Bioaccumulation and lack of toxicity of octachlorodibenzofuran (OCDF) and octachlorodibenzo-p-dioxin (OCDD) during an early-life stage test with zebra fish (*Brachydanio rerio*)

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1. Introduction

Compared to 2,3,7,8-TCDD, octachlorodibenzo-p-dioxin (OCDD) and octachlorodibenzofuran (OCDF) are considered much less toxic as both have been attributed a I-TEF of 0.001. While, ecotoxicological studies with 2,3,7,8-TCDD showed both bioaccumulation and toxic effects in laboratory tests [1,2], studies with OCDD or OCDF in juvenile or adult fish exposed via water revealed no toxicity, despite significant bioaccumulation[3,4].

Meanwhile, there seems to be a significant difference between the environmental fate of OCDF and OCDD. In the aquatic compartment, as well as in terrestrial organisms and humans, the levels of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are generally dominated by OCDD and not by OCDF [5-9].

To further evaluate the potential impact of OCDD and OCDF on aquatic life their toxicity and bioaccumulations were determined in a 32-days flow-through test with early-life stages of the zebra fish (*Brachydanio rerio*). This test system has shown to be the most sensitive for 2,3,7,8-TCDD. The study was conducted according to OECD guideline 210[10] and OECD GLP guidelines[11]. To maintain the test substance concentration at the maximum possible soluble level, a flow-through system with generator columns was used.

2. Materials and methods

OCDD and OCDF were obtained from C.N. Schmidt via Cambridge Isotope Laboratories (purity = 99 %). Parent zebra fish (*Brachydanio rerio*) were obtained from a commercial supplier and females and males were kept in separate aquaria filled with reconstituted water (1.0 mM $CaCl_2.2H_2O$, 0.25 mM $MgSO_4.7H_2O$, 0.38 mM $NaHCO_3$ and 0.039 mM KCl). At the day of test initiation two males and one female were put together in a spawning aquarium and the eggs were added to the test solutions within 2 hours after spawning. OCDD and OCDF were coated on a support material (Chromosorb G AW) at a concentration of 0.1 mg/g using stock solutions in

hexane. A control batch was also prepared. Six glass columns (length = 13 cm, internal diameter = 0.8 cm) were filled with 2 gram support material. Reconstituted water was pumped through each column and was delivered to 2-litre glass beakers via Teflon tubes. Two glass beakers (A and B) were used for each treatment. Test solution volumes in glass beakers were kept at 1.6 - 1.8 litre and the target flow rate was 6 - 9 litre per day per glass beaker. Two days after initiation of test solution delivery, 40 zebra fish eggs were added to each glass beaker. Eggs of replicates (A and B) originated from different females.

Each day the number of dead and living eggs/larvae was recorded. After one day of exposure, the number of living eggs was reduced to 20 per glass beaker. At day 7, 14, 21, 28 and 32 (test termination) the behaviour and appearance of the surviving larvae were recorded. Free-swimming larvae were mainly fed with nematodes (day 5 - 13) and brine shrimp nauplii (day 11 - 30) several times a day. At test termination the surviving larvae were killed with the anaestheticum Hypnodil and the weight of each larva was determined.

Hatching times of eggs were analysed with survival analysis techniques[12]. The cumulative number of hatched eggs during the study days was converted to interval censored time data per egg. The computer program SAS PROC LIFEREG was used to obtain estimates for the Median Effective Time (MET) of hatching. It was assumed that the logarithm of the hatching time was exponentially distributed.

Test solutions (0.4 - 0.6 litre) were collected when passing the overflow and were analysed with GC-MS. Sample weight and sampling period were recorded and used to determine flow rates. OCDD and OCDF solutions were collected on day 1, 4, 6, 8, 11, 15, 18, 22, 25, 29 and 32 and control solutions were sampled on day 1, 8, 15, 22 and 29. A glass stirring bar, a standard solution ($^{13}C_{12}$-OCDD and $^{13}C_{12}$-OCDF in acetonitrile) and 65 ml acetonitrile was added to each Erlenmeyer flask before sampling. Samples were autostirred and extracted with C-18 columns (J.T. Baker). The C-18 columns were dried and eluted with hexane. Hereafter n-tetradecane and a recovery standard solution (1,2,3,6,7,8-$^{13}C_{12}$-HxCDD in n-tetradecane) were added. The hexane was removed with nitrogen until a final keeper volume of 20 µl and this solution was used for GC-MS analysis.

At test termination the surviving fish were pooled and analysed with GC-MS to determine the content of OCDD, OCDF and potential dechlorinated degradation products. Both pooled fish samples were dried with anhydrous sodium sulphate, homogenised, spiked with an internal standard solution and extracted with toluene. N-tetradecane was added, the toluene was evaporated to keeper volume and finally hexane was added. Two disposable Silica and Alumina columns were coupled, prewashed with hexane and the sample was pumped onto the Silica column. Hexane was pumped through both columns and the Alumina column was disconnected, washed with hexane/dichloromethane (v:v = 98:2) and eluted with hexane/dichloromethane (v:v = 1:1). The eluate was spiked with n-tetradecane and the solvents were removed until keeper volume. A standard solution of $^{13}C_6$-1,2,3,4-TeCDD in n-tetradecane was added to the extract. After homogenisation and dilution with toluene this final extract was used for GC-MS analysis.

Extract volumes of 1 µl (test solutions) and 10 µL (fish samples) were used for GC-MS analysis. All samples were analysed using a GC-MS of Hewlett Packard (HP 5890 series II/ HP 5971 A).

The GC was equipped with a DB-5 MS capillary column, length 60 m, internal diameter 0.25 mm, df 0.25 µm from J and W. Samples were injected using a PTV injector CIS III from Gerstel.

3. Results

Test conditions

Measured pH, oxygen content and temperature of control solutions during the study ranged between 7.4-7.7; 7.7-8.6 mg/l and 23.2-25.2 °C, respectively. Mean flow rates of tests solutions and results of chemical analysis of test solutions are presented in Table 1. The analytical results of all samples were corrected for the recovery. Mean recoveries were 76 % ($^{13}C_{12}$-OCDD) and 77 % ($^{13}C_{12}$-OCDF), respectively. A significant variation in exposure concentration occurred from day 5 - 18, which was attributed to adsorption of OCDD and OCDF to nematodes, added in this period as feed for the zebra fish. During the second part of the test a decrease in the OCDD and OCDF concentration was found which could have been due to the increased fish biomass.

Toxicity

The mortality of eggs between day 0 and day 1 was strongly dependant on the parent female but not on OCDD or OCDF exposure (Table 1). No mortality of eggs was observed after day 1 and all eggs hatched normally during the experiment. A pairwise comparison of the control group with the OCDD and OCDF groups revealed no significant difference in Median Effective Time of hatching of the eggs ($p > 0.85$, two-sided).

Neither OCDD nor OCDF had an effect on larval survival of zebra fish (Table 1). No aberrant behaviour or appearance of the zebra fish larvae was observed throughout the test. A comparison of control data with OCDD data ($p = 0.98$) and OCDF data ($p = 0.46$) revealed no statistically significant difference in weights of the larvae at test termination.

Table 1: Summarized results of the early-life stage test with zebra fish

Glass beaker	*Mean flow rate (litre per day)*	*Geometric mean concentration*[A] *(ng/l)*	*Mortality (%) of eggs between day 0 and day 1*	*MET*[B] *(hours)*	*Survival (%) of larvae*[C]
control A	7.2	< 4	35.0	85.0	85 (14.8)
control B	5.7	< 4	2.5	89.1	95 (14.4)
OCDD A	6.5	33 (10-107)	45.0	83.6	95 (13.7)
OCDD B	6.0	31 (10-232)	5.0	85.0	100 (15.4)
OCDF A	6.4	32 (8-138)	32.5	82.3	95 (13.5)
OCDF B	6.2	36 (8-149)	5.0	85.0	100 (14.4)

[A] Between brackets the lowest and highest measured concentration are given

[B] Median Effective Time of hatching

[C] Between brackets the mean weight (mg) of surviving larvae at test termination is presented

Bioconcentration

Concentrations of OCDD and OCDF in zebra fish were measured at the end of the 32 days of exposure. Concentrations in pooled larvae tissues were based on wet weight and were equal to 61 µg/kg (OCDD) and 94 µg/kg (OCDF), respectively. The recovery of $^{13}C_{12}$ internal standards in

the extracts of zebra fish ranged from 80 - 96 % (OCDD) and 81 - 102 % (OCDF). Compared to geometric mean concentrations of OCDD and OCDF in test solutions, the bioconcentration-factors were 2 x 10^3 and 3 x 10^3, respectively. No other PCDDs nor PCDFs were detected in the exposed fish (limit of quantification = 1 µg/kg).

4. Discussion and conclusions

Early-life stages of fish are very sensitive to the effects of PCDDs and PCDFs. These life-stages are in general susceptible to chemicals and the bioaccumulation potential of lipophilic compounds like PCDDs and PCDFs in yolk is high. Studies with 2,3,7,8-TCDD and early-life stages of fish revealed adverse effects at concentrations of 0.1 ng/l[1] and 0.4 ng/l[2].

The results of our study with OCDF and OCDD, performed at the highest water soluble concentrations, showed no adverse effect on the early-life stages of the zebra fish despite that measured concentrations in zebra fish were 61 (OCDD) and 94 (OCDF) µg/kg. Previous publications showed already that relatively high concentrations of OCDD and/or OCDF administered to juvenile and adult fish via dietary and aqueous exposure produced no detectable adverse effects on fish[3,4,13,14].

During the early-life stage study OCDD and OCDF could potentially be taken up by fish directly from the water phase or via feed (due to adsorption). In all laboratory studies with only dietary exposure, the concentration of OCDD/OCDF in fish was significantly lower than in feed and thus no biomagnification occurred[3,4,13,14]. Therefore uptake of OCDD/OCDF from the water phase was probably the most important exposure route during our study. The bioconcentration factor of 2000 to 3000 was in agreement with a 21-days aqueous exposure study with guppies that revealed a bioconcentration factor of 2000[15].

In our laboratory early-life stage study, concentrations in zebra fish of 61 (OCDD) and 94 (OCDF) µg/kg were reached which is much higher than concentrations in field collected fish. For example, concentrations in bream and perch from the harbour of Hamburg (Germany) ranged between 1.4 - 10.5 (OCDD) and < 0.6 - 8.3 (OCDF) ng/kg[16]. Concentrations of OCDD were slightly higher than concentrations of OCDF. In 15 fish samples collected in the Netherlands the highest OCDF concentration was 2 ng/kg, while the highest OCDD content in 19 fish samples was 8 ng/kg (personal communication W. Traag, RIKILT, Wageningen).

The small differences in bioaccumulation between OCDD and OCDF observed in this study does not explain the difference in levels between OCDD and OCDF observed in the food chain and in human tissues. Indeed, OCDF is generally a minor contributor of the PCDD/PCDF load in the food chain, while OCDD is commonly the dominant congener. Human food samples of different animal origins show a factor ~ 10 between OCDD and OCDF levels[5,6] while levels in human milk[7,8] and adipose tissues[8,9] consistently show a factor 10 - 100 between OCDD and OCDF levels.

This is more remarkable, given the similar bioconcentration of OCDD and OCDF observed in a 9-month feeding study in rats[17]. A possible explanation could be that human exposure to OCDD and OCDF via the consumption of fish is low compared to other sources. Rappe (1991)[18] showed that there was no significant difference in human tissue levels of either OCDD and OCDF between high fish consumption and no fish consumption groups.

The higher levels of OCDD in human tissues could be due to the predominance of OCDD in the terrestrial foodchain contaminated via air through dry deposition. As it is known that OCDF is more sensitive to photochemical degradation[19,20,21] differences may be explained by differences in atmospheric fate. Furthermore the relative bioavailability of OCDD and OCDF in different parts of the foodchain may be different.

In conclusion, although concentrations of OCDD and OCDF in early-life stages of fish were several orders of magnitude higher than concentrations in field collected fish, no toxicity of OCDF and OCDD was found during the fish early-life stage test which is considered the most sensitive test with 2,3,7,8-TCDD. Furthermore laboratory fish experiments show a lack of biomagnification of OCDD and OCDF. Therefore adverse effects of these compounds on the aquatic environment are not expected. As a consequence, their impact on the aquatic environment should not be evaluated on the basis of I-TEFs which were predominantly based on toxicity data derived from mammals. The low level of OCDF in human tissues further questions the relevance of the presence of OCDF in the environment for human health.

5. References

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