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Elaborating chironomid deformities as bioindicators of toxic sediment stress: the potential application of mixture toxicity concepts

Angelo C. Vermeulen

Vermeulen, A. C., Freshwater Biological Laboratory, Helsingørsgade 51, DK-3400 Hillerød, Denmark
Present address: Laboratory for Ecology and Aquaculture, Zoological Institute, Catholic University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

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Morphological deformities in chironomid larvae represent sublethal effects of exposure to heavy metals, organochloropesticides and other organic xenobiotics. Because chironomid larvae live in close contact with sediment and often feed on organic detritus and algae associated with it, morphological deformities exhibit a substantial potential as monitoring tool for assessing sediment toxicity. Pollutants rarely occur singly and in most contaminated ecosystems biota are exposed to complex chemical mixtures. Chironomid deformities occurring in field populations may therefore be considered as responses to the concurrent action of different toxicants. The main objective of this review paper is to offer a conceptual framework on mixture toxicity conducive to further elaboration of chironomid deformities as a monitoring tool for contaminated sediments. Current knowledge of chironomid deformity induction is compiled and joint toxicity concepts and mixture models reviewed. A selection is presented of studies dealing with joint effects of heavy metals and pesticides, two classes of deformity-inducing compounds, and factors determining their joint toxicity are discussed.

1. Introduction

Wide variety of chemicals often acts simultaneously upon biota in contaminated ecosystems (Statham & Lech 1975, Hellawell 1986, De March 1988, Calamari & Vighi 1992, Kraak 1992). Inadvertent combinations of chemicals occur in the environment because many compounds may persist for long periods, while others are discharged repeatedly (Marking 1985). Direct effluent discharges and agricultural runoff water most often contain complex mixtures of contaminants (Spehar et al. 1978, Mohan et al. 1986, Spehar & Fiandt 1986, Warne et al. 1989). Pesticides are frequently used in combination to increase their efficacy (Howell et

al. 1964, Levot 1994). Degradation and transformation processes (e.g. hydrolysis, photolysis, oxidation), and chemical interactions may produce new compounds, and hence contribute to the complexity of the total toxic burden. Many aquatic organisms are exposed to contaminant mixtures during their entire life cycle or at least throughout the most important parts of it. For this reason, combination effects have been widely recognised as an important aspect of ecotoxicological assessment of chemicals (Murphy 1980, EIFAC 1987, Enserink et al. 1991).

Considering the wide diversity of man-made compounds accumulated in the environment, complete chemical characterisations are time-consuming, expensive and most often require specialised equipment (Giesy & Hoke 1989). Moreover, using chemical and physical measurements, the overall effect of a polluted ecosystem on its biota, may not be fully and easily assessed (Rosenberg & Resh 1993). Certitude that all toxic compounds in a sample are identified, may practically never be achieved, and hence, chemical measurements may lead to an underestimation of the total toxicity. Because of synergistic and antagonistic interactions, the combination of chemicals may result in an enhanced or reduced toxicity, further complicating the interpretation of chemical analyses (Giesy et al. 1988, Giesy & Hoke 1989, Warwick 1988).

Biological indicators provide the potential for direct observation of the overall effect of environmental contaminants by virtue of their role in aquatic ecosystems (Warwick 1988). Morphological deformities in chironomid larvae (Diptera, Chironomidae) offer promising biological indicators for the assessment of contaminated sediments. Such abnormalities represent sublethal effects and can be considered as early warning signals for environmental degradation by chemical contaminants (Warwick 1990c). The larvae of most chironomid species dwell in sediment and feed on organic matter and mineral particles with their associated microfauna and flora. Because of their benthic feeding habits, these larvae may be exposed to contaminated sediments throughout their entire larval stage. Results from field studies by subsequent workers strongly indicate a relationship between increased incidence of deformation and toxic sediment stress (e.g. Hamilton & Saether 1971, Köhn & Frank 1980, Warwick 1980ab, Wiederholm 1984, van Urk et al. 1985, Warwick, 1985, Dickman et al. 1990, Dermott 1991, Janssens de Bisthoven & Van Speybroeck 1994). Although most of the data in these studies are semi-quantitative and remain largely circumstantial, more substantial evidence was offered in recent field investigations concentrating on the relationship between deformities and contaminant concentrations in sediments (heavy metals: Janssens de Bisthoven et al. 1995, Vermeulen in prep.), and in larvae (PAHs: Dickman et al. 1992; heavy metals: Janssens de Bisthoven et al. 1992, 1995). In laboratory experiments, chironomid deformities were induced by heavy metals (Kosalwat and Knight 1987, Janssens de Bisthoven 1995), organochloropesticides (Hamilton & Saether 1971, Warwick 1985, Madden et al. 1992) and other organic xenobiotics (coal-derived oil: Cushman 1984, xylene: Janssens de Bisthoven 1995). *In situ* such compounds are often encountered in combinations, and hence chironomid deformities may represent responses to complex contaminant mixtures (Hamilton & Saether 1971, Dickman et al. 1992, Janssens de Bisthoven et al. 1992).

In order to utilise the full potential of chironomid deformities as biological screening tool, toxicants and groups of toxicants that produce particular morphological deformities need to be further identified, relationships between dose, severity and frequency of deformities further established (Johnson et al. 1993), and possible synergistic and antagonistic interactions between causal compounds investigated. The objectives of this paper are: 1) to offer a conceptual framework on mixture toxicity useful for further elaboration of chironomid deformities as screening tool for sediment toxicity, and 2) to illustrate the use of chironomid deformity screening by compiling current knowledge of their occurrence and induction.

2. Chironomid deformities: description and occurrence

Deformities can be defined as any morphological feature that departs from the normal configuration, excluding effects from mechanical wear (Warwick 1988, Madden et al. 1995). Breakage and abrasion are common phenomena caused by physical contact of the mouthparts with mineral particles and organic material (Janssens de Bisthoven & Ollevier

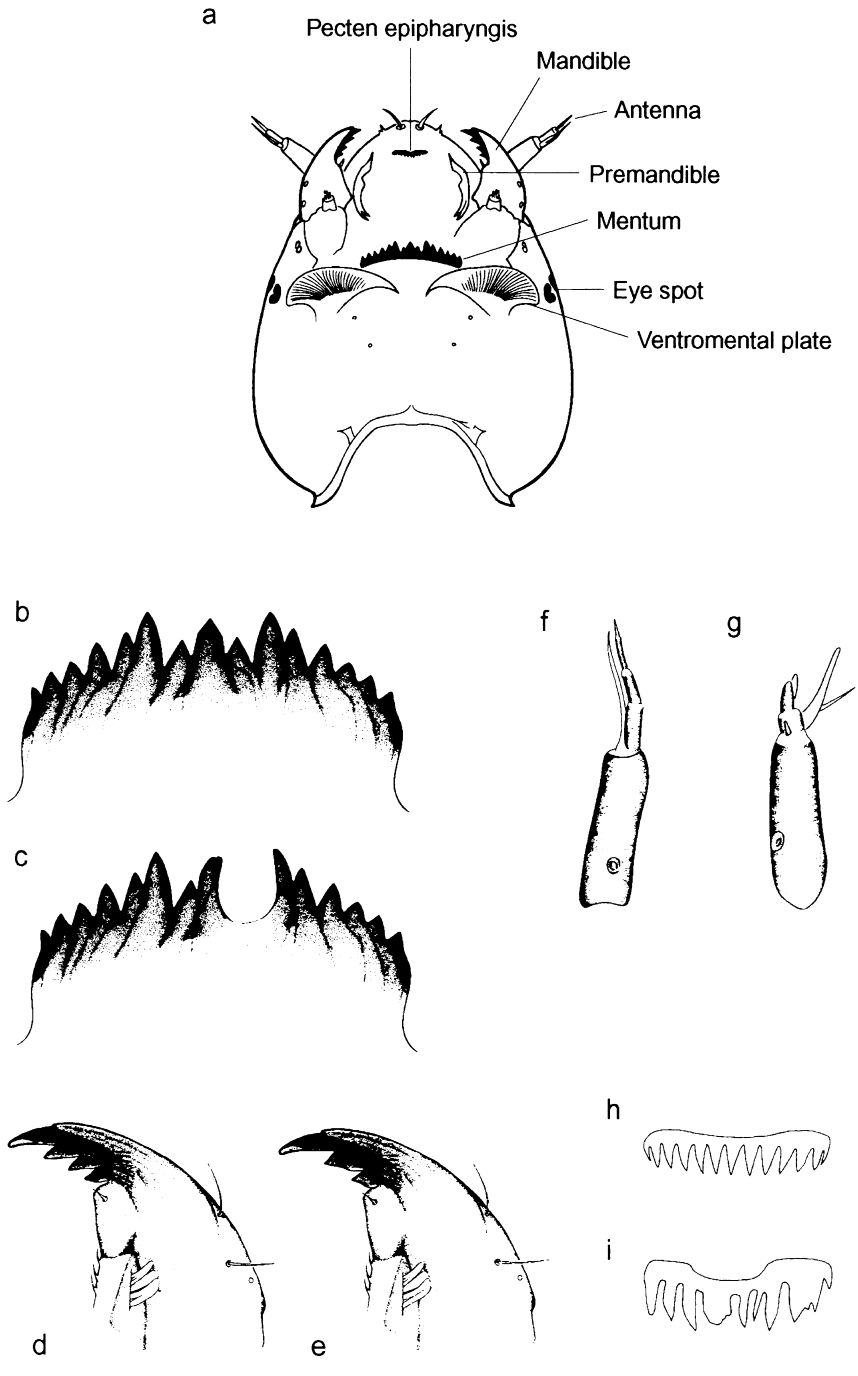

Fig 1a–i. — a: Schematical repsentation of the ventral view of a *Chironomus* head capsule (adapted from Oliver et al. (1978), — b: normal mentum, — c: deformed mentum showing a mentum gap or so-called Köhn gap, — d: normal mandible, — e: mandible missing one inner tooth, — f: normal antenna, — g: deformed antenna, — h: normal pecten epiphagrynis, — i: deformed pecten epipharyngis with fused teeth. All illustrations are composite drawings based on photograps, except f and g (adapted from Madden et a. 1992). Nomenclature follows Saether (1980).

1989, Vermeulen et al. 1994) and therefore should not be included in deformity screening.

Morphological deformation may range from mildly abnormal mouthparts to grotesque thickening and fusing of all body structures (Warwick 1988). Deformities have been reported in several chironomid species (references in Johnson et al. 1993 & Janssens de Bisthoven 1995), but *Chironomus* (belonging to the subfamily of the Chironominae) is the genus which seems to be most susceptible to morphological deformation. The basic morphology of a *Chironomus* head capsule together with some examples of common mouthpart and antennal deformities is presented in Fig. 1. Mouthpart deformities include minor abnormalities such as split, reduced and asymmetric teeth, missing and additional teeth, extensive gaps, fusions and complete distortions of the original morphology. Especially the mentum (or analogous ligula for the subfamily of the Tanypodinae) seems to display a wide variety of different deformity types (Tennessen & Gottfried 1983, Warwick 1989, 1990b, 1991, Warwick & Tisdale 1988). Antennal deformities most often concern fusion, loss or displacement of the distal segments and sensory organs (Warwick 1985). Thickening of body and head capsule walls and deformation of the chitinous claws on the parapods has also been reported (Brinkhurst et al. 1968, Hamilton & Saether 1971, Warwick 1988). For a detailed treatment of larval morphology and nomenclature, Saether (1980) and Cranston (1995) should be consulted.

Many aquatic ecosystems are polluted with contaminants originating from domestic sewage, agricultural runoff and industrial effluents (Frank et al. 1982, Muirhead-Thomson 1987, Wild & Jones 1991). Airborne compounds and polluted rain water may even contaminate areas lying relatively isolated from human activity (Hebert et al. 1994). Hence, clean areas bearing chironomids are difficult to find, hampering the determination of natural background levels of deformities. However, using subfossil material, these natural background levels were estimated to vary between 0 and 0.8% (Warwick 1980b, Wiederholm 1984, Klink 1985). In slightly contaminated areas receiving "limited" inputs of man-made chemicals and used as references in field studies, deformity levels vary between 0 and 8% (Wiederholm 1984, Warwick 1985, Van de Guchte & Van Urk 1989, Dermott 1991, Dickman et al. 1992, Madden et al. 1992, Van Urk et al. 1992, Bird, 1994). Differences in investigated structures, chironomid species and degree of contamination probably account for the variation of these reference levels.

Table 1 gives an overview of the xenobiotics present in different locations where high incidences of chironomid deformities were observed. In these field studies, heavy metals and several organic xenobiotics such as pesticides, PAHs and PCBs are frequently referred to as causal compounds. No relationship was found between deformities and organic loading, and therefore, organic enrichment is believed not to be causative (Hamilton & Saether 1971, Hare & Carter 1976, Nguyen Thi Hong 1992, Lenat 1993). Most of the field studies listed in Table 1 deal with the genus *Chironomus* (Chironominae), and to a lesser extent with the genus *Procladius* (Tanypodinae). Mouthpart deformities were most often observed, antennal deformities less frequently, and thickening of the body wall and deformation of other structures were only reported in a few cases.

3. Chironomid deformities: different causal compounds

Hamilton and Saether (1971) exposed larvae of *Chironomus tentans* to ranges of Aldrin, DDE, DDT, DDD, 2,4-D, 2,4-T, a number of PCBs and nitrilotriacetic acid (NTA). For all compounds high mortalities were reported, but only for the DDE exposure one severely deformed larvae was observed. However, antennae were not screened and when Warwick (1985) investigated the same larvae for antennal deformities, he found 25% of the larvae with abnormal antennae after exposure to 1 µg/l DDE. With increasing concentrations this incidence decreased again, revealing an inverse relationship. Warwick assumed that the observed relationship represented a right-hand side portion of the total bell-shaped response curve of antennae to DDE. In spite of the absence of any response to most compounds and the unexpected response to DDE, this investigation provided the first clear indication that deformities could be induced by a specific contaminant. Several subsequent laboratory investigations were carried out since then, mainly focused on heavy metals and organochloropesticides.

Table 1. Pollutants referred to as possible causal agents for chironomid deformation in subsequent field studies. AN = deformed antennae, MP= deformed mouthparts, PI = heavy pigmentation of the head capsule, PO = deformed posterior body structures (posterior parapodia, anal tubuli, procerci), WA = thickened head capsule and/or body wall.

Pollutants	Deformity	Location	Authors
Industrial pollutants	MP, WA	Okanagan Lake and Skaha Lake, British Columbia	Saether, 1970
Industrial and agricultural pollutants	MP, PI, WA	Lake Erie, Canada; Okanagan Lake and Skaha Laka, British Columbia	Hamilton and Saether, 1971
Industrial pollutants	MP	Parry Sound, Georgian Bay, Canada	Hare and Carter, 1976
Industrial pollutants (heavy metals)	MP	Teltowkanal, Berlin, Germany	Köhn and Frank, 1980
Industrial and agricultural pollutants	MP	Bay of Quinte, Lake Ontario, Canada	Warwick, 1980ab
Pb, Hg, As, Co, Cyanide, a-BHC, 2,4-D, 2,4-DP, 2,4,5-T, aldrin, lindane	MP	Pasqua Lake, Saskatchewan, Canada	Warwick, 1980c
Heavy metals	MP	12 Swedish lakes	Wiederholm, 1984
Heavy metals and organic micropollutants	MP	Rine River catchment, the Netherlands	van Urk et al., 1985
Industrial and agricultural pollutants	AN	Tobin Lake, Saskatchewan, Canada	Warwick, 1985
Heavy metals, radionuclides (U, Th) and elevated water temperatures	MP	Port Hope Harbour, Ontario, Canada	Warwick et al., 1987
Industrial and agricultural pollutants	MP, AN	Tobin Lake, Saskatchewan, Canada	Warwick and Tisdale, 1988
Cd, Pb, Cu, Hg, fluoranthene, benzo(a)pyrene and indenopyrene	MP	Lake Ketelmeer, the Netherlands	Kerkum and van Urk, 1989
Pb, Cu, Zn, Hg, As, DDT, TDE, dieldrin and endrin	MP	Murray and Darling Rivers, Australia	Pettigrove, 1989
Cd, Cu and Zn	MP	Dommel River, the Netherlands	van de Guchte and van Urk, 1989
Heavy metals, radionuclides, agricultural pollutants and domestic sewage	MP, AN	Sites in eastern and western Canada	Warwick, 1989
Industrial, agricultural and domestic pollutants	MP	Niagara River catchment Canada	Dickman et al. ,1990
Cd, Cu, Hg, As, DDE, HCB, PCBs, PAHs	MP, AN	St. Lawrence River, Canada	Warwick, 1990a
Cd, Cu, Hg, As, DDE, HCBs, PCBs, PAHs and domestic sewage	MP, AN	Lac St. Louis, Laprairie basins, Canada	Warwick, 1990b, 1992
Petrochemical pollutants : eg. Pb, Hg, benzene and derivates, chloroethanes, PCBs, PAHs	MP, AN, PS	St. Clair River, Canada	Dermott, 1991

(Contd.)

Table 1. contd.

Pollutants	Deformity	Location	Authors
Cd, Pb, Cu, Zn, Hg, Ni, As, Cr, Co, PAHs and radionuclides (U, Th)	MP, AN	St. Lawrence River, Port Hope Harbour, S. Indian Lake - Notigi Reserv., Tobin Lake and Last Mountain Lake, Canada	Warwick, 1991
PAHs	MP	Niagara River catchment and Lake Huron, Canada	Dickman et al., 1992
Heavy metals (Cu, Pb) and pesticides	MP	Dyle River, Belgium	Janssens de Bisthoven et al., 1992
Cd, Pb, Cu, Hg, fluoranthene, Heavy metals and hydrocarbons Pollutants from textile and light industry, agricultural pollutants and domestic sewage	MP MP MP, AN	Lake Vossemeer, Buffalo River, Yamaska River, Quebec,	van Urk et al., 1992 Diggins et al., 1993 Bird, 1994
Industrial and domestic pollutants	MP, AN	Nairobi River, Kenya	Janssens de Bisthoven and Van Speybroeck, 1994
Heavy metals (Cd, Pb, Cu, Zn, Hg), agricultural pollutants and domestic sewage	MP	3 Danish streams	Vermeulen et al., in prep.

By exposing *Chironomus decorus* larvae to substrate bound copper, a clear positive relationship was found between the copper concentration and the incidence of deformation of the pecten epipharyngis (Kosalwat and Knight, 1987). No mentum or mandible deformities were observed, and no deformities were induced with copper present in aqueous form. Grootelaar et al. (1988) carried out experiments with *Chironomus riparius* and sediments spiked with cadmium, zinc and copper. High mortality rates were observed, but no mentum deformities were induced. In other experimental studies with *Chironomus riparius*, Janssens de Bisthoven (1995) could demonstrate the deformity-induction capacity of cadmium, zinc and copper, and to a lesser extent of lead. Abnormalities were observed in the mentum, mandibles, pecten epipharyngis, premandibles and antennae. Low frequencies of deformities encountered in the control conditions were attributed to stress factors present in food and substrate. Concentration-response relationships were not always linear and were depending on the structure and deformity type investigated.

In experiments by van de Guchte and Maas-Diepeveen (1988) with pentachlorophenol (PCP) and Dieldrin, no deformities could be induced in *Chironomus sp.* larvae. Madden et al. (1992) induced deformities in *Chironomus sp.* larvae using DDT and Dacthal®. A clear positive relationship between increasing DDT concentrations and mentum deformities was observed. There was no effect of DDT on antennal deformities. Dacthal® induced both mentum and antennal deformities, but clear dose-response curves could not be detected.

Cushman (1984) exposed *Chironomus reductus* larvae to different concentrations of coal-derived oil in experimental ponds. While medial mentum deformities showed a weak positive relationship with oil concentrations, the incidence of overall mentum deformities in the coal liquid conditions did not differ significantly from that in the control conditions. By exposing *Chironomus riparius* instar 1 or 2 larvae (instar not clear) acutely to xylene (72h), mentum deformities were induced in an experiment by Janssens de Bisthoven (1995).

An overview of the experiments together with details on contaminant concentrations, deformity types and their occurrence is presented in Table 2. At present, experimental data on chironomid deformity induction seem rather inconsistent. The ex-

periments with copper illustrate this well: used in three different experiments, different results were obtained. This inconsistency can be attributed to differing screening resolutions and to differing experimental parameters such as temperature, water characteristics (hardness, pH), contaminant concentration ranges, exposure source (substrate bound or aqueous), test species and developmen-

Table 2. Causal compounds for chironomid deformities as reported in induction experiments. Concentrations are expressed as mg/kg for exposure to substrate bound contaminants, and in µg/l (or ml/m3) for exposure to aqueous forms of contaminants. Max.% = maximum percentage deformed larvae. Dose-resp. = dose-response relationship: P = positive, N = negative, A = absent, brackets are used to indicate weak relationships, concentration ranges are indicated for partial relationships.

Compound	Concentrations	Test species	Deformity*	Max. %	Dose-resp.
Heavy metals					
Copper[1]	21, 878, 1808, 2636 mg/kg	*Ch. decorus*	Pecten epipharyngis	62	P
Copper[2]	0, 10, 100 µg/l	*Ch. riparius*	Mentum, mandibles, pecten epipharyngis, premandibles and antennae	28	P
			Mentum (Köhn gaps)	6.5	P
Cadmium[2]	0, 3, 9, 27 µg/l	*Ch. riparius*	Mentum (small split medial tooth)	11	P
			Mentum (large split medial tooth)	13	A
			Mandibles (missing tooth)	3.5	(P)
			Premandibles	3.5	N (> 0 µg/l)
Zinc[2]	0, 10, 100 µg/l	*Ch. riparius*	Mentum (split medial tooth)	30	N (> 0 µg/l)
			Mentum (additional tooth)	3	(P)
			Pecten epipharyngis	11	(P)
			Premandibles	7	P
			Antennae	8	(P)
Pesticides					
DDE[3]	1, 10 µg/l	*Ch. tentans*	"Severely deformed" (*fide* Warwick, 1985; antennal deformities not included)	< 10 (1 larva)	
DDE[4]	1, 5, 10, 15, 20 µg/l	*Ch. tentans*	Antennae	25	N
DDT[5]	0, 1, 5, 10 µg/l	*Chironomus sp.*	Mentum	60	P
Dacthal®[5]	0, 0.2, 2, 10, 100 µg/l	*Chironomus sp.*	Mentum	17	A
			Antennae	18	N (> 0.2 µg/l)
Other organic compounds					
Synthetic crude oil[6]	0, 15, 75, 375 ml oil/m3	*Ch. decorus*	Mentum (medial deformities)	1.9	P
Xylene[2]	0, 10, 100 µl/l	*Ch. riparius*	Mentum	33.3	P

* Nomenclature follows Saether (1980) and Warwick and Tisdale (1988).

1. Kosalwat and Knight (1987), 2. Janssens de Bisthoven (1995), 3. Hamilton and Saether (1971), 4. Warwick (1985), 5. Madden et al. (1992), 6. Cushman (1984).

tal stage at which the larvae were exposed (eggs or larvae) (Janssens de Bisthoven, 1995). Also, experimental designs were often restricted with few replicates, test animals and controls, and limited concentration ranges.

The importance of food and temperature conditions for the induction of deformities was demonstrated in a study by Parren et al. (1993). Deformities were induced with contaminated sediments from the Laan River in Belgium using two temperatures (13 and 18°C) and two food conditions (5 and 30 mg Tetramin per larva). Only in the high temperature condition deformity percentages were higher than in the control condition (diatomaceous earth): in the high food regime, the incidence was comparable with field data (10%), in the low food condition the incidence was lower. The induction of deformities seemed to be enhanced by a higher metabolic rate (temperature effect), as well as by a higher food availability. It was suggested that deformities were induced after ingestion of contaminants associated to food particles. This is in accordance with the results from Kosalwat and Knight (1987) discussed earlier.

Experimentally induced deformities are generally weaker and the number of deformed larvae is generally lower than in natural populations from contaminated sites (Janssens de Bisthoven 1995). Nevertheless, high mortality rates in most experiments indicate that this cannot be attributed to a decreased bioavailability of the contaminants. A combination of physical, chemical and biological environmental parameters is probably responsible for the observed higher frequencies in field studies (Grootelaar et al. 1988). In this context mixtures of chemicals may play an important role in deformity induction (Hamilton & Saether 1971, Janssens de Bisthoven et al. 1992, Dickman et al. 1992).

Janssens de Bisthoven et al. (1992, 1995) determined the concentrations of cadmium, lead, copper and zinc in *Chironomus* larvae from several locations in the polluted Dyle Basin and Dommel River in Belgium, and compared levels in normal larvae with those in deformed larvae. In most cases metal levels were significantly higher in deformed larvae than in normal larvae. The populations without significant differences between normal and deformed larvae for their trace metal contents, may have been exposed to the action of other organic xenobiotics. A similar investigation was carried out by Dickman et al. (1992) in which levels of PAHs in the body tissues of *Chironomus* larvae from a site in the Welland River in Canada (Niagara River watershed) were measured. Higher levels of the PAHs were found in deformed larvae compared to normal larvae and suggested that PAHs may act together (synergistically) to effect the observed deformities. In both studies it was concluded that deformed larvae represented individuals more often exposed to higher local contaminant concentrations than their apparently normal counterparts.

4. Chironomid deformities as contaminant-specific responses

Chironomid deformities involve a wide range of different types of morphological aberrations (e.g. descriptions and illustrations in Warwick & Tisdale 1988, Warwick 1990b). Hamilton and Saether (1971) suggested that different types of deformities may be referable to different contaminants. The first attempt to investigate the contaminant specificity of deformities systematically, was a study in the polluted Dyle River in Belgium by Janssens de Bisthoven (1988, 1990). More recently, 20 more Belgian lowland rivers have been included in the study and deformities were correlated with contaminant concentrations in the sediment (Janssens de Bisthoven et al. 1995). Although many deformity types were rather pollutant-aspecific, some deformity types showed significant correlations with contaminant concentrations and may prove useful in identifying the presence of specific compounds in contaminated sediments. In a study carried out in Denmark in which the effect of a paper mill effluent on chironomid deformation was investigated, diffuse domestic and agricultural pollution could be distinguished from industrial point source pollution (heavy metals) using multivariate deformity patterns (Vermeulen et al. in prep.). The proportional occurrence of the different deformity types seemed to shift consistently according to the type of pollution. Especially mentum gaps (or so-called Köhn gaps) seemed to have a high discriminative power: they were encountered in all sites receiving heavy metal inputs, but only in a few sites (in low numbers) receiving domestic sewage and agricultural run-off. This may imply that mentum gaps are specifically induced by heavy metals, which is in

accordance with other studies (Köhn & Frank 1980, Janssens de Bisthoven 1995). Most other deformity types in the study by Vermeulen et al. (in prep.) seemed to be pollutant-aspecific and were encountered in all sampling locations. These results were confirmed by literature data: some of the aspecific deformities (e.g. split medial mentum tooth) were experimentally induced with organochloropesticides (Madden et al. 1992), as well as with heavy metals (Janssens de Bisthoven 1995). Contaminant specificity of deformities may prove advantageous for its analytical capacities. However, for a clear-cut validation of the hypotheses derived from these field studies, laboratory research should be conducted, systematically investigating and characterising causal compounds of all deformity responses.

5. Basic concepts and additive toxicity of heavy metals and pesticides

Contaminant-aspecific responses may be induced by several compounds acting together. Considering both the diversity and the general occurrence of heavy metals, organochloropesticides and other organic xenobiotics causing deformities, we may assume that exposure to mixtures, rather than to single compounds, is responsible for elevated levels of chironomid deformities in field situations. This may explain the differences observed between laboratory and field studies (Hamilton & Saether 1971, Dickman et al. 1992, Janssens de Bisthoven et al. 1992). Here, the following questions seem most pertinent to us: 1) how to relate a deformity response to causal compounds acting simultaneously?, and 2) do synergistic and antagonistic effects occur between the causal compounds? To deal with these questions, a short review of mixture toxicity is presented.

The toxicity of chemical mixtures not only includes the simple summation of the activities of the different components, but also covers the full range between the general terms antagonism and synergism. Terminology on mixtures used in literature is rather confusing, some terms are defined differently by different authors and used inconsistently. Based on Sprague's (1970) interpretation we will use the terms "synergism", "simple addition" and "antagonism" in this paper to distinguish between the three basic types of joint toxicity. "Simple addition" signifies strict summation of the individual effects. "Synergism" signifies that the combination produces an effect greater than that expected from strict summation, and "antagonism" that it produces a smaller effect.

Apart from the nature of the components of a mixture, joint toxicity is determined by many other factors such as mixture concentration, water hardness, temperature, duration of exposure, test species, etc. This is illustrated in Table 3 which gives an overview of selected studies on mixture toxicity of heavy metals and pesticides. Heavy metals and pesticides are two common and widespread groups of pollutants (Leland & Kuwabara 1985, Nimmo 1985, Tessier et al. 1994). Many studies are available on their joint toxicity, comprising a multitude of test species, endpoint parameters and experimental conditions (see e.g. extensive overview given by EIFAC, 1987).

The acute joint toxicity of copper and zinc mixtures for fish are a historically well-known example of synergism (Bandt 1946, Doudoroff 1952). Lloyd (1961) investigated their joint toxicity for rainbow trout in more detail and concluded that synergism only occurred in conditions of low water hardness, and relatively high mixture concentrations. These results were confirmed by Sprague (1964) and Sprague and Ramsay (1965) for young Atlantic salmon. Concentration-dependent mixture toxicity also occurred in a study where bacterial cultures were exposed to mixtures of the organophosphate pesticides monocrotophos and quinalphos (Bhaskar et al. 1992). McInnes and Calabrese (1977) reported the interaction of metals to be temperature-dependent. Kraak (1992) demonstrated that joint toxicity can also be time-dependent. When zebra mussels were exposed to mixtures of copper and cadmium, the metals displayed an antagonistic toxicity in short term experiments, but synergistic toxicity in chronic experiments.

The test species and the endpoint parameter are also factors determining joint toxicity. In short term experiments, Kraak (1992) observed antagonistic effects between copper and zinc on the filtration rate of zebra mussels. This contradicts the results on fish cited above. In three separate studies on the toxicity of binary mixtures of copper and mercury, three different responses were observed (Barnes & Stanbury 1948, Corner & Sparrow 1956, Moulder 1980). Different test or-

Table 3. Results from selected studies on mixture toxicity of heavy metals and pesticides.Mixtures with similar composition are grouped. Specific experimental conditions are mentioned if needed: water hardness expressed in mg/l $CaCO_3$ and mixture concentration in toxic units (TU; for an explanation of the toxic unit concept see text, section 7). conc.-dependent = concentration-dependent.

Composition	Joint toxicity	Effect	Duration	Organism	Reference
Heavy metals					
Cu + Zn	Synergistic	Mortality	Acute	Fish	Bandt, 1946
Cu + Zn	Synergistic	Mortality	Acute	Fish	Doudoroff, 1952
Cu + Zn • 320 mg/l $CaCO_3$; 15–20 mg/l $CaCO_3$: < 7 TU • 15–20 mg/l $CaCO_3$: > 7 TU	 Simply additive Synergistic	Mortality	Acute	Rainbow trout	Lloyd, 1961
Cu + Zn • 14 mg/l $CaCO_3$: 1TU • 14 mg/l $CaCO_3$: 2 + 5 TU	 Simply Additive Synergistic	Mortality	Acute	Atlantic salmon	Sprague, 1964
Cu + Zn • 20 mg/l $CaCO_3$: > 1 TU	Synergistic	Mortality	Acute	Atlantic salmon	Sprague and Ramsay, 1965
Cu + Zn	Synergistic	Avoidance response	–	Atlantic salmon	Sprague et al, 1965
Cu + Cd	Antagonistic	Filtration rate	Chronic	Zebra mussel	Kraak, 1992
Cu + Hg	Synergistic	Mortality	Acute	Copepod	Barnes and Stanbury, 1948
Cu + Hg	Simply additive	Mortality	Acute	*Artemia salina*	Corner and Sparrow, 1956
Cu + Hg	Antagonistic	–	–	Amphipod	Moulder, 1980
Cd + Zn	Antagonistic	Mortality	Chronic	Flagfish	Spehar et al., 1978
Cd + Hg	• Synergistic • Synergistic or antagonistic (conc.-dependent) • Synergistic	• Mortality • Byssus thread production • Filtration rate	Acute	Tropical green mussel	Mohan et al., 1986
Fe + Cu Fe + Ni	Mostly antagonistic	Growth	–	*Chlorella vulgaris*	Mallick et al., 1990
Cu + Ni	Synergistic				
Ni + Pb Ni + Cr Pb + Cr	Mostly antagonistic (Ni)	Growth	–	*Nostoc muscorum*	Rai & Raizada, 1989
Cu + Zn Cu + Cd Cd + Zn Cu + Zn + Cd	Antagonistic Synergistic Simply additive Simply additive	Filtration rate	Acute	Zebra mussel	Kraak, 1992
Cu + Zn + Cd	Synergistic	Mortality	Acute	Fathead minnow	Eaton, 1973
Cu + Zn + Ni	Simply additive	Mortality	Acute	Rainbow trout	Marking, 1977

(Contd.)

Table 3. contd.

Composition	Joint toxicity	Effect	Duration	Organism	Reference
Cu + Cd + Hg + Pb + Cr + As	• Synergistic • Antagonistic	• Mortality • Growth rate	• Acute • Chronic	Fathead minnow	Spehar and Fiandt, 1986
	• Simply additive	• Mortality Reproduction	• Acute Chronic	*Daphnia magna*	
Cu + Zn + Cd + Ni + Hg + Pb + Cr	Simply additive	Mortality and population growth	Chronic	*Daphnia magna*	Enserink et al.,1991
Organophosphates					
Malathion + Delnav	Synergistic	Mortality	Acute	Rainbow trout	Marking, 1977
Phosphamidon + methidathion	Simply additive or synergistic (ratio dependent)	Mortality	Acute	Lobster	Sprague and Ramsey, 1965
Monocrotophos + quinalphos	All responses (conc. dependent)	Growth and development	–	Bacteria	Bhaskar et al.,1992
Carbamates and organochlorines					
Carbaryl + dieldrin	Synergistic	Mortality	Acute	Rainbow trout	Statham and Lech, 1975
Other pesticides					
Atrazine + metribuzin	Simply additive	Growth and reproduction	Acute	*Chlorella fusca*	Altenburger et al., 1990
Amitrole + glufosinate-ammonium	Antagonistic				
Carbaryl + either rotenone, 2,4-D n-butyl ester or pentachlorophenol	Synergistic	Mortality	Acute	Rainbow trout	Statham and Lech, 1975
TFM + Bayluscide	Simply additive or synergistic	Mortality	Acute	Rainbow trout	Marking, 1977
Antimycine + dibrom	Antagonistic				
Antimycine + dibrom	Synergistic	Mortality	Acute	Bullhead Largemouth bass Yellow perch	Berger, 1971
Antimycine + KMnO4	Antagonistic	Mortality	Acute	Rainbow trout	Marking, 1977
Rotenone + piperonyl-butoxide or sulfoxide	Synergistic				

ganisms were used in each investigation: a copepod species, a brine shrimp and an amphipod species, and the effects of synergism, simple additivity and antagonism were reported, respectively. Mixtures of the pesticides antimycine and dibrom were synergistic to bullhead, largemouth bass and yellow perch (Berger 1971) but antagonistic to rainbow trout (Marking 1977). Even intraspecific variation in the response on mixture exposure has been observed. The degree of synergism between the pesticide deltamethrin and the commercial synergiser piperonylbutoxide for moth larvae was dependent on the pesticide resistance of each strain of test larvae (Ho et al. 1983).

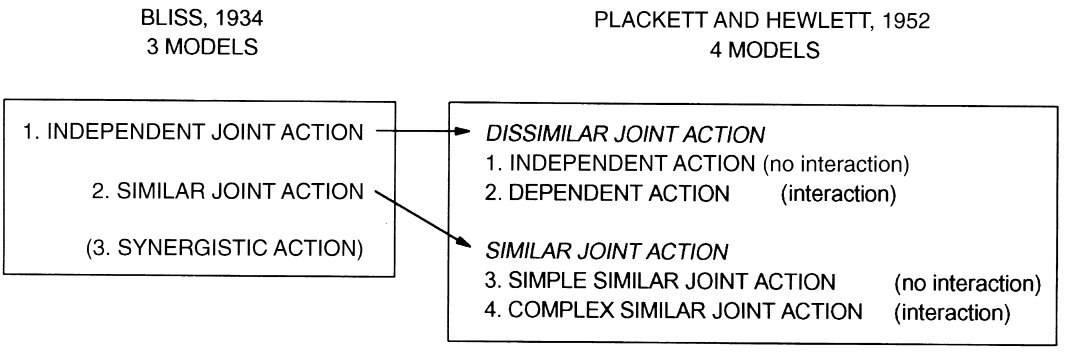

Fig. 2. Overview of the different mathematical models for joint toxicity as defined by Bliss (1934) and Plackett and Hewlett (1952). Arrows are used to indicate the relation between the models.

6. Mixture models describing different types of joint action

Sprague's (1970) use of the three categories: simple additivity, antagonism and synergism is in fact a classification based on final effects, not on underlying physiological mechanisms. Several attempts have been made to model the relationship between toxic effects of mixtures and their component concentrations, and distinguish between different types of physiological action. Bliss (1934) was the first with this kind of approach, although some of his ideas were anticipated by Trevan (1927). According to Bliss, the toxicity of a mixture may be predicted from the separate toxicities of the individual substances: these may either be 1) independent, 2) similar, or 3) synergistic or antagonistic. "Independent joint action" is defined as: "The poisons or drugs act independently and have different modes of toxic action...". Although their final effect is the same (e.g. mortality), the primary underlying physiological effects are different. "Independent" is used to indicate that there are no synergistic or antagonistic effects. "Similar joint action" is defined as: "The poisons or drugs produce similar but independent effects, so that one component can be substituted at a constant proportion for the other...". In this case, the physiological effects are the same for all components. In both mixture models correlations between susceptibilities are incorporated. When organisms susceptible to A are also susceptible to compound B, the susceptibilities are positively correlated; when organisms susceptible to A are not susceptible to B, the susceptibilities are negatively correlated. Only when the susceptibilities are negatively correlated, both models coincide with simple additivity. The fraction of the population responding to A, will not respond to B and vice versa, therefore the single toxicities of A and B can be summed in order to obtain the toxicity of the mixture for the whole population. When there is a zero or positive correlation, the susceptibilities overlap and the joint toxicity will be antagonistic. The third model is a model for "synergistic action", but is defined less precisely than the other two models. Since then the mathematical development of these mixture models has been revised and extended several times (references in Plackett & Hewlett 1952).

Plackett and Hewlett (1952) treated the subject in a more comprehensive manner and offered a more general set of mathematical models. They defined four types of joint action. Joint action is defined as "similar" if the sites of primary action of two chemicals are the same, "dissimilar" if these sites are different. Joint action is referred to as "interactive" if one chemical influences the biological action of the other (synergism or antagonism). If there are no interactions, the joint toxicity is simply additive. An overview of the different types of joint action as defined by Bliss (1934) and Plackett and Hewlett (1952) is presented in Fig. 2.

In a review on simple similar joint action and independent action, De March (1987) remarked that relatively few data have been applied to these models. In a hypothetical experiment, she tried to describe the results with common variations of both models corresponding with various modes of action. The results could be significantly described in terms of any of the assumed modes of

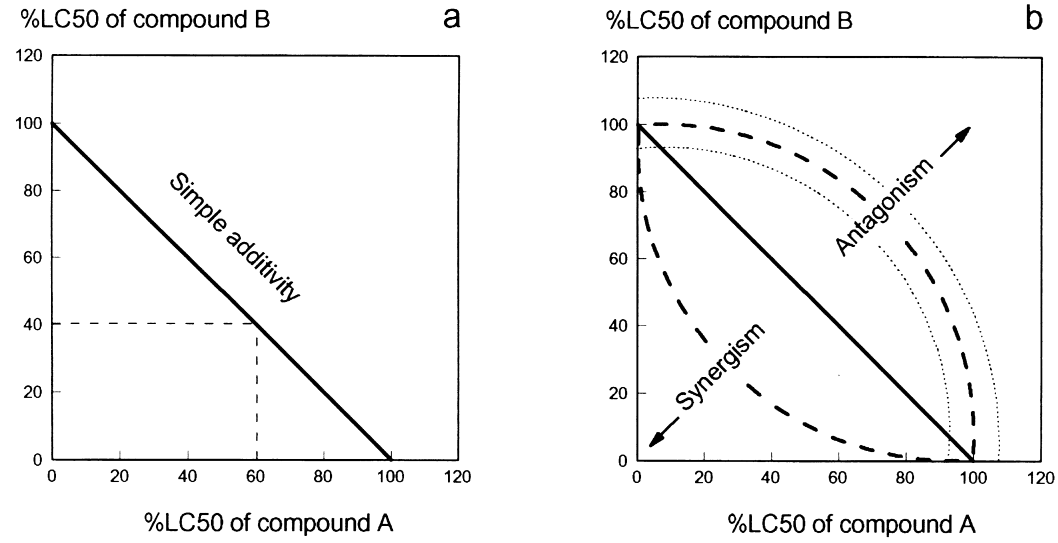

Fig. 3a–b. — a:Isobole representing simple additivity: a mixture containing e.g. 60% of the LC50 of compound A and 40% of the LC50 of compound B has the same effect as the LC50 of one component applied alone (100%). — b: Synergism and antagonism: the LC50 of the mixture is respectively smaller or greater than expected from simple additivity; a confidence belt may be added (shown for antagonism) to determine if deviation from simple additivity is significant. Modified from Marking 1985, Altenburger et al. 1990.

action and any attempts for further discrimination between modes of action seemed unpractical because of the extensive experimental design required. De March (1987) suggested keeping only one of the models and its expanded forms.

7. Determination of mixture potency: the toxic unit model

Apart from models describing mixture effects as a function of component concentrations, more analytical models have been constructed in order to investigate the type and degree of joint effect of chemicals. Loewe and Muischnek (1926) developed a simple method for analysing results from chemical mixture experiments for pharmacological use. Their "isobologram method" displayed simple additivity, synergism and antagonism in an easy interpretable graph. The isobole (GR: isos = equal; bolos = blow or strike) is a line representing different proportions of two compounds yielding the same effect, e.g. 50% mortality. The concentrations of the single components are expressed as fractions of their toxicities, in e.g. as a fraction of the LC50 (Fig. 3). If experimental results can be represented as a line connecting 100% LC50 of compound A with 100% LC50 of compound B, then toxicity is simply additive: if 50% of the LC50s of both components is present, the effect will be the same as with 100% of the LC50 of only one component. One component can be substituted for the same concentration of the other component, concentrations expressed as fractions of the LC50s. When the results are situated in the left area under the simple additivity isobole, the joint toxicity is synergistic. This means that e.g. only 30% of the LC50s of both components is necessary to attain a mortality of 50%. Likewise, the area to the right, above the simple additivity isobole, represents antagonistic toxicity. Altenburger et al. (1990) studied the combined effects of pesticides in algal biotests using the isobologram method. They calculated statistical confidence intervals and added a confidence belt to the additivity line to decide whether deviation from the additivity line was systematic or due to chance (Fig. 3b).

An important limitation of isobolographics is the extensive input of data required, as stated by several authors (Tammes 1964, Akobundu et al.

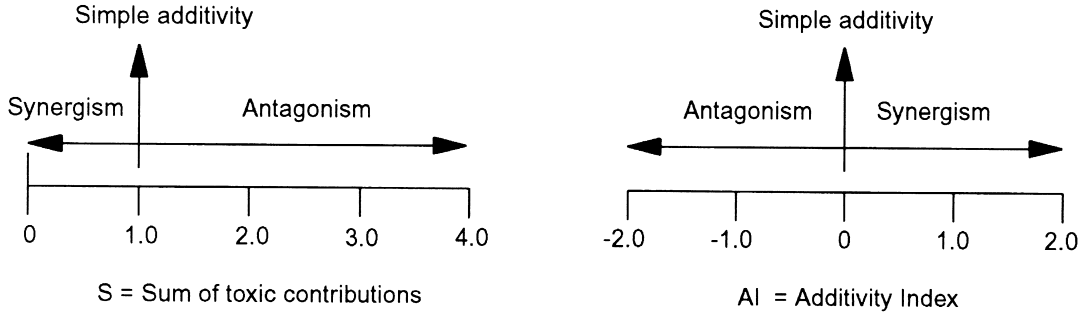

Fig. 4. The sum of toxic contributions of the components of a mixture is a measurement of joint toxicity with 1.0 as reference point and an asymmetrical scaling (left). The additivity index is a measurement of joint toxicity with zero as a referencce point and a symmetrical scaling (right). Modified from Marking and Dawson 1975.

1975, Murphy 1980, *fide* Altenburger et al. 1990). The restriction to binary mixtures is another limitation of this method for ecotoxicological investigations (Altenburger et al. 1990). Nevertheless, the isobole concept has been the basis for further development of methods for assessing mixture toxicity.

The "toxic unit model" compensates for some of the limitations of the isobologram method. The toxic strength of a concentration of a chemical (C) can be expressed in toxic units (TU):

$$\text{number of TU} = C/\text{toxicity} \quad (1).$$

If the toxicity is expressed as the LC50, then:

$$\text{number of TU} = C/LC50 \quad (2).$$

Assuming simple additivity in a mixture, the strengths of individual compounds can be summed as toxic units, and the expected total toxicity can be calculated (e.g. percentage mortality) and compared with the observed toxicity of the mixture. If the observed toxicity is different from the expected toxicity, synergistic or antagonistic effects occur, according to the direction of the deviation (Lloyd 1961, Sprague 1964, Sprague & Ramsay 1965, Brown 1968, Brown & Dalton 1970, Enserink et al. 1991, Kraak 1992). The sum of toxic contributions (S) of the components of a mixture is calculated according to the formula (toxicities expressed as LC50s):

$$S = (Am/Ai) + (Bm/Bi) \quad (3),$$

where: A and B = chemicals, i = LC50 of A and B applied as single components, m = concentrations of A and B in a binary mixture causing 50% mortality.

If the sum of toxic contributions of components A and B is equal to 1.0, the toxicity is simply additive. If it is smaller or greater than 1.0, the toxicity is respectively synergistic or antagonistic. This index is useful, except that values greater than 1.0 are not linear with values less than 1.0 (Fig. 4). For binary mixtures, this method is basically similar to the isobologram method.

Several toxicity indices were further derived from this method (Marking & Dawson 1975, Könemann 1980) and among them the Additivity Index (AI) from Marking has several advantages. The mathematical details for the calculation of this index can be found in Marking and Dawson (1975) and Marking (1977). After assigning zero as a reference point and establishing linearity for formula (3), the following formulas are obtained:

$$\text{for } S < 1.0 \quad AI = (1/S) - 1.0,$$
$$\text{for } S > 1.0 \quad AI = S(-1) + 1.0 \quad (4).$$

An AI of 0 means simple additivity, an AI smaller or greater than 0, respectively antagonistic or synergistic toxicity (Fig. 4). As illustrated in Fig. 4, S and AI show an inverse relationship: antagonism is indicated by a relatively high S value and a low AI value, while the opposite is true for synergism.

The meaning of this index value can be better understood by calculating the corresponding magnification factor referred to as the "Toxicity Enhancement Index" (TEI) which is in fact the ratio of the actual toxicity of the mixture to the toxicity

expected from simple addition. If the TEI of a mixture is 2, it means that the toxicity from the mixture has doubled. The following examples will illustrate the method to derive the TEI from the AI (Marking, 1977):

Additivity Index	Toxicity Enhancement Index
9	10×
1	2×
0	1×
− 1	1/2×
− 9	1/10×

The method can be summarised as follows: AI > 0: add one to the AI, AI < 0: add one to the absolute value of the AI, and reciprocate this number.

Confidence limits for an AI are calculated using the 95% confidence limits of the toxicity values (in our example LC50s). The range is derived by selecting those values yielding the greatest deviation from the AI. When the upper limits for Am and Bm, and the lower limits for Ai and Bi are put in formula (3), the largest possible value for S is obtained. Because of the inverse relationship between S and AI, this value corresponds with the lower limit for the AI. Similarly, the lower limits for Am and Bm, and the upper limits for Ai and Bi are used to calculate the upper limit of the AI.

For mixtures of more than two chemicals, formula (2) can be expanded by adding extra terms :

$$S = (Am/Ai) + (Bm/Bi) + (Cm/Ci) + \ldots \quad (5).$$

8. The use of mixture toxicity concepts in chironomid deformity studies

An investigation of several major rivers in Ireland (Harper et al. 1977) showed the presence of BHC residues at concentrations as low as 10 to 23 ng/l. Laboratory studies showed major reductions of the survival time of mayfly nymphs when exposed to a concentration of 100 ng/l. With a concentration of 10 ng/l slight effects on survival could already be observed. If these values are compared with the magnitude of concentrations of heavy metals in polluted environments, ranging from µg/l to mg/l, it is clear that concentrations of contaminants cannot just be summed directly. Determination of toxic units is needed when summing concentrations of different pollutants without neglecting low, but in fact deleterious, concentrations of contaminants. The toxic unit concept offers a useful alternative by making different magnitudes of effective concentrations comparable and additive. A hypothetical example with two compounds will illustrate this. Suppose the effective concentrations to induce 10% deformities (EC10) for two compounds A and B respectively are 1 µg/l and 100 mg/l. Choosing to express toxicities as EC10s, each concentration corresponds to one toxic unit. Excluding synergistic and antagonistic effects, the presence of 1 µg of compound A and 100 mg of compound B in 1 litre (corresponding to 2 TU) will produce 10% + 10% = 20% deformities. The deformity response can be related to the amount of toxic units, but not to the direct sum of contaminant concentrations.

In fact, an investigation of joint toxicity using the toxic unit model deals with our two basic questions by 1) making summation of different magnitudes of concentrations possible, and 2) offering an objective and comparative method to analyse synergistic and antagonistic effects of causal compounds. The Additivity Index from Marking and Dawson (1975) seems an appropriate adaptation of the toxic unit concept: a) confidence limits are of great importance when comparing toxicities of different mixtures, b) the index has a linear scaling and zero as a reference point, and therefore, is easy to use, and c) the calculation of corresponding TEI values facilitates interpretation.

9. Discussion

The sediment is a sink for pollutants and can be an important contributor of contaminants in the environment even long after discharges have ended (Miles & Harris 1971). Many contaminants of concern in aquatic ecosystems tend to associate preferentially with the sediment and suspended particulate material rather than being maintained in solution, although this behavior varies in extent between individual contaminants (Phillips & Rainbow 1993). Several trace metals (e.g. aluminium, iron, lead, manganese), organochlorine pesticides and most hydrocarbons are distinctly hydrophobic in nature, and therefore tend to be found almost completely in the particulate-associated fraction. Other chemicals, such as organophosphate compounds, monocyclic aromatic compounds and the trace

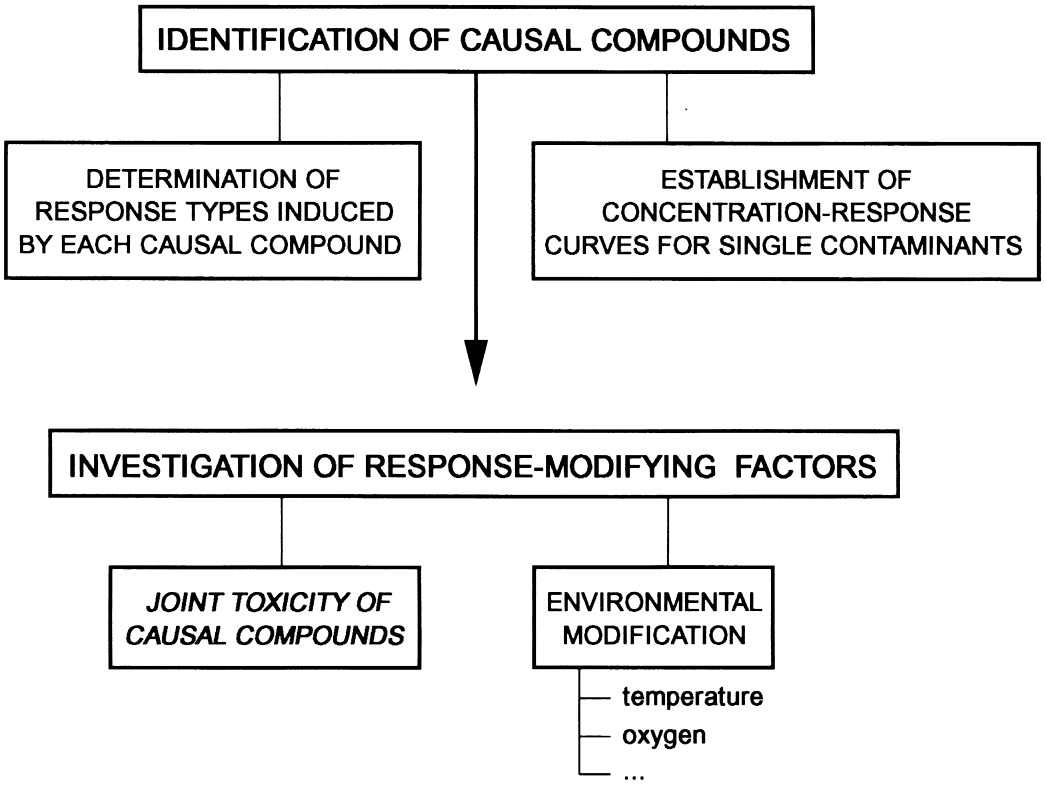

Fig. 5. Generalized framework showing essential experimental procedures needed for an optimalisation of the use of chironomid deformities as bioindicators of sediment toxicity.

metals arsenic, cadmium and selenium, exhibit much higher aqueous solubilities and are maintained in solution to a greater extent. Hence uptake from contaminants accumulated in the sediment by benthic organisms may occur both from solution (pore water) and from the ingestion of particulate matter (Giesy et al. 1988, Schloesser 1988, Giesy & Hoke 1989). Deformities in benthic chironomid larvae seem an appropriate biological screening tool for detecting adverse sediment conditions and assessing compliance with sediment quality criteria.

Because of the wide variety and general occurrence of causal agents, chironomid deformities occurring in field situations may be considered as responses to many contaminants acting concurrently. Hence, they can be used as biological indicators of overall sediment toxicity, next to more common biological indicators such as development rate, growth rate and community structure. Moreover, deformities are caused exclusively by chemical contaminants and therefore accurately indicate toxic compounds in sediments. Adverse habitat conditions modify the response (Parren et al. 1993), but do not seem to be causative. Growth and development retardation, on the other hand, are provoked both by food shortage and environmental contamination, with possible interferences of both factors. Although this limitation can be overcome in lab tests, these parameters do not seem very practical for field surveys.

Field surveys of benthic community structure using presence-absence data, diversity and density measurements are often used, but have several limitations. The absence of macroinvertebrates does not necessarily implicate sediment toxicity as the causal factor: differences among populations may be caused by differing physico-chemical parameters and the occurrence of short-term chemical and physical stressors (pH, temperature, alkalinity) may affect community structure without leaving

toxic residues in the sediment (Giesy et al. 1988, Giesy & Hoke 1989). The Belgian Biotic Index (BBI) is a diversity index based on macroinvertebrate community structure mainly oriented to eutrophication and saprobicity problems (De Pauw & Vanhooren 1983). However, the presence of toxicants in the sediment might modulate the BBI as well, and therefore the BBI might be designated as an index of "global pollution" (De Pauw et al. 1986, De Pauw and Vannevel 1991). Studies by Nguyen Thi Hong (1992) and Janssens de Bisthoven (1995) demonstrated that the occurrence of chironomid deformities in Belgian lowland rivers was independent from the BBI, implying that deformities generate exclusive information pertaining to the toxicity of aquatic sediments, information not detectable with classic biotic diversity measurements.

While growth and development reduction are most useful in lab tests and community structure analyses in field studies, chironomid deformities have the advantage of being both useful in field and laboratory studies, and even in studies intermediate between lab and field by exposing lab cultures *in situ* to contaminated sediments (Van de Guchte, pers. comm.). However, since it is likely that no one test will suffice to assess sediment toxicity, a combination of bioindicators will probably be required (Giesy et al. 1988, Warwick 1990c). The combined use of field surveys, laboratory bioassays and chemical analyses of contaminated sediments offers an integrated approach to assess sediment contamination (Triad: Chapman 1986), and the use of chironomid deformities seems very promising in the application of this Triad concept (van de Guchte 1994, Canfield et al. 1995).

However, because of a lack of experimental background data, many studies using chironomid deformities remain rather superficial and do not optimally utilise all the information included in deformity responses. The experimental requirements needed to establish a complete and reliable deformity screening method, are presented in Fig. 5. Summarised, the necessary investigations include: identification of causal agents, investigation of contaminant-specific responses, establishment of dose-response curves, investigations of environmental modification of deformity responses, and studies of the joint toxicity of the causal compounds.

Several recent studies on chironomid deformities integrate the subject in a more ecological context (van Urk et al. 1992, Diggins & Stewart 1993). Such studies illustrate the usefulness of chironomid deformities as tools for screening environmental degradation in an integrated approach together with diversity indices, density measurements and life cycle studies, and demonstrate their potential in offering a more complete and ecologically relevant picture of aquatic ecosystem health.

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