Phthalate Esters: Heartrate Depressors in the Goldfish*

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Phthalic acid esters are common commercial plasticizers added to plastics such as polyvinyl chloride to make them more flexible. Over the past few years they have been recognized as environmental pollutants affecting life forms. JAEGER and RUBIN (1970) found them in tissues from two patients who had received transfusions of blood stored in containers made of polyvinyl chloride. Other investigators have found them in animals, fish, water, soil, and air (TABORSKY, 1967; MAYER et al., 1972; HITES, 1973; THOMAS, 1973).

We encountered phthalate esters while isolating a heartrate-depressing factor from water in which goldfish and carp had been kept under crowded conditions (PFUDERER et al., 1974a). Our assay (FRANCIS et al., 1974) involved adding the suspected sample to goldfish water and looking for a change in the heartrate of the fish by monitoring them with implanted electrodes. Since goldfish and carp showed essentially the same response, we used the larger fish (carp) to locate the factor in a particular tissue. Carp liver produced a significant lowering of the goldfish heartrate (Table 1) when residues from chloroform extracts of the tissue were added to goldfish water. However, chromatographic analysis showed that the depression was caused by phthalate esters present in the carp tissues (PFUDERER et al., 1974b), presumably absorbed from the lake water the carp had lived in. We have qualitatively identified di-2-ethylhexyl phthalate, di-n-butyl phthalate, and benzylbutyl phthalate in the tissues of carp taken from Watts Bar Lake, part of the TVA system (PFUDERER et al., 1974b). MAYER et al. (1972) have shown that phthalates are present in fish taken from water that contains such esters.

When the three phthalate esters were tested individually for their effects on heartrate, dilutions of sonicated suspensions of both di-n-butyl and benzylbutyl were active (Table 2), but di-2-ethylhexyl phthalate, at the concentration we used, was not active during a 10-minute assay. The decrease in heartrate occurred within 30 seconds with dibutyl phthalate at 12 ppm, but took several minutes with benzylbutyl phthalate and with lower concentrations (<12 ppm) of dibutyl phthalate. The positive effect of the benzylbutyl phthalate at 200 ppm was probably caused by the small amount of di-n-butyl phthalate present. Since dibutyl phthalate is much more water-soluble than the other two compounds, and di-2-ethylhexyl phthalate is the least soluble of the three, differences in water solubility may be involved in the ability of the compounds to reach their site of action.

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TABLE 1

Localization of Heartrate-Lowering Activity in the Carp

Tissue	Weight used* (grams)	Mean heartrate of goldfish† (beats/min)
Heart	0.97	100 ± 10
Liver	3.75	66 ± 10
Brain	0.80	100 ± 10
Kidney	2.65	114 ± 10
Gut	29.47	127 ± 15
Remainder of fish		126 ± 15
$CHCl_3$ residue (control)		128 ± 10

(From PFUDERER, WILLIAMS, and FRANCIS, 1974a)

*Starting weight, before homogenization and chloroform extraction. The organs were homogenized in two parts, by weight, of 0.1 M potassium phosphate buffer (pH 6.8), and the solution was extracted twice with 50 ml $CHCl_3$. The $CHCl_3$ was separated and removed by rotoevaporation, and the $CHCl_3$ residue was equilibrated with 75 ml distilled H_2O at 24°C. This sample was then added to the assay chamber. A goldfish, whose heartrate had previously been recorded, was immersed in the sample and heartrate data was monitored for 20 min. The average at 8–10 min was used for the assay.

†The heartrate-depressing activity was found principally in the liver, with perhaps some activity in the brain, heart, and kidney. The heartrate could be measured with a precision of ~2 beats/min, but individual fish varied considerably in their response to a given solution. A drop of 20 beats/min was occasionally encountered when control solutions were used, but larger decreases (e.g., from 120 to a steady 90 beats/min) rarely occurred unless a positive sample was present.

TABLE 2

Heartrate-Lowering Activity of Phthalate Esters

Tissue	Concentration used* (ppm)	Mean heartrate of goldfish (beats/min)
Di-n-butyl phthalate†	0.5	130 ± 15‡
	1	105 ± 10
	5	60 ± 10
	12	48 ± 10
Di-n-butyl phthalate plus 0.20 mg atropine per ml	12	120 ± 5
Di-2-ethylhexyl phthalate	200	87 ± 5‡
Benzylbutyl phthalate	100	105 ± 10‡
	200	40 ± 5

*The fish were immersed in 75 ml of a stable sonicated emulsion of the phthalate ester at the indicated concentration, and the heartrate was recorded as in Table 1.

†The di-n-butyl phthalate was 99.94% pure by gas chromatography, containing one other supposed ester of similar boiling point, and no detectable butanol or other low molecular weight impurities.

‡No apparent change from resting level.

The benzylbutyl phthalate contained 1.5% di-n-butyl phthalate and 0.3% of one other supposed ester of similar boiling point by chromatographic analysis, but no detectable butanol, benzyl alcohol, or other low-boiling substance.

If we assume that dibutyl phthalate is the only heartrate depressant in these fish tissues, the observed heartrates shown in Table 1 lead to an estimate of phthalate levels on the order of 100 ppm in both liver and brain tissue. These values seem high, and therefore this initial premise may not be true.

Heartrates lowered by the phthalates were returned almost to the resting level when the fish were put through two or more changes of fresh water. Atropine at 0.2 mg/ml, even in the presence of phthalate, reversed the effect of phthalate in about 4 minutes and usually returned the heartrate to a level close to the normal resting level. Since atropine is a parasympathetic blocking agent and control of heartrate in the goldfish is primarily through the vagus (RANDALL, 1966), a parasympathetic nerve, the phthalate esters probably affect the heartrate indirectly through the nervous system.

Phthalate esters are found as contaminants in lake and river water samples at concentrations on the order of parts per billion (MAYER et al., 1972; METCALF et al., 1973), i.e., lower than the concentrations we have found to be active. However, as they pass through the food chain of aquatic organisms they are concentrated many thousandfold, and concentrations in fish do reach levels comparable to those we have tested (MAYER and SANDERS, 1973; YU and PERLMUTTER, 1970). Moreover, aquatic organisms such as Daphnia and zebra fish show a remarkable sensitivity to chronic doses of the phthalate esters at the range of parts per billion (YU and PERLMUTTER, 1970). Many of the symptoms produced by phthalates, which include decreases in growth and reproduction, are similar to the effects of crowding in aquatic organisms (PFUDERER et al., 1974a; YU and PERLMUTTER, 1970), leading us to speculate that the nervous system may be the target in all such cases, as it appears to be in the case of heartrate depression. MILKOV et al. (1973) have recently reported that humans chronically exposed to concentrations of phthalate esters also suffer deleterious effects, primarily to the nervous system.

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