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Acute Toxicity and Bioaccumulation of Lindane in Gudgeon, *Gobio gobio* (L.)

C. Marcelle* and J. P. Thome

Laboratory of Animal Morphology, Systematics and Ecology, Zoology Institute, University of Liège, Belgium

Lindane and endosulfan are the only organochlorine insecticides still usedin Belgium. A recent survey showed its wide distribution in fresh water ecosystems (THOME & THOME, 1982). Many authors have determined the LC50 of lindane for different species of fish; a review of these studies may be found in ULMANN (1972) and in PORTMANN (1979). The fish used as experimental material in these acute toxicity tests are generally bred species such as trout, salmon, bluegill... often selected for the facility of their maintenance in aquariums. CABRIDENC & BOUCHINET (1982) suggested, the use of species more representative of the ecosystems, for toxicity tests.

Accordingly, we chose a wild and widely distributed species, the gudgeon (*Gobio gobio* L.) for our experiments. In this paper are reported the results of acute toxicity tests conducted with lindane on gudgeons in order to determine a LC50 (96 h). Lindane contents in muscles, brain and liver were measured at the end of this experiment and factors of bioaccumulation of lindane were calculated.

MATERIAL AND METHODS

Gudgeons (*Gobio gobio* L.) obtained from a local fishing store were held for 3 weeks in a 300 l glass tank containing well aerated tap water. They were fed with Chironomidae larvae and fresh water crustaceans (*Gammarus pulex*). During the 96 h exposure they were not fed.

For the acute toxicity tests, a dynamic system of water replacement was used ('flow through system'). It consisted in a series of 120 l glass tanks, one per pesticide concentration, in which water containing the dissolved pesticide was replaced at a constant rate (5.4 l h^{-1}). Lindane (99.9 %) was dissolved in methanol.

Ten gudgeons were place in each tank. The temperature of water was maintained at 12.2 $\pm$ 0.3°C. The physico-chemical parameters of water measured with a kit Aqua Merck were pH = 8; total hardness : 200-220 ppmCaO ; nitrites : 0.05 to 0.1 ppm; nitrates : 25 to 30 ppm; NH_4^+ : not detected. Dead or moribund fish were recorded every 24 h for four days. After a 96 h exposure, the fish were sampled and their brain, liver

(*) Present address : Rue du Mont, 24, B-4458 Fexhe-Slins.

and muscles (upper part of the body, behind the dorsal fin) were dissected out and weighed. Lindane was immediately extracted from the fresh material, or subsequently from frozen material.

Lindane was extracted from water by using extraction micro columns of 1 ml (C-18 Bond Elut, Analytichem International, Harbor City, USA), particulary convenient for pesticide extractions from water. The columns were placed under vacuum. Before extraction, each column was wetted by passing 1 ml of methanol. One hundred ml of water to be analyzed was allowed to pass through the column for lindane adsorption. The most polar compounds retained on the cartridge were eliminated by eluting with 0.1 ml of methanol : water (1 : 1). This procedure was useful when water to be analyzed was very polluted. Lindane was then eluted with hexane to a final volume of 1 ml. This extraction method gave us excellent and rapid results (mean recovery percentage of 104 %, 5 extractions could be performed in 10 minutes).

Lindane extraction from tissues and organs was performed according to the EPA method (1980) for extraction of pesticides, slightly modified by using acetone instead of acetonitrile. Satisfactory results were obtained taking into account the small size of the samples (30 to 200 mg fresh weight).

A Carlo Erba 4130 gas chromatograph equipped with a Ni ^{63} ECD was used in this study. The extracts were analyzed on a 25 m x 0.3 mm ID OV1 glass capillary column (Column temperature programmed 60° to 240° at 5°/min; hydrogen carrier gas 1 ml/min; Make up gaz : Ar : CH_4 (90 : 10) : 30 ml/min); injector 250°C; detector : 275°C).

The LC50 was determined using a SAS programme (1979) at the computing Centre of the University of Liège. Analysis of variance including Levene's tests for equality of variance were performed with BMDP 7D programs (DIXON and BROWN, 1979).

RESULTS AND DISCUSSION

The graphic method for the determination of LC50, as described in MATSUMURA (1975), gave a value of 72.7 ppb; the SAS programme applied on the same data calculated a LC50 value of 74.3 ppb with a confidence interval of 42.3 to 196.5 ppb (for $p = 0.05$). The computer-derived curve in Figure 1 allows estimations of mortality with various lindane concentrations.

A comparison of this LC50 value of about 75 ppb with values obtained with other species (PORTMANN, 1979) showed that the gudgeon had a moderate sensitivity of lindane : more resistant than the most sensitive species such as rainbow trout (LC50 ± 22 ppb) but less than relatively resistant species such as the goldfish (LC50 ± 156 ppb). The gudgeon sensitivity is similar to that of bluegill and carp.

Table 1 gives the lindane contents measured in different tissues and organs at the end of the acute toxicity tests.

Table 1 shows that the standard errors of the means were increasing proportionally to the lindane contents in organs. A transformation of the decimal data was used to normalize their distribution (DAGNELIE, 1970). The Levene's tests for equality of variances demonstrated that the logarithmic transformation correc-

tly normalized the data and allowed the further use of parametic statistics.

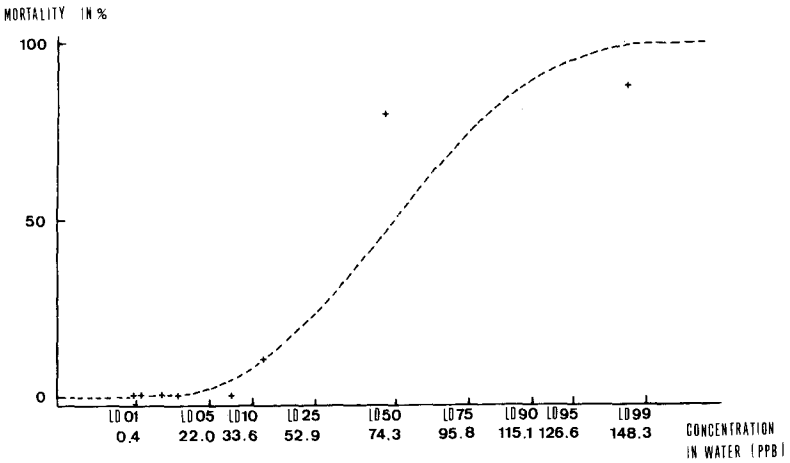

Figure 1 : Mortality induced in the gudgeon population after 96 hours of exposure to different lindane concentrations in water.

Lindane concentrations in water were determined once a day during the 96 h exposure. Results reported in Table 1 shows that the flow through system used for the experiment maintained a very stable concentration of lindane in each aquarium.

Lindane contents measured in liver, brain and muscles increased with lindane concentrations in water (Table 1). This increase seemed relatively moderate until the 28.5 ppb of lindane concentration in water; beyond this concentration, it became sharper especially in brain and liver. In our experiment, this lindane concentration of 28.5 ppb in water was a treshold beyond which the accumulation of lindane in tissues and organs induced an increase of mortality (see also figure 1).

Table 1 : Lindane content (in ppb) of different tissues and organs at the end of an acute toxicity test (96 h. exposure).

Lindane concentrations in water (ppb)[b]	Lindane contents[b] Muscles	liver	brain
0[a]	11 ± 2	24 ± 4	37 ± 7
0.22 ± 0.08	20 4	46 6	129 27
1.13 0.03	22 2	224 43	145 18
7.5 0.26	87 18	254 33	108 10
28.5 0.35	13 4[c]	187 43	149 16
37.0 1.30	193 43	2160 696	899 206
72.0 4.71	1068 157	5005 621	3527 605
142.0 4.04	593 121[c]	8257 1556	9005 2416
Total number	50	47	47

a : contents measured just before the experiment
b : mean ± standard error of the mean
c : values obtained after a storage of 3 months in deep freezer.

The existence of this threshold was also obvious when the concentration factors were calculated for each tissue or organ (figure 2).

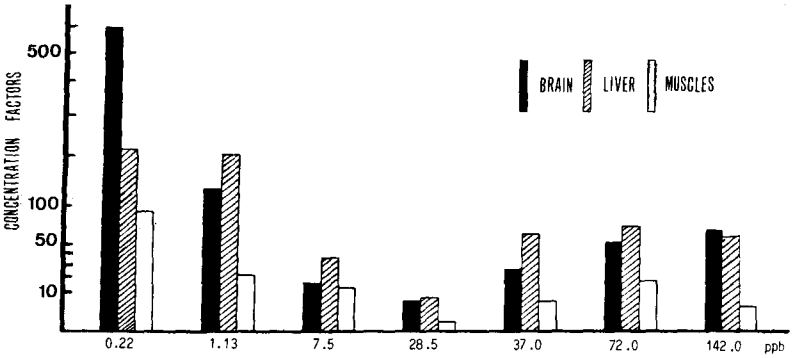

Figure 2 : Concentration factors calculated for brain, liver and muscles of gudgeons, after 96 hours of exposure to different lindane concentrations in water.

The bioaccumulation dramatically decreased between 0.22 and 28.5 ppb of lindane in water but increased beyond this threshold concentration (figure 2). SCHIMMEL et al. (1977), in the same type of experiment, did not find differences in the bioaccumulation factors of organs according to the concentrations of lindane in water as we did, although the factors reported here are in the same range (5 to 600) as reported in literature (MATSUMURA, 1977; PORTMANN, 1974; KANAZAWA, 1980). We determined the relations between the lindane concentrations in water and the logarithms of lindane concentrations in the analyzed organs or tissues. Results in Figure 3 were based on the higher correlation coefficients for the different tested relations.

We found a very highly significant linear correlation between both variables in the case of brain and a very highly significant logarithmic correlation in the cases of liver and muscles. These results implied that the decimal lindane concentrations in brain exponentially increased with lindane concentrations in water. The lindane concentrations in muscles and liver, when also expressed in decimal values, linearly increased with the lindane concentrations in water. Brain seemed thus to accumulate lindane at a slower rate than liver and muscles when the lindane concentrations in water were low. This observation could be an indication of the presence of a blood-brain barrier preventing to a certain extent the lindane penetration into brain. The presence of such a barrier effective against organochlorine insecticide penetration in brain has been previously established in mammals (MATSUMURA 1975). This barrier, however, seemed to become ineffective at higher lindane concentrations in water (above 30 ppb) as the pesticide concentration in brain very rapidly increased beyond this threshold level in water (see also above). It would be interesting to investigate the structural features of the poisoned organs in order to see whether a threshold could also be demonstrated at the cellular or subcellular level as far as the damages are concerned.

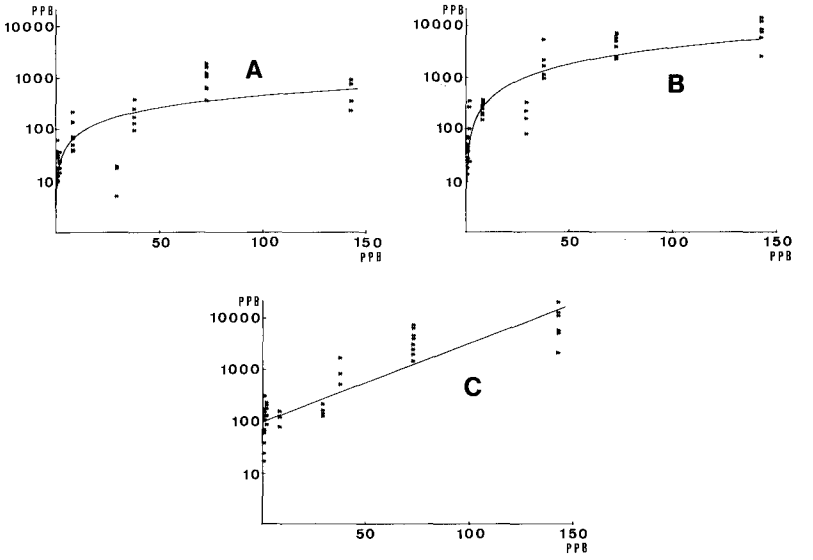

Figure 3 : Lindane concentrations in muscles (A), liver (B) and brain (C) vs lindane concentrations in water; on x axis: decimal lindane concentrations in water, on y axis : neperian logarithmic concentrations of lindane in organs or tissues. The equations of the regression curves were

for muscles : $y = 0.7794 \ln x + 2.5604$
$r = 0.82$ $(n = 50)$

for liver : $y = 1.0774 \ln x + 3.3261$
$r = 0.92$ $(n = 47)$

for brain : $y = 0.0355 x + 4.5779$
$r = 0.90$ $(n = 47)$

A new method for extraction of lindane from water is described in this paper. A LC50 (96 h.) experiment was conduced using a wild species, the gudgeon. The values found were 72-74 ppb. After a 96 h exposure, the lindane concentrations in brain, liver and muscles were determined. Concentrations in brain and liver reached 8-9 ppm for a lindane concentration in water of 142 ppb. Concentrations in muscles were generally lower. A 30 ppb lindane concentration in water corresponded to a rose rising of both mortality and lindane concentration in the organs, whilst concentration factors were the lowest at this concentration.

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