data on the other individual components of the mixture. True synergism of chemicals is a property which can be valuable for a specific commercial use (insecticide, herbicide, etc.); consequently agricultural and pharmaceutical chemical companies have conducted many tests with mixtures of chemicals and with formulations searching for synergism. There are few commercial pesticide treatments which have a patent protection based on synergism. This is a good indication of the scarcity of useful synergism. Consequently, testing for synergism is a discouraging scientific or commercial endeavour. This is not to conclude that even the limited synergism or toxicity which can occur is not important in the protection of species.

From the foregoing it can be seen that the ratio of the synergist to the synergized chemical is important. It appears that most synergism usually involves a narrow ratio of the ingredients to each other and a narrow range of minimum concentrations of the mixture.

Lloyd (this volume) reviewed the literature on special toxicity tests for chemical mixtures as related to physicochemical conditions of the test water. He discussed the effects of abiotic factors such as ionization, solubility, adsorption, sequestering agents, dissolved oxygen, and temperature on the toxicity to fish. Prediction of toxicity of mixtures of chemicals from these variables is discussed in relation to their possible negative or additive joint toxicity, and to test methodology. Lloyd states that, 'The available evidence suggests that the toxicity of each chemical in a *mixture* will be affected by these variables to the same extent as for the *single* chemical.'

Another problem is the difficulty in obtaining accurate replicate toxicity tests that are adequate in sensitivity to detect small changes in the effects of even a few of the many variables, whether biotic or abiotic. This problem is prevalent in laboratory tests, and far more so in field tests where variables are less controllable. This, of course, is a problem which has received much attention and has resulted in the development of rigorous toxicity test methods from organizations such as the Environmental Protection Agency, Fish and Wildlife Service, etc., in the United States, other world and country governmental environmental organizations, as well as standardization groups such as the

American Society for Testing and Materials. Evidence to support or 'verify' laboratory test conclusions for field prediction must await more complete analysis of the variables in field tests, gathered from extensive experiments in order to gain confidence in extrapolation.

4 OTHER CONSIDERATIONS

The foregoing tests for synergism do not include the chronic effects or the effect on the various life-stages of organisms or interactions between organisms such as those in microcosms. Chronic effects can be shown when the detoxifying mechanisms of species tested are slowed down by any of the chemicals in the mixtures. Various life-stages of organisms such as the egg stage can be more (or less) susceptible than others to one or more chemicals in the mixture. Chemicals of short residual toxicity can act differently when mixed with those of longer residual toxicity.

The careful selection of test methods and organisms and concentrations of chemicals chosen for use also play a large part in the possibility of demonstrating synergism or antagonism with chemical mixtures.

5 CONCLUSIONS

Considerations for testing the joint action of chemicals in mixtures should be based on the proper selection of test methods, environmental conditions, and suitable surrogate species of organisms representing different biochemical reactions and ecosystems. The test species can be chosen from taxonomically distantly related species of known sensitivity to benchmark chemicals. For synergism tests the species should be sensitive to chemicals having different modes of action. The test species need not necessarily be any different from the surrogate species chosen for individual chemicals. True synergism is easily detected by comparison of the toxicity of the mixture with the toxicity from double the concentration of each component tested separately.

The likelihood of significant synergism occurring in nature, after the chemicals have been distributed in rivers, soil, or air, are somewhat remote because of (a) the dilution factor to concentrations below 1 ppm, (b) the change in ratio of one chemical to another, (c) physical and chemical buffering properties of the many natural chemicals in these environments, (d) competitive and varying sorption by the media encountered, and (e) the volatilization and degradation properties of each individual chemical in a mixture. The laboratory data base for this tentative conclusion needs confirmation from additional field data to test its scientific accuracy for predictive purposes.

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