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# Kinetic Studies on the Elimination of Different Substituted Phenols by Goldfish (*Carassius auratus*)

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Phenols are pollutants in aquatic environments. According to BONI (1965), FLEROV + ERSHOV (1974) and KOBAYASHI + AKITAKE (1975 a) fish are able to excrete phenol rapidly. Kinetic studies are of interest because there are two different phases of elimination of phenols from goldfish.

FORSTER + GOLDSTEIN (1969) reported that the gills of fishes ostensibly do not provide an efficient way of elimination even for readily diffusible foreign substances. According to ADAMSON + SIEBER (1974) foreign compounds are only excreted to a limited degree across dogfish gill. The aim of this paper is to investigate whether this applies to phenols too.

KOBAYASHI + AKITAKE (1975 a,b) reported that phenol and pentachlorphenol are excreted by goldfish at different rates. Therefore it is necessary to explore the relation between the chemical structure of phenols and their elimination by fish.

## MATERIALS AND METHODS

Labeled compounds: [U-$^{14}$C] - phenol (368 Ci/mg), [G-$^{3}$H]-3,5 diethylphenol (37mCi/mg), The Radiochemical Centre Amersham; [U-$^{14}$C] - 4 - aminophenol (125 Ci/mg), Hoechst; [U-$^{14}$C] - 3 - nitrophenol (100 Ci/mg), Schering.

Determination: Radioactivity was measured by liquid scintillation counting.

Application: Phenols were administered intraperitoneally (0,25 mL, 10 mg/kg). With the exception of unsubstituted phenol, the substances were solved in one volume Cremophor EL [Sigma, Germany] and three volumes water. To prevent the oxidation of 4 - aminophenol ascorbic acid was added. The average body weight of goldfish used in these experiments was 37 $\pm$ 6 g. Three goldfish were placed in a 10-L glass tank containing 3 L tap water and kept at 20° C.

Routes of excretion: Gills and cloaca are to be considered as routes of excretion. To determine the relative importance of the two routes it was necessary to separate the eliminated substances. For that purpose rubber tubes made out of a condom (Fromms) were used. The end

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of the reservoir of the condom was cut off and the condom pulled from the head over the body until its anterior edge fitted tightly between dorsal fin and pectoral fin. The posterior part of the tube was filled with 2o mL water and knotted at the end. The fishes were able to swim during the experiments.
The separation of the nonpolar excretory products from the polar was carried out by continuous liquid-liquid extraction (pH 6,5; diethylether).

Pharmacokinetics: A two-compartment-model (Fig.1) was used to describe the levels of the phenols in goldfish (DOST 1968, GLADTKE + v. HATTINGBERG 1973).

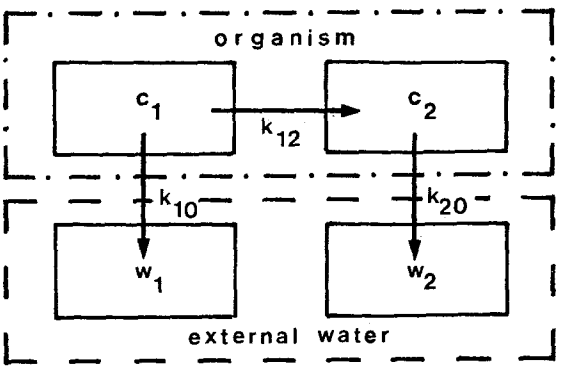


Figure 1. Diagram of the two-compartment-model

$c_1$ = concentration in compartment I (non polar, presumable unconjugated phenols)

$c_2$ = concentration in compartment II (polar, presumable conjugated phenols)

$k_{1o}$ = rate constant of elimination from compartment I

$k_{2o}$ = rate constant of elimination from compartment II

$K_{12}$ = rate constant of transfer from compartment I to compartment II

$w_1$ = concentration in external water (non polar)

$w_2$ = concentration in external water (polar)

The concentrations in both compartments can be described by the equation

$$\frac{d\, c_1\,(t)}{dt} = -\,(k_{1o} + k_{12})\; c_1(t) \qquad (1)$$

and $$\frac{d\, c_2\,(t)}{dt} = -\,k_{2o}\; c_2(t) + k_{12}\; c_1(t) \qquad (2)$$

It is a system of homogenous differential equations. After integration with $c_1\ (t=0) = C$ and $c_2\ (t=0) = 0$ follows:

$$c_1(t) = C\, e^{-(k_{10} + k_{12})\, t} \qquad (3)$$

$$c_2(t) = C \frac{k_{12}}{k_{20} - k_{10} - k_{12}} \left[e^{-(k_{10} + k_{12})t} - e^{-k_{20}t}\right] \qquad (4)$$

From this follows for the total concentration in the organism:

$$C(t) = \frac{C\ (t = 0)}{k_{20} - (k_{10} + k_{12})} \left\{(k_{20} - k_{10}) \exp\left[-(k_{10} + k_{10})t\right] - k_{12} \exp\left[-k_{20}\, t\right]\right\} \qquad (5)$$

The measured concentration CE (t) was fitted to the function C (t) by variation of the parameter $k_{10}$, $k_{20}$ and $k_{12}$; using least square fitting. A computer pdp 11/10 (digital) was used in the Physics Department of Mainz.*

## RESULTS AND DISCUSSION

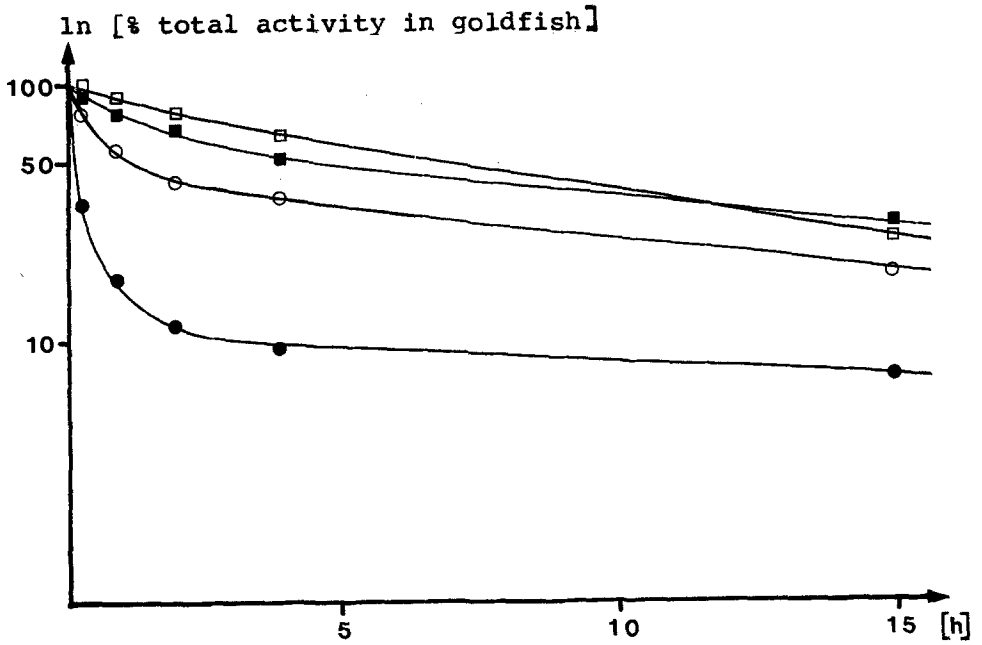


Figure 2. Retention of the phenols in goldfish during culture in water after i.p. injection (10 ppm)

● = Phenol, O= 3 - nitrophenol, ■= 3,5 - diethylphenol

□ = 4 - aminophenol

*The authors thank Dr. W. Ritter for solving the mathematical problem

Figure 2 shows the change in amounts of phenols in goldfish. After fitting CE (t) on C (t) in equation (5) the rate constants of elimination in table 1 are found.

TABLE 1

Rate constants of the elimination of phenols from goldfish [$h^{-1}$]

| | $k_{10}$ | $k_{20}$ | P |
|---|---|---|---|
| phenol | 3,15 | 0,02 | 28,8 |
| 3 - nitrophenol | 1,54 | 0,06 | 100 |
| 3,5 - diethylphenol | 0,57 | 0,07 | 2188 |
| 4 - aminophenol | 0,20 | 0,08 | 0,7 |

P = Partition coefficient n - octanol/water (FUJITA et al. 1964)

The smaller the molecular weight and the more polar the phenols are the better they can be eliminated from compartment I. The elimination from compartment II shows the reverse correlation. 4 - aminophenol represents an exception.
Using the system described above it was not possible to separate renal and biliary elimination. Considering the results of SCHAWALLER (1977) that substances excreted via the bile appear in the water not sooner than 6 h after i.p. injection, the activities eliminated by cloaca within 4 h can be identified as renal products.
If we consider that the activities present in the gut and bile are eliminated from the organism, then the results of table 2 follow.

TABLE 2

Elimination of phenols from goldfish within 4 hours (% activity eliminated)

| | branchial | | | renal | | biliary |
|---|---|---|---|---|---|---|
| | total | non polar | polar | total | non polar | polar | total |
| PH | 95 | 92 | 3 | 3 | 1 | 2 | 2 |
| NP | 78 | 61 | 17 | 5 | 2 | 3 | 17 |
| DP | 52 | 47 | 5 | 18 | 7 | 11 | 30 |
| AP | 33 | 9 | 24 | 26 | 5 | 21 | 41 |

PH = Phenol, NP = 3-nitrophenol,
DP = 3,5-diethylphenol, AP = 4-aminophenol

Table 2 shows that gills are responsible for the quick elimination of phenols from goldfish. The assumption

of FORSTER + GOLDSTEIN (1969) and ADAMSON + SIEBER (1974) that foreign compounds are only excreted to a limited degree across the gills does not apply to phenols.
The more polar the phenols are and the smaller their molecular weight the better they can be eliminated across the gills. 4-aminophenol is an exception.
According to STEIN (1971) one additional aminogroup reduces the likelihood of the transfer from aqueous phase to the membrane by a factor 12 - 20.
The amount of the renally eliminated phenols is correlated with the lipophilic properties in the order: phenol, 3 - nitrophenol and 3,5 - diethylphenol. 4 - aminophenol again is an exception.
The biliary excretion shows similar results.
According to KOBAYASHI et al. (1976) for the conjugated phenols the renal excretion appears to be a minor route compared with both the branchial and biliary excretion.
The example of 3,5 - diethylphenol shows, that does not apply to all phenolic compounds.

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