Combined action of pesticides towards aquatic organisms

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A review of available literature from 1972 - 1998

J.W. Deneer

Alterra-rapport 004

Alterra, Green World Research, Wageningen, 2000

ABSTRACT

J.W. Deneer, 2000. *Combined action of pesticides towards aquatic organisms; a revoew of available literature from 1972 - 1998.* Wageningen, Alterra, Green World Research. rapport 004. 46 p.; 1 Appendix.

Literature from 1972 to 1998 was critically reviewed and used to assess to which degree the concept of 'concentration addition' (CA) is able to correctly predict the acute toxicity of mixtures of pesticides towards aquatic organisms. For more than 90% of 202 mixtures in 26 studies CA predicted the observed toxicity within a factor 2. There were no apparent differences between mixtures of compounds with similar or dissimilar toxic modes of action. Deviations from CA did occur, but were mostly limited in extent. Combinations of an organophosphorus ester or a carbamate with either another organophosphorus ester or a synthetic pyrethroid were identified as the mixtures most frequently leading to deviations from CA.

Keywords: aquatic organisms, mixture toxicity, pesticides

ISSN 1566-7197

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Project 81183 [rapport 004/HM/07-2000

Contents

Preface

Pesticides are introduced into the environment with the purpose of destroying pests and protecting crops. In most agricultural areas several different crops are grown at the same time on neighbouring fields, which often results in the presence of a broad range of pesticides in local surface waters. Moreover, since transport of pesticides can also proceed via the atmosphere, pesticides not used in the area itself may be found in the surface water. The use of pesticides thus results in the presence of mixtures of toxicologically very active substances in the surface water in agricultural areas during the growing season.

Understandably, the study of joint toxicity of pesticides has mostly been concerned with the action of mixtures of pesticides towards target pest organisms, in an effort to improve the efficacy of commercially available products. Only little is known about the combined action of pesticides towards non-target aquatic organisms like fish, algae and waterfleas. Information upon which to base a choice of either concentration addition or response addition to predict the joint (non-interactive) toxicity is only seldom available, since knowledge about the modes of action of pesticides in non-target organisms is often lacking.

When trying to predict the toxicity of a given mixture of compounds the choice of using either the concentration addition (CA) or the response addition (RA) model will often be influenced by the amount of information available. When using CA the information needed consists of the median toxicity values (LC_{50}'s) of the substances for the organism of choice, since the concentration of each compound has to be expressed as a fraction of its LC_{50}. Using RA, not only the LC_{50}'s of all compounds have to be known, but additional information about the steepness of the dose-response curve of each compound is needed. If this information is lacking, the use of RA is not possible (except in the special situation where the concentration of each compound equals its LC_{50}). Unfortunately, even today many papers reporting LC_{50} values for aquatic organisms do not include information about the steepness of the dose-response curve, resulting in the inability to assume RA in calculations of mixtures containing the compounds involved. The most suitable way of predicting the joint toxicity of pesticides appears, therefore, to be based on CA. The aim of the literature review described in this report is to assess the reliability of CA based predictions on the basis of experimental results reported in scientific literature from 1972 – 1998.

This study was carried out in the framework of a research project on ecotoxicological risks of pesticide mixtures in freshwater ecosystems. Partners within this research project were Wageningen University (Department of Aquatic Ecology and Water Quality Management), the National Institute of Public Health and the Environment (RIVM), the National Institute for Inland Water Management and Waste Water Treatment (RIZA) and Alterra. The work presented in this report was financially supported by research programma 359 of the Dutch Ministry of Agriculture, Nature

Management and Fisheries. Results of the study were first published in Pest Management Science Vol. 56, pp. 516 – 520, for which the copyright is owned by the Society of Chemical Industry. They are reproduced here as chapter 1 with kind permission of John Wiley & Sons on behalf of SCI.

Appendix 1: Summaries of individual mixture toxicity studies of pesticides used in the present evaluation of combination toxicity of pesticides.

Title Toxicity evaluation of single and chemical mixtures of Roundup, Garlon-3A, 2,4-D and Syndets surfactant to channel catfish (*Ictalusus punctatus*), bluegill sunfish (*Lepomis macrochirus*), and crawfish (*Procambarrus* spp.)

Author(s) Abdelghani, A.A., P.B. Tchounwou, A.C. Anderson, H. Sujono, L.R. Leyer and A. Monkiedje

Source Environ. Toxicol. Water Qual. 12, 237-243 (1997).

Test method Semi-static.

GLP No.

Test substance Roundup (35.6% glyphosate), Garlon-3A (31.8% trichlopyr), 2,4-D (39.3% 2,4-dichlorophenoxyacetic acid), Syndets (80% polyethylene glycol alkyl ether, anionic surfactant).

Vehicle None.

Test species Juvenile channel catfish (*Ictalurus punctatus*) 2-3 inches long, juvenile bluegill sunfish (*Lepomis macrochirus*) 2-3 inches long and crawfish (*Procambarus* spp.), which were declawed after capture. All animals were acclimated for 3-4 weeks before use in the experiments. Mixture toxicity experiments were carried out with bluegill sunfish only.

No. of fish 10 animals per 10 l glass aquarium.

Concentrations At least 5 test concentrations and a control. 3 Replicates per concentration.

Conditions Semi-static, solutions were changed daily. Dechlorinated tap water, total hardness 128 ± 5 mg/l, alkalinity of 62 ± 2 mg/l, pH 7.5 ± 0.5, dissolved oxygen 7.6 ± 0.4 mg/l. A 12 hour light/12 hour darkness photoperiod was used.

Study duration 96 hours.

Observations Mortality after 0, 24, 48, 72 and 96 hours of exposure.

Physical meas. During testing: none. Temperature, pH, oxygen, hardness and alkalinity were measured in the acclimation water.

Results

Chemical	LC50 (mg a.i./l)					
	Channel catfish		Bluegill sunfish		Crawfish	
	48 h	96 h	48 h	96 h	48 h	96 h
Surfactant	2.4 ± 0.1	2.3 ± 0.1	1.9	1.9	22 ± 8	15 ± 6
Roundup	5.5 ± 0.7	4.9 ± 0.4	4.5 ± 0.3	4.4 ± 0.2	32650 ± 5284	21633 ± 4706
Garlon-3A	123 ± 7	109 ± 7	94 ± 3	91 ± 8	9060 ± 601	6398 ± 341
2,4-D	224 ± 21	181 ± 33	313 ± 20	266 ± 38	1436 ± 634	750 ± 7

Mixture toxicity was assessed using blugill sunfish only. Mixture composition at 50% effect:

Roundup + Garlon	1.24 + 0.11 = 1.35 TU
Roundup + 2,4-D	1.15 + 0.09 = 1.24 TU
Surfact. + Roundup + Garlon	0.68 + 0.32 + 0.03 = 1.03 TU
Surfact. + Roundup + 2,4-D	0.77 + 0.38 + 0.03 = 1.18 TU

Conclusions

For the fish species the order of toxicity was Roundup > Garlon > 2,4-D. For crawfish the order of toxicity was 2,4-D > Garlon > Roundup.
Mixture toxicity was close to concentration addtion.

Rev. note

1. The combination of Garlon and 2,4-D, which presumably act through similar mechanisms in fish, was not tested.
2. No actual concentrations were measured in the test vessels, all calculations are based on nominal concentrations.

Title	Determination of LC50 of pirimor and nuvacron on *Tilapia nilotica* fish.
Author(s)	Abdel-Nasser, M.
Source	Assiut. Vet. Med. J. 25, 132-139 (1991).
Test method	Static.
GLP	No.
Test substance	Pirimor (50% pirimicarb), Nuvacron (400 g/l monocrotophos).
Vehicle	None.
Test species	*Tilapia nilotica*, 6-8 cm length, 35-50 g body weight. Fish were transferred to the test vessels and acclimated for 2 weeks before the start of the experiments. Mixture toxicity experiments were carried out after determinations of LC50 of individual compounds.
No. of fish	8 animals per 20 l glass aquarium.
Concentrations	Five test concentrations and a control. No replicates per concentration.
Conditions	Static, solutions were changed daily. Tap water, total alkalinity of 30 – 38 mg/l as $CaCO_3$, pH 6.4 – 6.8, temperature 23 ± 2^{o}C, aerated with compressed air. Details about the photoperiod are not given.
Study duration	Not given, presumably 96 hours.
Observations	Not given.
Physical meas.	During testing: none.
Results	The 96-hour LC50 of Pirimor and Nuvacron were 145 mg/l (95% confidence limits: 94 – 223 mg/l) and 35 mg/l (95% confidence limits: 28 – 44 mg/l) resp. Combination toxicity was tested using a mixture containing equitoxic amounts of both compounds. The mixture causing 50% mortality (LC50: 33.3 mg/l, 95% confidence limits: 23 – 49 mg/l) consisted of 26.8 mg/l Pirimor and 6.5 mg/l Nuvacron, and was 2.7 times more toxic than expected on the basis of concentration addition.
Conclusions	The equitoxic mixture of the carbamate pirimicarb and the organophosphorus ester monocrotophos was approx. 2.7 times more toxic to *Tilapia nilotica* than predicted by concentration addition.
Rev. note	The duration of the test is not given, which makes it difficult to compare results to other studies. In view of the fadt that a static test system was used, the duration of the test was probably 96 hours.

Title	Eva.luation of the isobologram method for the assessment of mixtures of chemicals.
Author(s)	Altenburger, R., W. Bödeker, M. Faust and L.H. Grimme.
Source	Ecotoxicol. Environ. Saf. 20, 98-114 (1990).
Test method	Static.
GLP	No.
Test substance	Amitrole, atrazine, glufosinate-ammonium, metribuzin (all 99% purity). Mixtures of atrazin + metribuzin and of amitrole + glufosinate-ammonium were tested at various compositions (including the pure compounds).
Vehicle	For the atrazin+metribuzin mixtures acetone was used, which was always less than 0.1% in the test medium. For amitrole+glufosinate no vehicle was used.
Test species	*Chlorella fusca* var. *vacuolata* Shih. Et Krauss, cultured at 10^6 cells/ml at 28.0±0.5°C.
Concentrations	Six mixtures metribuzin+atrazin (100:0, 90:10, 75:25, 50:50, 25:75, 0:100) and five mixtures amitrole+glufosinate (100:0, 13:87, 5:95, 2:98, 0:100) were tested (all concentration ratios are given on a molar basis).
Conditions	Algeal growth medium, temperature 28.0±0.5°C, aerated with water-saturated, CO_2-enriched (1.5-2.0% v/v) compressed air. Light intensity of 22-33 kLux, photon flux density of approx. 370 $\mu E\ m^{-2}\ s^{-1}$ at the water surface. Standard cell density at the start of the exposure 10^6 cells/ml.
Study duration	24 Hours.
Observations	Cell volume growth and cellular reproduction during a one-generation cycle. Samples were taken at t=0, t=14 hours and t=24 hours.
Physical meas.	During testing: none.
Results	The mixtures of metribuzin+atrazin (similar mode of action) closely corresponded to concentration addition, the mixtures of amitrole+glufosinate (dissimilar mode of action) were slightly less toxic than predicted by CA. Mixture toxicity was analyzed using isobolograms.
Conclusions	The authors conclude that the usefullness of isobolograms depends on the possibilities of statistical evaluation, i.e. deciding whether a given effect significantly differs from the effect predicted by CA. They attempt to achieve this by using 95% confidence intervals of effect concentrations and constructing a 95% confidence belt around the CA isobole. If the confidence interval of the experimental effect concentration does not overlap the confidence belt of the CA isobole, then CA is not a valid assumption.
Rev. note	Primary aim of the paper is the evaluation of the usefulness of isobolograms for the analysis of the combined toxicity of mixtures of compounds. The authors themselves indicate that the proposed method will be difficult to employ when dealing with mixtures of more than 2 compounds.

Title	Regulations for combined effects of pollutants: consequences from risk assessment in aquatic toxicology.
Author(s)	Altenburger, R., W. Bödeker, M. Faust and L.H. Grimme.
Source	Food Chem. Toxicol. 39, 1155-1157 (1996).
Test method	Static.
GLP	No.
Test substance	14 Pesticdes ( ), 5 surfactants ( ) and 137 binary mixtures of these compounds.
Vehicle	None.
Test species	*Chlorella fusca* var. *vacuolata* Shih. Et Krauss, cultured at 10^6 cells/ml at 28.0±0.5°C.
Conditions	Algal growth medium, temperature 28.0±0.5°C, aerated with water-saturated, CO_2-enriched (1.5-2.0% v/v) compressed air. Light intensity of 22-33 kLux, photon flux density of approx. 370 $\mu E\ m^{-2}\ s^{-1}$ at the water surface. Standard cell density at the start of the exposure 10^6 cells/ml.
Study duration	24 Hours.
Observations	Algal reproduction. Analysis of combination toxicity, i.e. deviations from predictions based on the concentration addition and the response addition models, is performed using IPQ (Index of Prediction Quality).
Results	Approx. 80% of all pesticide/pesticide mixtures were in agreement with the predictions derived from the concept of concentration addition (CA), while 43% were in agreement with the prediction based on response addition (RA). Hence, CA is a better predictive model than RA for the toxicity of binary combinations of the pesticides studied. Approx. 60% of pesticide/surfactant mixture agreed with predictions by CA, 46% agreed with predictions by RA.
Conclusions	The authors conclude that concentration addition leads to reasonable predictions for the combination effects on integral effect levels, irrespective of the pharmacological action of the mixture components.
Rev. note	Unfortunately the authors do not present detailed results of the tests conducted. It is e.g. not clear which combinations were tested and which were not. No mention is made of which compounds were actually tested, only that mixtures were selected consisting of compounds with either similar or dissimar modes of action.

Title	Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*.
Author(s)	Bailey, H.C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic, T. Shed.
Source	Environ. Toxicol. Chem. 16, 2304-2308 (1997).
Test method	Static, US EPA 600/4-90/027, Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (1991).
Test substance	Diazinon (99%), chlorpyrifos (99%) and mixtures of constant concentration ratios of these compounds were tested in laboratory water and in 2 types of natural water.
Concentrations	Nominal concentrations of diazinon: 0.05, 0.10, 0.20, 0.40 and 0.80 μg l^{-1}. Nominal concentrations for chlorpyrifos: 0.008, 0.016, 0.033, 0.066 and 0.132 μg l^{-1}. Nominal concentrations of diazinon and chlorpyrifos in the mixtures: 0.05/0.008, 0.10/0.016, 0.20/0.033, 0.40/0.066 and 0.80/0.132 μg l^{-1}. Four replicates for each solution. Only initial concentrations were measured. All effect concentrations were calculated on the basis of measured (initial) concentrations.
Vehicle	Methanol, < 0.1%.
Test species	*Ceriodaphnia dubia* neonates, < 24 h at the start of the exposure.
Conditions	Temperature 25±1°C. 16 Hour light/8 h dark photoperiod. Animals were not fed during the exposure. Dissolved oxygen (7.6-8.4 mg l^{-1}), pH (7.40-8.23), conductivity (290-320 μS cm^{-1}) were measured at the start and at the end of the exposure. Hardness (80-100 mg l^{-1} as $CaCO_3$) and alkalinity (100-120 mg l^{-1} as $CaCO_3$) were measured at the start of the experiment in the dilution water. Temperature (25-26°C) was measured daily.
Study duration	48 Hours for 2 tests in laboratory water, 96 h for 2 other tests in laboratory water, 72 h for tests in natural waters.
Observations	Survival, daily observations. Combination toxicity was evaluated on the basis of summation of toxic units.
Results	Control survival always > 95% LC50's are given in the Table below.

Type of water, duration	LC50 (μg l^{-1}) of single compounds		LC50 (TU) of compounds in mixtures	
	Diazinon	Chlorpyrifos	Diazinon	Chlorpyrifos
2x Laboratory, 48 h	0.28 (0.19 – 0.46)	0.065 (0.055 – 0.078)	0.70	0.48
2x Laboratory, 96 h	0.34 (0.27 – 0.38)	0.054 (0.040 – 0.071)	0.69	0.41
1x Natural 1, 72 h	0.33 (0.28 – 0.38)	0.107 (0.091 – 0.123)	0.45	0.33
1x Natural 2, 72 h	0.43 (0.36 – 0.50)	0.068 (0.056 – 0.080)	0.58	0.47

The LC50 of chlorpyrifos in natural waters was somewhat higher than found in laboratory water, which may have been the result of binding of chlorpyrifos to fine particulate matter. The sum of Toxic Units observed in the mixtures corresponding to 50% mortality ranged from 0.78-1.18 TU. Mixtures containing 0.95-1.20 total TU's resulted in 40-65% mortality.

Conclusions	The authors conclude that diazinon and chlorpyrifos exert concentration additive toxicity to *C. dubia*.

Title	Acute and chronic toxicity of rice herbicides thiobencarb and molinate to Opossum shrimp (*Neomysis mercedis*).
Author(s)	Bailey, H.C.
Source	Marine Environ. Res. 36, 197-215 (1993).
Test method	Continuous flow.
Test substance	Thiobencarb, molinate (both technical grade) and mixtures of these compounds.
Concentrations	Exposure was continued until no mortality was observed over a 48 h period, which resulted in a test duration of 18 days for thiobencarb and 28 days for molinate. Exposure concentrations were measured 2 times per week. Effect concentrations were calculated on the basis of measured concentrations.
Vehicle	None.
Test species	Opossum shrimp, *Neomysis mercedis*, caught in the wild, reared at 16-20°C.
Conditions	A 16 Hour light / 8 h dark photoperiod was used. Animals were fed daily during the exposure. Dissolved oxygen (7.6-9.4 mg l^{-1}), pH (8.0-8.9), conductivity (3400-3900 µS cm^{-1}) and temperature (16-20°C) were measured daily. Test solutions were replaced continuously at a rate of 2 replacements per day.
Study duration	Mixture toxicity was evaluated after 7, 14 and 28 days of exposure.
Observations	Survival, daily observations. Combination toxicity was evaluated on the basis of summation of toxic units.
Results	Control survival always > 95% LC50's are given in the Table below.

Test duration (days)	LC50 (µg l^{-1}) of single compounds, 95% confidence limits		LC50 (TU) of compounds in mixtures	
	Thiobencarb	Molinate	Thiobencarb	Molinate
7	214 (174 – 258)	2530 (1870 – 3560)	0.33	0.82
14	91 (52 – 137)	820 (570 – 1040)	0.26	0.94
18	53 (32 – 100)	-	-	-
28	53 (assumed value)	230 (150 – 340)	0.17	1.26

Conclusions	The author concludes that Thiobencarb and Molinate (2 thiocarbamates) exhibited no significant interactive effects.
Rev. note	1. Apart from mixture toxicity acute and chronic toxicity of the single compounds were assessed. Since no mixture toxicity was evaluated in these tests they are not considered here. 2. The 28-day LC50 value for Molinate was assumed to be the same as the 18-day value. This assumption does not affect the conclusion of the study.

Title Toxicity of the herbicides diquat and endothall to goldfish.

Author(s) Berry Jr., C.R.

Source Environ. Pollut (Series A) 34, 251-258 (1984).

Test method Static.

Test substance Diquat, endothall and a 1:1 (concentration) mixture of these compounds.

Concentrations 23 – 98 mg l-1 diquat, 32 – 200 mg l-1 endothall and mixtures of 23 – 74 mg l-1 of each herbicide. Exposure concentrations were not verified through analytical measurements. Effect concentrations are based on nominal concentrations.

Vehicle None.

Test species Goldfish, *Carassius auratus*, acclimatized for at least 2 weeks prior to use.

Conditions Dechlorinated tap water was used to prepare dilutions. Total hardness was 50 mg l^{-1}, pH was "circumneutral". Tests were run at 20-23^{o}C and aeration was provided by bubbling air through a glass tube into the test vessels. The frequency of measurement of physical conditions is not given. A 14 Hour light/10 h dark photoperiod was used.

Study duration 96 Hours.

Observations Survival, daily observations. Combination toxicity was evaluated on the basis of summation of toxic units.

Results

Compound	96-h LC50 (mg l^{-1}) (95% confidence limits)	LC50 (mg l^{-1}) of compounds in mixtures	LC50 (TU's) of compounds in mixtures
Diquat	85 (76 – 95)	64	0.75
Endothal	372 (340 – 406)	64	0.17

Conclusions The author concludes that diquat and endothal (2 herbicides) exhibited no significant interactive effects and that their joint toxicity is described by concentration addition.

Rev. note 1. Apart from acute mortality tissue damage was also investigated. Since the findings were not discussed quantitatively no mixture toxicity was evaluated in these tests and they are not considered here.

Title	Combined effects of alachlor and atrazine on benthic algal communities in artificial streams.
Author(s)	Carder, J.P., K.D. Hoagland.
Source	Environ. Toxicol. Chem. 17, 1415-1420 (1998).
Test method	Artificial streams, dosed once with solutions of the test substances.
Test substance	Alachlor, atrazine and 2 mixtures of these compounds.
Concentrations	5 μg l-1 alachlor, 90 alachlor, 12 atrazine, 150 atrazine, or combinations of alachlor+atrazine. All solutions were tested in duplicate. Exposure concentrations were verified through analytical measurements 24 h and after 4 weeks of exposure. Calculated effect concentrations are based on nominal concentrations.
Vehicle	None.
Test species	Benthic algae, collected from a natural system.
Conditions	Stream water collected from a natural sediment-dominated stream. Conductivity (0.89-1.40 mS cm^{-1}), pH (8.8±0.1), dissolved oxygen (9.0±0.3 mg l^{-1}), total N (1.0-1.6 mg l^{-1}), total P (0.08-0.12 mg l^{-1}) and temperature (17.4±0.4°C) were measured when sampling algae, at 24 h, 1, 2 and 4 weeks after herbicide addition.
Study duration	28 Days.
Observations	Cell density, cell biovolume and relative abundance of the 6 dominant taxa. Samples were taken 24 h, 1, 2, and 4 weeks after herbicide addition.
Results	The biovolume of algae after 1, 2 and 4 weeks after treatment was decreased at all treatment levels. Significant decreases were observed in all treatments with high atrazine concentrations (HiAt, HiAt+LoAl, HiAt+HiAl) at 1, 2 and 4 weeks. Significant decreases were also observed in all treatments with low atrazine concentrations (LoAt, LoAt+LoAl, LoAt+HiAl) at 1 and 4 weeks post-treatment, but in none of the 3 treatments with low atrazine concentrations at 2 weeks post-treatment. Single compound treatment with low concentrations of alachlor did not significantly affect biovolume at 1, 2 and 4 weeks post-treatment. Single compound treatments with high concentrations of alachlor did not significantly affect biovolume at 1 and 2 weeks post-treatment, but did cause a reduction in biovolume 4 weeks post-treatment.
Conclusions	The author concludes that the lack of significant synergistic effects may be attributed to the herbicides (alachlor and atrazine) having unique modes of action, but that it is also possible that no interaction was observed because atrazine and alachlor impact algal communities over different time scales, i.e. that effects caused by alachlor needed longer to develop than the effects caused by atrazine.
Rev. note	1. The experiment was carried out as a 3x3 factorial design, which makes it unfeasible to analyse combination toxicity in terms of agreement with predictions on the basis of concentration addition (CA). However, the results are not indicative of a strong interaction between atrazine and alachlor, and are therefore considered to be in agreement with CA.

Title	Toxicity of sediments containing atrazine and carbofuran to larvae of the midge *Chironomus tentans*.
Author(s)	Douglas, W.S., A. McIntosh and J.C. Clausen.
Source	Environ. Toxicol. Chem. 12, 847-853 (1993).
Test method	Static. Sediment slurry (3.2% clay, 7.0% silt, 89.8% sand; organic matter 4.85% by weight) was spiked with atrazine and/or carbofuran. Water was added and the suspended matter was allowed to settle, after which the midge larvae were added.
Test substance	Atrazine, carbofuran and mixtures of these compounds.
Concentrations	5 Nominal concentrations of carbofuran (0, 25, 45, 83 and 150 μg carbofuran kg^{-1} dry weight), 4 nominal concentrations of atrazine (0, 5, 10, 20 mg atrazine kg^{-1} dry weight); for the mixtures all combinations of these concentrations were used (complete block design). Five replicates for each exposures were used.
Vehicle	None. Acetone was used to prepare stock solutions, but was drawn off under vacuum.
Test species	Second-instar larvae of the midge, *Chironomus tentans*, 20 larvae per beaker.
Conditions	250 Ml sediment slurry, brought to 950 ml total in a 1 l all glass beaker; 20 larvae per beaker; temperature 23±2°C; 16:8 h light:dark cycle; pH: 7.5-8.5; dissolved oxygen: 4.9-8.3 mg l^{-1}; alkalinity from 35-70 mg l^{-1} as $CaCO_3$; hardness 28-68 mg l^{-1} as $CaCO_3$; in the combination toxicity experiments the observed ranges in physical parameters (values given) were usually wider than observed in the single compound experiments.
Study duration	10 Days.
Observations	Mortality after 10 days of exposure, determined at the end of the exposure period.
Results	For atrazine no LC50 could be calculated (less than 50% mortality at highest exposure). The 10-day LC50 for carbofuran was 47.9 μg kg^{-1} dry weight (95% confidence limits: 43.9-52.1 μg kg^{-1} dry weight), equivalent to 11.8 μg l^{-1} (10.9-17.1 μg l^{-1}) in the interstitial water. ANOVA indicated that the presence of both pesticides together did not change the toxicity more than would be expected from adding the effects of each pesticide separately.
Conclusions	The author conclude that ANOVA is a suitable technique for studying the joint toxicity of chemicals. No interactions have been observed for the mixture of carbofuran and atrazine in the present study.
Rev. note	1. The experiment was carried out as a 3x3 factorial design, using ANOVA for identifying possible interactions. This technique is not really able to analyse combination toxicity in terms of Toxic Units, as is done when using procedures involving Toxicity Indices and similar ways of analyzing data. However, the results are not indicative of a strong interaction between atrazine and alachlor. On the basis of mortality and concentration data that the authors present it is obvious that mortality does not deviate substantially from what would be expected on the basis of concentration addition. 2. The authors statement that "… did not change the toxicity more than would be expected from adding the effects of each pesticide separately" is misleading since neither concentration addition or response addition involve "adding the effects" (if algebraic summation is meant).

Title	Synergistic action of organophosphorus pesticides on fish, *Oreochromis mossambicus*.
Author(s)	Durairaj, S. and V.R. Selvarajan.
Source	J. Environ. Biol. 16, 51-53 (1995).
Test method	Semi-static, renewal of solutions every 24 hours.
Test substance	Quinalphos and phentoate (both technical grade, 90-95% purity).
Concentrations	Pesticides were mixed 1:1 in acetone stock. Fish were exposed to nominal overall concentrations of 0.40, 0.45, 0.50, 0.55, 0.60 and 0.65 mg quinalphos+phentoate l^{-1}. Choice of concentration was based on previous work where the LC50 of quinalphos was established at 14.5 mg l^{-1} and the LC50 of phentoate at 2.5 mg l^{-1}. No replicates. No chemical analysis, effect concentrations are based on nominal initial concentrations.
Vehicle	Acetone.
Test species	Fish, *Oreochromis mossambicus* (Peters), 5±2 g, 7 ± 2 cm at the start of the exposure.
Conditions	Ten fish per concentration, renewal of solutions at 24, 48, 72 and 96 h. Details on phyical conditions (pH, temperature, light etc.) are not given.
Study duration	96 Hours.
Observations	Mortality, every 24 h; overall mortality was determined at the end of the exposure period (96 h).
Results	The 96-h LC50 of the mixture was 0.51 mg l^{-1}. This mixture contained 0.255 mg l-1 of each pesticide, and hence 0.018 TU quinalphos and 0.10 TU phentoate.
Conclusions	The mixture was approx. 10 times more toxic than predicted by concentration addition.
Rev. note	1. No details on physical conditions are given, which makes it unfeasible to determine the reliability of the experiment. 2. Evaluation of mixture toxicity is based on LC50 values determined in a previous study. Although the LC50 values were determined by the same authors and presumably in the same laboratory, such an approach increases the uncertainty in the quantitative analysis of combination toxicity experiments. 3. The mixture is far from equitoxic. Based on Toxic Units, the concentration of phentoate is approx. 5 times higher than the concentration of quinalphos.

Title	Apparent potentiation of the cotton defoliant DEF by methyl parathion in the mosquitofish.
Author(s)	Fabacher, D.L., J.D. Davis and D.A. Fabacher.
Source	Bull. Environ. Contam. Toxicol. 16, 716-718 (1976).
Test method	Static.
Test substance	DEF (S, S, S-tributyl phosphorotrithioate), methyl parathion and one mixture of these compounds.
Concentrations	DEF: 0.5 mg l^{-1}; methyl parathion: 5 mg l^{-1}; mixture: 0.5 mg l^{-1} DEF + 5 mg l^{-1} methyl parathion. Six replicates. From previous studies using mosquitofish the 24-h LC50 of DEF was 0.8 mg l^{-1}, and the 48-h LC50 of methyl parathion was 13 mg l^{-1}.
Vehicle	Acetone, 1.05 ml l^{-1} water.
Test species	Mosquitofish *Gambusia affinis*.
Conditions	Twelve fish per aquarium, containing 6 l of dechlorinated tap water. No renewal of solutions. Details on phyical conditions (pH, temperature, light etc.) are not given.
Study duration	24 Hours.
Observations	Mortality after 24 h.

Results

Compound, concentration	24-Hour mortality
DEF, 0.5 mg l^{-1}	0%
Methyl parathion, 5 mg l^{-1}	8%
DEF 0.5, mg l^{-1} + Methyl parathion, 5 mg l^{-1}	89%

Conclusions	The mixture contained approx. 0.63 Toxic Unit DEF and 0.39 Toxic Unit Methyl parathion, 1.02 TU in all, and caused 89% mortality. This is slightly more than would be expected on the basis of concentration addition. The authors conclude that “These data indicate an apparent pronounced increase in the toxicity of DEF by methylparathion in mosquitofish.”
Rev. note	1. No details on physical conditions are given, which makes it unfeasible to determine the reliability of the experiment. 2. TU’s are based on LC50 determined in separate studies (1 in the same laboratory, the other taken from the literature and performed in another laboratory). Therefore there is some uncertainty about the number of Toxic Units present in the mixture. 3. The authors conclusion that there is an “apparent pronounced increase in the toxicity of DEF by methyl parathion in mosquitofish” is premature since no information on the dose-response relationships for the 2 compounds is given. If the compounds have a steep dose-response relationship then the outcome of the mixture study is in agreement with concentration addition. 4. The drawback of this type of block design, using only a single exposure concentration of each toxicant, is a complete lack of knowledge about the dose-response relationship, which makes a reliable interpretation of combination toxicity impractical.

Title	Additive effects of herbicide combinations on aquatic non-target organisms.
Author(s)	Faust, M., R. Altenburger, W. Boedeker and L.H. Grimme.
Source	Sci. Tot. Environ. Supplement 1993, 941-952 (1991).
Test method	Static.
Test substance	Nine herbicides: atrazine, simazine, chlorotoluron, methabenzthiazuron, bentazone, metazachlor, tri-allate, glyphosate (isopropylamine salt) and 2,4-D. All compounds were of analytical standard quality (98-99% purity). Of these 9 herbicides 29 binary mixtures were composed, 21 consisting of compounds with dissimilar modes of action and 8 consisting of compounds with similar modes of action.
Concentrations	At least 11 concentrations for single compounds, at least 6 concentrations for mixtures. The spacing between the concentrations was adjusted to the shape (steepness) of the dose-response curve.
Vehicle	Acetone, always less than 0.1 ml l^{-1} water.
Test species	Unicellular green alga, *Chlorella fusca*.
Conditions	Algal growth medium, temperature 28.0±0.5°C, aerated with water-saturated, CO_2-enriched (1.5-2.0% v/v) compressed air. Light intensity of 22-33 kLux, photon flux density of approx. 370 $\mu E\ m^{-2}\ s^{-1}$ at the water surface. Standard cell density at the start of the exposure 10^6 cells/ml.
Study duration	24 Hours.
Observations	Inhibition of reproduction after 24 h.
Results	For 25 of the 29 binary mixtures the response closely adhered to concentration addition. For 4 mixtures slight deviations from concentration addition were found; methabenzthiazuron/simazine and methabenzthiazuron/metazachlor were slightly more toxic than predicted by CA, whereas metazachlor/bentazon and glyphosate/2,4-D were slightly less toxic. However, differences between predicted and experimental EC50 values never exceed a factor of two.
Conclusions	The toxicity of all 29 mixtures (8 with similar modes of action, 21 with dissimilar modes of action) corresponded to Concentration Addition.
Rev. note	1. The authors reach very high precision in their analysis due to a very close spacing of the tested concentrations and a high degree of reproducibility of the response of the algae tested.

Title	Algal toxicity of binary combinations of pesticides.
Author(s)	Faust, M., R. Altenburger, W. Boedeker and L.H. Grimme.
Source	Bull. Environ. Contam. Toxicol. 53, 134-141 (1994).
Test method	Static.
Test substance	2 Fungicides (anilazine, prochloraz), 2 insecticides (lindane, parathion) and 8 herbicides (bentazone, chlorotoluron, methabenzthiazuron, metazachlor, simazine, tri-allate, glyphosate (isopropylamine salt), 2,4-D). Of these compounds 38 binary mixtures were composed, none of which consisted of herbicide/herbicide combinations.
Concentrations	At least 11 concentrations for single compounds, at least 6 concentrations for mixtures. The spacing between the concentrations was adjusted to the shape (steepness) of the dose-response curve.
Vehicle	Acetone, always less than 0.1 ml l^{-1} water.
Test species	Unicellular green alga, *Chlorella fusca*.
Conditions	Algal growth medium, temperature 28.0±0.5°C, aerated with water-saturated, CO_2-enriched (1.5-2.0% v/v) compressed air. Light intensity of 22-33 kLux, photon flux density of approx. 370 $\mu E\ m^{-2}\ s^{-1}$ at the water surface. Standard cell density at the start of the exposure 10^6 cells/ml.
Study duration	24 Hours.
Observations	Inhibition of reproduction after 24 h.
Results	For 35 of the 38 binary mixtures the response closely adhered to concentration addition. For 3 mixtures slight deviations from concentration addition were found; prochloraz/2,4-D was approx. 3 times less toxic than predicted by CA, anilazine/prochloraz was approx. 2.1 times less toxic than predicted by CA and anilazine/tri-allate was approx. 3 times more toxic than predicted by CA. For the 35 other combinations differences between predicted and experimental EC50 values were within a factor of two.
Conclusions	The toxicity of all 35 of the 38 mixtures corresponded to Concentration Addition.
Rev. note	1. The authors reach very high precision in their analysis due to a very close spacing of the tested concentrations and a high degree of reproducibility of the response of the algae tested.

Title	The effect of certain intrinsic and extrinsic variables on the acute toxicity of selected organophosphorus insecticides to the mummichog, *Fundulus heteroclitus*.
Author(s)	Fulton, M.H. and G.I. Scott.
Source	J. Environ. Sci. Health B26, 459-478 (1991).
Test method	Semi-static, 24-h renewal of solutions.
Test substance	Azinphos-methyl, acefate and mixtures of azinphos-methyl / fenvalerate, azinphos-methyl / endosulfan, acephate / fenvalerate.
Concentrations	In mixtures the concentrations were selected to be representative of the LC50 ratios of the individual compounds.
Vehicle	Acetone, concentration in test solution not given, but equal for all exposure concentrations.
Test species	Mummichog, *Fundulus heteroclitus*, a marine fish species.
Conditions	5 Liter glass aquaria, filled with either mid range salinity (20 mg l^{-1}) or low salinity (5 mg l^{-1}), temperature 20-25^{o}C. Light:dark cycle of 12:12 h. Ten fish were exposed to each concentration; two separate groups of 5 fish, consisting entirely of either male or female fish, were kept in 1 aquarium of 5 l. Other physical conditions (pH, hardness, oxygen levels) are not given and were probably not determined during the tests.
Study duration	96 Hours.
Observations	Mortality.
Results	The toxicity of the mixture of azinphos-methyl and endosulfan corresponded to concentration addition (CA) at low salinity and was slightly more toxic at high salinity. The toxicity of the mixture of acephate and fenvalerate was slightly less than predicted by CA at low salinity and corresponded to CA at high salinity. The toxicity of the mixture of azinphos-methyl and fenvalerate corresponded to CA both at low and at high salinity. Deviations from CA predicted toxicity were slight (less than a factor 2) for all mixtures, except for the acephate / fenvalerate mixture at low salinity (2.25 Toxic Units to evoke 50% effect). For the latter mixture the 95% confidence limits largely overlapped the 0.5 – 2 TU range which indicates CA.
Conclusions	The toxicity of all 6 mixtures studied (3 mixtures at 2 salinity levels each) corresponded to Concentration Addition.
Rev. note	1. Physical conditions prevailing during the tests are not fully described. 2. The LC50 values used for endosulfan and fenvalerate were taken from literature and not determined in the present study. This may in part explain some of the observed small deviations from CA.

Title	Joint toxicity of mixtures of 8 and 24 chemicals to the guppy (*Poecilia reticulata*).
Author(s)	Hermens, J. and P. Leeuwangh.
Source	Ecotoxicol. Environ. Saf. 6, 302-310 (1982).
Test method	Semi-static, 24-h renewal of solutions.
Test substance	26 Chemicals, mostly pesticides, from which 5 mixtures of 8 compounds and 1 mixture of 26 compounds were composed. Chemicals were selected on the basis of a presumed difference in mode of action.
Concentrations	At least 5 test concentrations for each compound or mixture; concentrations increased geometrically with a factor of 1.8 or 3.2 (factor not given). Mixtures contained identical fractions of the LC50 of each compound, i.e. they were equitoxic.
Vehicle	Acetone, 100 $\mu l\ l^{-1}$.
Test species	Guppy, *Poecilia reticulata*.
Conditions	1 Liter glass jars, relatively soft (25 mg l^{-1} as $CaCO_3$) reconstituted water, temperature 22 ± 1^oC. Light:dark cycle not given. Ten fish were exposed to each concentration. Oxygen levels and pH are not given.
Study duration	14 Days.
Observations	Mortality.
Results	For all 5 mixtures containing 8 compounds the observed toxicity was quite close to the toxicity predicted by concentration addition. These mixtures contained 1.1-1.7 Toxic Units at 50% mortality. The mixture containing 24 compounds was somewhat less toxic than predicted by concentration addition, containing 2.4 Toxic Units at 50% mortality.
Conclusions	The toxicity of mixtures of toxicants with diverse modes of action is near concentration addition. The minimum toxicity, depending on the hydrophobicity of the chemicals, can only slightly contribute to the final toxic action of the studied mixtures of 8 and 24 chemicals.
Rev. note	1. Physical conditions during the tests are not fully described.

Title	Method for assessiment of toxicity or efficacy of mixtures of chemicals.
Author(s)	Marking, L.L. and V.K. Dawson.
Source	Investigations in Fish Control 67, 1-7 (1975).
Test method	Not given.
Test substance	Malathion and Delnav (dioxathion).
Concentrations	Not described in the paper, a reference describing the determination of LC50 is given.
Vehicle	Not given.
Test species	Rainbow trout, *Salmo gairdneri*.
Conditions	Temperature 12^{o}C. Other physical conditions are not given, a reference describing the determination of LC50 is given.
Study duration	96 Hours.
Observations	Mortality.
Results	The mixture of malathion and dioxathion (Delnav) is much (approx. 7 times) more toxic than expected on the basis of concentration addition.
Conclusions	Malathion and dioxathion act highly synergistic against fish. These chemicals are known to increase each others toxicity towards insects as well.
Rev. note	1. Physical conditions prevailing during the tests are not fully described. 2. The paper deals mainly with the usefulness of the proposed Toxicity Index, and does not extensively discuss the phenomenon of joint toxicity of pesticides.

Title	Toxicity of paired mixtures of candidate forest insecticides to rainbow trout.
Author(s)	Marking, L.L. and W.L. Mauck.
Source	Bull. Environ. Contam. Toxicol. 13, 518-523 (1975).
Test method	Static.
Test substance	1 Carbamate (Zectran), 3 organophosphates (Dylox, Volaton and Guthion), a pyrethrum extract (SBP-1382) and a pyrethroid (RU-11679).
Concentrations	Not described in the paper, a reference describing the determination of LC50 is given.
Vehicle	Acetone, concentration not given.
Test species	Rainbow trout, *Salmo gairdneri*.
Conditions	Temperature 12°C. Other physical conditions are not given, a reference describing the determination of LC50 is given.
Study duration	96 Hours.
Observations	Mortality.
Results	Of the 20 binary equitoxic mixtures tested, 9 were slightly less than additive and 2 were slightly more toxic than additive. Of the 20 mixtures tested none deviated more than a factor 2 from concentration addition.
Conclusions	The joint toxicity of the 20 tested binary combinations of pesticides is described reasonably well by concentration addition.
Rev. note	1. Physical conditions prevailing during the tests are not fully described.

Title	Toxicity of mixtures of phosphamidon and methidathion to lobsters (*Homarus americanus*).
Author(s)	McLeese D.W. and C.D. Metcalfe.
Source	Chemosphere 2, 59-62 (1979).
Test method	Semi-static, renewal of solutions after 48 h.
Test substance	Phosphamidon, methidathion and 3 mixtures using different concentration ratios of these organophosphorus insecticides.
Concentrations	Not described for single compounds; for mixtures concentrations within a test series increased with a factor of 2.
Vehicle	Acetone, 1 ml/30 l.
Test species	Lobster, *Homarus americanus*, weighing at least 450 g.
Conditions	2 Lobsters per jar of 30 l. Temperature 20^{o}C. Other physical conditions are not given.
Study duration	96 Hours or longer when no 50% mortality had been reached after 96 h.
Observations	Mortality.
Results	The 3 mixtures tested (P:M ratios of 1 : 1.5, 1 : 4.4 and 2 : 1) appeared to be slightly more toxic than predicted by concentration addition. However, 2 of the mixtures did not deviate more than a factor 2 from concentration addition.
Conclusions	The joint toxicity of the binary combinations of pesticides does not deviate substantially from concentration addition. The toxicity of 2 of the 3 mixtures tested were within a factor 2 of concentration addition predicted toxicity.
Rev. note	1. Physical conditions prevailing during the tests are not fully described.

Title	Application of toxicity identification evaluation procedures to the ambient waters of the Colusa basin drain, California.
Author(s)	Norberg-King, T.J., E.J. Durhan and G.T. Ankley.
Source	Environ. Toxicol. Chem. 10, 891-900 (1991).
Test method	Not given.
Test substance	Methylparathion, carbofuran and mixtures (1:3, 1:1 and 3:1) these compounds.
Concentrations	Not given
Vehicle	Methanol, maximum concentration not given.
Test species	*Ceriodaphnia dubia*.
Conditions	No experimental details are given.
Study duration	48 Hours.
Observations	Mortality.
Results	The 48-h LC50 of both compounds was 2.6 µg l^{-1}. The toxicity of the 3 mixtures tested (M:C ratios of 1 : 3, 1 : 1 and 3 : 1) was close to what was predicted by concentration addition. The mixtures causing 50% mortality after 48 h contained 0.88 – 1.08 Toxic Units, which closely corresponds to the CA-predicted 1.0 Toxic Unit.

Concentration Ratio Methylparathion : Carbofuran	Concentration (Toxic Units)	
	Methylparathion	Carbofuran
1:1	0.50	0.38
3:1	0.27	0.81
1:3	0.77	0.25

Conclusions	The joint toxicity towards *Ceriodaphnia* of the binary combinations of methylparathion and carbofuran does not deviate from concentration addition.
Rev. note	1. No details about experimental setup or physical conditions prevailing during the tests are given.

Title	Acute toxicity of equitoxic binary mixtures of some metals, surfactants and pesticides to the freshwater amphipod *Gammarus italicus* Goedm.
Author(s)	Pantani, C., P.F. Ghetti, A. Cavacini and P. Muccioni.
Source	Environ. Technol. 11, 1143-1146 (1990).
Test method	Static.
Test substance	Azinphos-methyl, methyl-parathion and an equitoxic mixture these compounds.
Concentrations	Equitoxic fractions of the LC50, 6 concentrations per series, conc. factor not given.
Vehicle	Acetone, maximum concentration not given.
Test species	*Gammarus italicus*.
Conditions	Temperature 8^{o}C, no oxygenation. Hardness and alkalinity 240 mg l^{-1} and 55 mg l^{-1} as $CaCO_3$ resp.
Study duration	48 Hours.
Observations	Mortality.
Results	The 48-h LC50 of azinphos-methyl was 1.0 $\mu g\ l^{-1}$, the 48-h LC50 of methylparathion to *Gammarus* was 6.3 $\mu g\ l^{-1}$. The mixtures causing 50% mortality after 48 h contained 0.79 Toxic Units
Conclusions	The toxicity of the mixtures tested corresponded to concentration addition.
Rev. note	1. Only very little details about experimental setup are given.

Title	Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model.
Author(s)	Pape-Lindstrom, P.A. and M.J. Lydy.
Source	Environ. Toxicol. Chem. 16, 2415-2420 (1997).
Test method	Static.
Test substance	Atrazine was combined with methoxychlor, methylparathion, trichlorfon, malathion, chlorpyrifos and mevinfos. Methylparathion was also tested in combination with methoxychlor.
Concentrations	5 Concentrations per test compound, 3 replicates of each concentration. Mixtures were equitoxic, and contained 0.5, 0.75, 1.0, 1.5 and 2.0 TU in total.
Vehicle	Acetone (DMSO for atrazine), 0.5 ml l^{-1} for single compounds and 1 ml l^{-1} when testing binary mixtures.
Test species	*Chironomus tentans*, fourth instar larvae.
Conditions	10 Larvae in 1 l, containing 20 g silica sand and 1 l of moderately hard standard reference water. pH: 7.8 – 8.1, dissolved oxygen 80 – 95%, conductivity 350 – 370 µS cm^{-1}, temperature 19 – 21°C.
Study duration	96 Hours.
Observations	Mortality.
Results	The toxicity of mixtures of atrazine and methylparathion and chlorpyrifos were slightly (less than a factor 2) more toxic than expected on the basis of concentration addition. The toxicity of mixtures of atrazine and trichlorfon and malathion were more than a factor 2 more toxic than expected on the basis of CA. The toxicity of all other mixtures was within a factor 2 of what was expected on the basis of CA.

Compounds	Concentration needed for 50% mortality (Toxic Units; 95% confidence limits in parentheses)
ME + A	1.67 (1.38 – 2.25)
ME + MP	1.02 (0.85 – 1.19)
	0.94 (0.80 – 1.08)
ME + MP + A	0.80 (0.69 – 0.90)
	0.95 (0.84 – 1.09)
A + MP	0.59 (0.51 – 0.65)
A + TR	0.26 (0.21 – 0.32)
A + MA	0.36 (0.29 – 0.43)
A + CH	0.58 (0.50 – 0.64)
A + MEV	1.34 (1.14 – 1.63)

A: Atrazine; CH: chlorpyrifos; MA: malathion;
ME: methoxychlor; MEV: mevinfos; MP: methylparathion;
TR: trichlorfon;

Conclusions

Atrazine enhances the toxicity of several organophosphorus insecticides. The authors propose an explanation for this phenomenon, based on the observation that only the organophosphorus compounds which undergo metabolic activitation through oxidation become more toxic in the presence of atrazine. Assuming that atrazine enhances both Mixed Function Oxidase (MFO, metabolic activation) and esterase (metabolic breakdown) activity, the metabolic activation of the organophosphorus insectides is accellarated in the presence of atrazine, thus causing the presence of larger concentrations of toxic metabolites. This is partly counterbalanced by the enhanced breakdown (esterase activity). For compounds like mevinfos, which are not affected by the enhanced metabolic activation but are affected by the increase in breakdown the toxicity should be decreased, which is indeed observed.

Rev. note

Title	Combined action of carbaryl and phenthoate on a freshwater fish (*Channa punctatus* Bloch).
Author(s)	Rao, K.R.S.S., K.S.P. Rao, I.K.A. Sahib and K.V.R. Rao.
Source	Ecotoxicol. Environ. Saf. 10, 209-217 (1985).
Test method	Semi-static, daily renewal of solutions.
Test substance	Carbaryl, phentoate and mixtures (1:3, 1:1 and 3:1) of these compounds.
Concentrations	Five concentrations per test series; carbaryl 6-12 mg l^{-1}, phentoate 0.3-0.6 mg l^{-1}. Mixtures contained 0.6-1 mg l^{-1} pesticide.
Vehicle	Acetone, maximum concentration 1 ml per 15 l.
Test species	Adult snakehead fish (also known as murrel), *Channa punctatus*, 20±4 g
Conditions	12 fish per aquarium of 15 l; pH 7.1±0.2, hardness 140±20 mg l^{-1}, temperature 26±2^{o}C.
Study duration	48 Hours.
Observations	Mortality.
Results	The 48-h LC50 of carbaryl was 8.7 mg l^{-1} (95% confidence limits: 7.9 – 9.5 mg l^{-1}). The 48-h LC50 of phentoate was 0.47 mg l^{-1} (95% confidence limits: 0.31 – 0.63 mg l^{-1}).

The toxicity of the 3 mixtures tested (C:P ratios of 1 : 3, 1 : 1 and 3 : 1) varied with composition. The mixture with a C:P ratio of 3:1 needed only 0.47 Toxic Units to cause 50% mortality after 48 h, and is therefore slightly more than twofold as toxic toxic as predicted by concentration addition. The other 2 mixtures do not differ more than twofold from CA-predicted toxicity.

Concentration Ratio Carbaryl : Phentoate	Concentration (Toxic Units)	
	Carbaryl	Phentoate
1:1	0.04	0.78
3:1	0.07	0.40
1:3	0.02	1.14

Conclusions	The joint toxicity of carbaryl and phentoate towards the fish *Channa punctatus* is dependent on the ratio of the concentrations at which both compounds is present, varying from concentration addition at C:P ratios of 1:3 and 1:1 to enhanced toxicity at a C:P ratio of 3:1.
Rev. note	1. According to the authors the toxicity at a C:P ratio of 3:1 indicates “a marked synergism”. However, there were no replicates used. The joint toxicity at this ratio is only slightly more than twofold higher than predicted by concentration addition. In view of the variation in acute toxicity tests using fish, the conclusion that there is “marked synergism” can hardly be justified.

Title	Acute toxicity of Garlon 4 and Roundup herbicides to Salmon, *Daphnia*, and trout.
Author(s)	Servizi, J.A., R.W. Gordon and D.W. Martens.
Source	Bull. Environ. Contam. Toxicol. 39, 15-22 (1987).
Test method	Static.
Test substance	Garlon 4 (61.6% butoxyethyl ester formulation of triclopyr), Roundup (48% isopropylamine salt of glyphosate + 15% Mono818 surfactant).
Concentrations	Five concentrations per test series; carbaryl 6-12 mg l^{-1}, phentoate 0.3-0.6 mg l^{-1}. Mixtures contained 0.6-1 mg l^{-1} pesticide.
Vehicle	None.
Test species	Sockeye salmon (*Oncorhynchus nerka*) fry (0.22 g wet weight, 2.9 cm average length) and fingerlings (4.5 g wet weight, 7.1 cm average length); 10 fry (0.22 g l^{-1} biomass) or 5 fingerlings (0.4 g l^{-1} biomass) were used per test concentration. Tests were performed in glass aquaria of 10, 20 or 50 l; volume was adapted to keep biomass always below 0.5 g l^{-1}. *Daphnia pulex* (<24 h at the start of exposure) was tested in 30 ml glass beakers, 5 animals per beaker. Tests with daphnids were carried out in triplicate. Fry of rainbow trout (*Salmo gairdneri*) (0.33 g wet weight, 3.4 cm average length) and fry of coho salmon (*Oncorhynchus kisutch*) (0.29 g wet weight, 3.4 cm average length) were tested in aquaria of 30 l.
Conditions	Water used for tests with sockeye fry and fingerlings and *Daphnia pulex* was taken from a lake at 37 m depth. pH 7.8, hardness 84 mg l^{-1}, alkalinity 60 mg l^{-1}, conductivity 168 µS cm^{-1}. Water used with rainbow fry and coho salmon fry was dechlorinated tap water. pH 6.3, hardness 3-4 mg l^{-1}, alkalinity 2-3 mg l^{-1}, conductivity 12 µS cm^{-1}. Temperature 4.5°C (sockeye fry and fingerlings), 15°C (rainbow trout fry and coho salmon fry), 21°C (*Daphnia pulex*).
Study duration	96 Hours.
Observations	Mortality.

Results

Species	96-Hour LC50 ($mg\ l^{-1}$)			
	Garlon 4	Roundup	Glyphosate	Mono 818
Sockeye fry	1.2	28.8	106*	2.6
Sockeye fingerling	1.4	27.2		
Daphnia pulex	1.2	25.5	962	2.0
Rainbow fry	2.2	26.7	106	3.2
Coho fry	2.2	42.0	106*	3.5

* Value not known, value for rainbow trout is used.

The toxicity of the formulated product Roundup is mainly due to the toxicity of the surfactant Mono 818, and only to a small extent caused by the active ingredient glyphosate.

Species	LC50 Roundup (mg l^{-1})	Conc glyphosate (TU)	Conc Mono818 (TU)
Sockeye fry	28.8	0.08	1.65
Sockeye fingerling	27.2	0.08	1.65
Daphnia	25.5	0.01	1.90
Rainbow fry	26.7	0.08	1.31
Coho fry	42.0	0.12	1.80

Conclusions

The toxicity of Roundup to Sockeye salmon, Coho salmon, rainbow trout and Daphnia pulex is mainly due to the presence of the surfactant Mono 818, and only to a small extent to the presence of glyphosate. The observed joint toxicity of glyphosate and Mono 818 does not differ more than twofold from what is expected on the basis of concentration addition.

Rev. note

1. For sockeye salmon and coho salmon (both fry and fingerlings) no LC50 value for glyphosate was established, and the LC50 value for rainbow trout was used. This makes the calculation of Toxic Units in the formulated product for these species less certain.

Title	Interaction effects of permethrin and atrazine combinations towards several non-target microorganisms.
Author(s)	Stratton, G.W.
Source	Bull. Environ. Contam. Toxicol. 31, 297-303 (1983).
Test method	Static.
Test substance	Permethrin, 88.6% (40:60 mixture of cis and trans isomers); atrazine, >95% pure.
Concentrations	Permethrin: 0, 0.5, 1.0, 2.0, 3.0 mg l^{-1} (growth), 0, 50, 100 mg l^{-1} (photosynthesis), or 0, 100 mg l^{-1} (nitrogenase activity). Atrazine 0, 0.01, 0.025, 0.05, 0.075, 0.1 mg l^{-1} (growth), 0, 0.05, 0.1, 0.2 mg l^{-1} (photosynthesis), or 0, 50, 100 mg l^{-1} (nitrogenase activity). Mixtures consisted of all combinations of concentrations used in the exposures to single compounds (factorial design).
Vehicle	Acetone, 0.1 ml l^{-1}.
Test species	Cyanobacter, *Anabaena inaequalis*.
Conditions	Initial cell density 6.5 10^4 cells ml^{-1} (photosynthesis and nitrogenase activity) or 0.32 10^4 cells ml^{-1} (growth). Temperature 22^{o}C, light intensity of 7000 lux, 12 h light-dark cycle.
Study duration	Growth test lasted 14 days; duration of tests on photosynthesis and nitrogenase activity are not given in the paper.
Observations	Growth, inhibition of photosynthesis, inhibition of nitrogenase activity.
Results	Permethrin and atrazine interacted in an additive manner when tested towards *Anabaena inaequalis* using growth, inhibition of photosynthesis or inhibition of nitrogenase activity as the effect parameters.
Conclusions	The joint toxicity of permethrin and atrazine towards Anabaena inaequalis corresponded to concentration addition for all three effects tested (growth, inhibition of photosynthesis and inhibition of nitrogenase activity).
Rev. note	

Title	Synergism in tertiary mixtures of pesticides.
Author(s)	Tripathi, A.M. and R.A. Agarwal.
Source	Chemosphere 35, 2365-2374 (1997).
Test method	Static.
Test substance	Decis (pyrethroid), sevin (carbamate) and MGK-264 (esterase inhibitor).
Concentrations	Five concentrations (0.05, 0.09, 1.0, 3.0 and 4.0 mg l^{-1}) of decis + sevin mixed in a ratio of 1:46 and 7 concentrations (0.007, 0.01, 0.03, 0.05, 0.09, 0.2 and 0.3 mg l^{-1}) of decis, sevin and MGK mixed in a ratio of 1:46:5, along with controls. The effect of 0.3 mg l^{-1} MGK-246 was assessed in a separate experiment. Six replicates for each concentration.
Vehicle	Acetone, 0.1 ml l^{-1}.
Test species	Adult freshwater snail, *Lymnae acuminata* (2.6 ± 0.3 cm).
Conditions	Glass aquaria containing 3 l dechlorinated tap water; 10 snails per aquarium. Temperature 26-29°C, light intensity of 7000 lux, 12 h light-dark cycle.
Study duration	96 Hours.
Observations	Mortality.
Results	The 96-h LC50 of Decis and Sevin were 0.44 mg l^{-1} and 4.88 mg l^{-1} resp. When applied in combination in a D:S ratio of 1:46 the LC50 of the mixture was 0.183 mg l^{-1}, containing 0.009 Toxic Units Decis and 0.04 Toxic Units Sevin. Adding MGK increased the toxicity of the mixture further (D:S:M 1:46:5) approx. tenfold (LC50 of the mixture 0.017 mg l^{-1}).
Conclusions	The toxicity of the pyrethroid Decis is enhanced when applied in combination with esterase-inhibitors like carbamaters (Sevin) or MGK-264.
Rev. note	

Title	Acute toxicity of mixtures of range management herbicides to cu tthroat trout.
Author(s)	Woodward, D.F.
Source	Journal of Range Management 35, 539-540 (1982).
Test method	Static.
Test substance	Dicamba, picloram, 2,4-D butylester, 2,4-D isooctylester, 2,4-D propylene glycol butylester.
Concentrations	Ratio between concentrations in tests of single compounds is not given. Concentrations above 50 mg l^{-1} were not used (LC50 given as > 50 mg l^{-1}). Mixtures tested were equitoxic.
Vehicle	In some tests acetone was used, maximum concentration not given.
Test species	Cutthroat trout fingerlings (0.4 – 0.8 g), *Salmo clarki*.
Conditions	Details are not given. Tests were performed according to "Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)".
Study duration	96 Hours.
Observations	Mortality.
Results	The 96-h LC50 of 2,4-D isooctyl ester and dicamba were > 50 mg l^{-1}. For these compounds the toxicity of mixtures could not be assessed reliably. For 2,4-D butyl ester and 2,4-D propylene glycol ester the joint toxicity with picloram was established. The equitoxic mixture of 2,4-D BE + picloram causing 50% mortality contained 1.20 Toxic Units, the equitoxic mixture of 2,4-D PGBEE + picloram causing 50% mortality contained 0.96 Toxic Unit.
Conclusions	The toxicity of 2,4-D butylester and 2,4-D propylene glycol ester in combination with picloram was well predicted by concentration addition. For all other mixtures tested no reliable assessment can be given.
Rev. note	1. No experimental details are given in the paper.