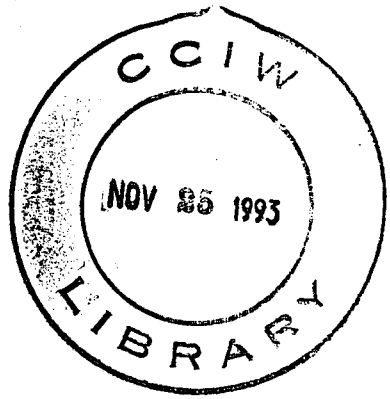

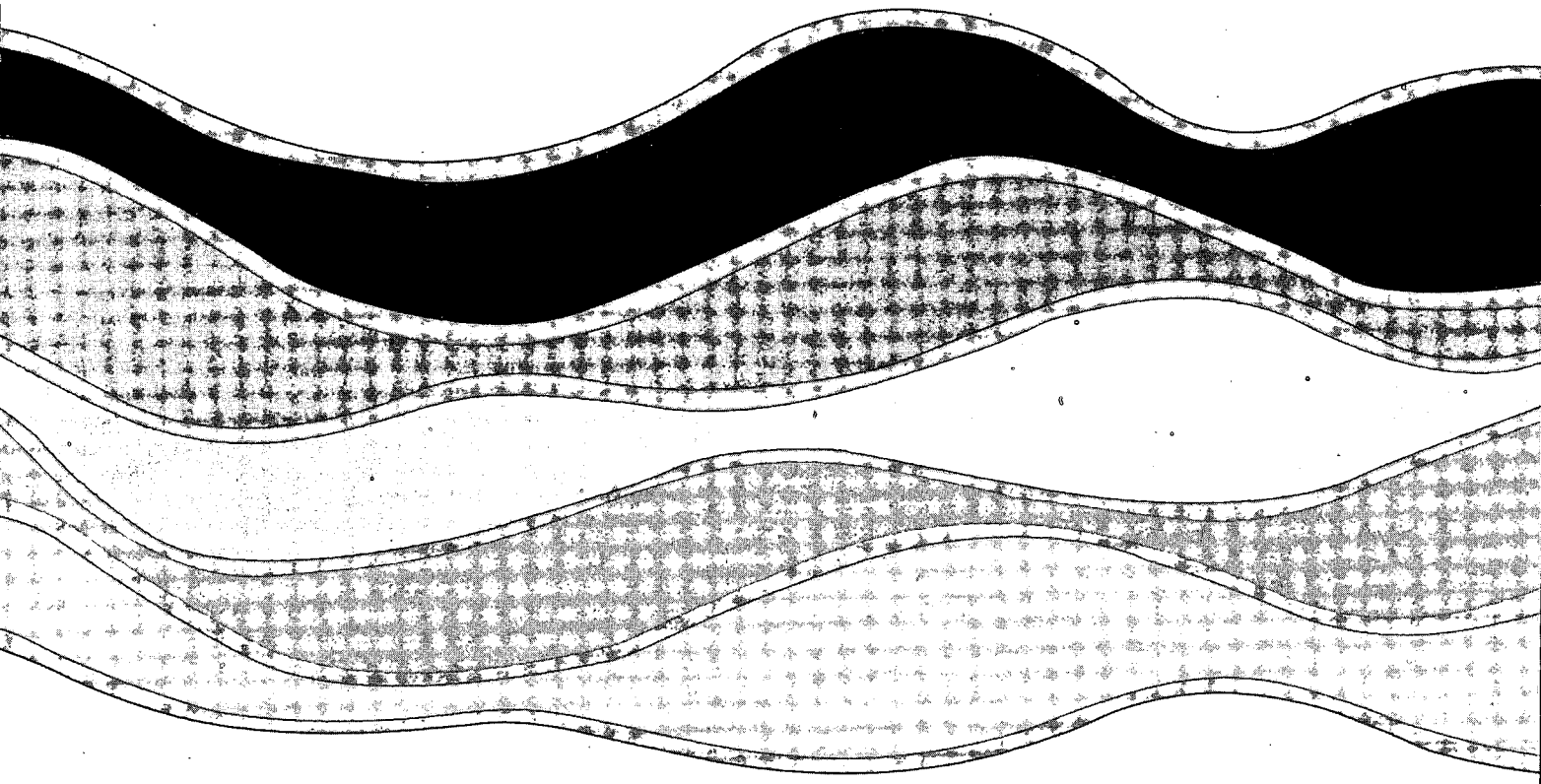

NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

Environment Canada Environnement Canada

Canada

NATIONAL DE RECHERCHE SUR LES EAUX
5050, BURLINGTON (ONTARIO) L7R 4A6

Division Control No. ECD-103B

BIOLOGICAL HALF-LIVES OF CHLORINATED DIBENZO-p-DIOXINS AND DIBENZOFURANS IN RAINBOW TROUT (SALMO GAIRDNERI)

A.J. Niimi and Barry G. Oliver

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National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

• Environment Canada

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A.J. Niimi and B.G. Oliver
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

Management Perspective

This paper indicates that chlorinated dioxins and dibenzofurans are poorly assimilated by fish from fish food. The large molecular size of these chemicals is likely the reason for the difficulty in transport across the stomach membranes. The small portion of these chemicals that are assimilated are quickly eliminated by the fish. These results help explain why the concentration of dioxins and dibenzofurans is so low in field fish (parts per trillion) even from sites which are fairly heavily contaminated with these chemicals. The low bioaccumulation potential of these compounds is indeed fortunate because of their extreme toxicity.

PÉRIODE BIOLOGIQUE DES DIBENZO-PARADIOXINES CHLORÉES ET DES DIBENZOFURANNES POUR LA TRUITE ARC-EN-CIEL (SALMO GAIRDNERI)

A.J. Niimi et B.G. Oliver
Centre canadien des eaux intérieures
Burlington (Ontario) Canada L7R 4A6

PERSPECTIVE-GESTION

Le document démontre que les dioxines chlorées et les dibenzofurannes sont mal assimilés par les poissons qui s'en nourrissent. La grosseur des molécules de ces substances chimiques explique probablement la difficulté qu'elles ont à passer à travers les membres de l'estomac. La faible quantité que les poissons arrivent à assimiler est rapidement éliminée. Ces résultats nous aident à comprendre pourquoi les poissons présentent une aussi faible concentration de dioxines et de dibenzofurannes (parties par billion), même dans les endroits où le taux de contamination est très élevé. Les possibilités restreintes de bioaccumulation de ces composés chimiques constituent vraiment un facteur favorable, en raison de leur très grande toxicité.

Biological half-lives of chlorinated dibenzo-p-dioxins and dibenzofurans in rainbow trout (Salmo gairdneri)

A. J. Niimi[1] and B. G. Oliver[2]

[1]Great Lakes Fisheries Research Branch, Canada Centre for Inland Waters, Burlington, Ontario, Canada. L7R 4A6

[2]Environmental Contaminants Division, Canada Centre for Inland Waters, Burlington, Ontario, Canada. L7R 4A6

Abstract - The biological half-lives of five dibenzo-p-dioxins and two dibenzofurans were determined in rainbow trout following a single oral exposure. Estimates ranged from 2 days for 2,7-dichlorodibenzo-p-dioxin to 43 days for 1,2,3,4-tetrachlorodibenzo-p-dioxin among the dioxins, and 12 days for octachlorodibenzofuran to 24 days for 3,6-dichlorodibenzofuran. No consistent relationship was shown between half-life and the degree of chlorination. Absorption efficiences varied from 2 to >30% although most values ranged from 5-16%.

Keywords - Half-life, Dioxin, Furan, Rainbow trout

INTRODUCTION

The chlorinated dibenzo-p-dioxins and dibenzofurans include some highly toxic substances whose effects on animals and environmental distribution has only recently received wide attention {1,2}. There are 75 polychlorinated dioxin and 135 polychlorinated furan congeners although most studies have focused on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) because of its toxicity {3,4}.

Information on the toxicology and kinetics of dioxins and furans on fish is limited. Contaminant surveillance studies have reported the presence of dioxin and furan congeners and isomers in aquatic organisms. Levels of 2,3,7,8-TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF) up to 150 ng/kg have been reported in fish from the Great Lakes {5-7}. Isomers of dichlorodibenzo-p-dioxin, tri-, and pentachlorodibenzofuran were reported to be present, but not hexa-, or heptachlorodibenzofuran in fish collected from waters or watersheds of the Great Lakes {8}. 2,3,7,8 TCDD was also shown to accumulate at different rates by several organisms in a model aquatic ecosystem {9}.

The toxicity of 2,3,7,8-TCDD has been shown in waterborne exposure studies where levels of 10-100 ng/L were lethal to salmon, trout, and pike {10,11}. A dietary exposure of 2 mg/kg 2,3,7,8-TCDD was lethal to trout after 33 days, but not at the 2 µg/kg dietary level {10}. Other studies would suggest related dioxin and furan congeners may be less toxic and less bioaccumulative than 2,3,7,8-TCDD. No detectable levels of di-, tri-, and tetrachlorodibenzofurans and only low levels of octachlorodibenzofuran were present in salmon fed diets containing 3-9 mg/kg of each congener for 140 days {12}. Only trace levels of octachlorodibenzo-p-dioxin were present in guppies fed on a 50 mg/kg diet for 70 days {13}. The biological half-life for 2,3,7,8-TCDD was estimated to be 58 days in trout and calculated to be in excess of 100 days in guppies {14,15}. The half-life of 1,3,6,8-TCDD has also been estimated to be 3-4 days in minnow

Demi-vie des dibenzo-p-dioxines chlorées et des dibenzofuranes chlorés dans la truite arc-en-ciel (Salmo gairdneri)

A.J. Niimi[1] et B.G. Oliver[2]

[1]Direction de la recherche sur les pêches dans les Grands Lacs, Centre canadien des eaux intérieures (Ontario) Canada L7R 4A6

[2]Division des contaminants environnementaux, Centre canadien des eaux intérieures, Burlington (Ontario) Canada L7R 4A6

Résumé - On a déterminé la demi-vie biologique de cinq dibenzo-p-dioxines et de deux dibenzofuranes dans la truite arc-en-ciel après une seule exposition par voie buccale. Les valeurs mesurées variaient de deux jours pour la 2,7-dichlorodibenzo-p-dioxine à 43 jours pour la 1,2,3,4-tétrachlorodibenzo-p-dioxine dans le cas des dioxines, et de 12 jours pour l'octachlorodibenzofurane à 24 jours pour le 3,6-dichlorodibenzofurane. Aucune relation applicable dans tous les cas n'a pu être établie entre la demi-vie et le degré de chloration. L'efficacité d'absorption variait de 2 p. 100 à plus de 30 p. 100, même si la plus plupart des valeurs étaient comprises entre 5 et 16 p. 100.

Mots clés - demi-vie, dioxine, furane, truite arc-en-ciel

and trout {16}.

It is apparent that further information on the kinetics of dioxins and furans in fish would be required to achieve a better understanding of their behavior in the aquatic environment. This study estimates the biological half-lives of five dibenzo-p-dioxins and two dibenzofurans in rainbow trout (Salmo gairdneri).

MATERIALS AND METHODS

The procedures used in this study were similar to those used to estimate the biological half-lives of polychlorinated biphenyl (PCB's) congeners in rainbow trout {17}. Fish averaging 1 kg each were held in three 660 L tanks at 10±1°C and fed once daily on a dry diet at 1% of body weight. After 6 weeks of acclimation, individual fish from one group received an oral dose of one ml fish oil in a gelatin capsule which contained 82 µg/ml 2,7-dichlorobidenzo-p-dioxin (2,7-DCDD), 38 µg/ml 1,2,4-trichlorobidenzo-p-dioxin (1,2,4-TCDD), and 30 µg/ml octachlorodibenzo-p-dioxin (OCDD). A second group received a one ml dose containing 37 µg/ml 2,3-dichlorodibenzo-p-dioxin (2,3-DCDD), 30 µg/ml 1,2,3,4-tetrachlorodibenzo-p-dioxin (1,2,3,4-TCDD), 115 µg/ml 3,6-dichlorodibenzofuran (3,6-DCDF), and 15 µg/ml octachlorodibenzofuran (OCDF). A third group of control fish received only fish oil. Each fish was anesthetized with MS-222 before the gelatin capsule was orally inserted into the stomach using a glass tube and plunger. The oil mixtures were prepared by dissolving dioxin and furan analytical standards (Analabs, North Haven, CT) in hexane, adding the solution to fish oil, then removing the hexane from the mixture using a 3-stage condenser.

Six fish each were sampled 5, 15, 35, and 70 days after receiving the oral dose for the exposed groups, and after 5, 35, and 70 days for the control group. All fish were frozen until analyses.

Residue levels in fish were determined according to the following procedure.

Whole fish samples were prepared by using a Hobart grinder and an Oster blender to obtain a representative sample. A 15 g subsample was placed in a 250 ml wide-mouth bottle with 105 ml concentrated HCl (preextracted 3 times with hexane) and 50 ml hexane then sealed with a teflon-lined cap. The sample was digested overnight at room temperature (about 16 h), then placed in a separatory funnel and the hexane layer recovered. The sample was extracted twice with 30 ml hexane and the aliquots combined. The extract was shaken vigorously with 30 ml of concentrated H_2SO_4 (BDH Aristar) to remove most of the lipids and allowed to sit for at least 2 h for the layers to separate. The hexane layer was dried through a column of Na_2SO_4 and evaporated to 1 ml using a Kuderna-Danish (KD) condenser. The 1 ml extract was passed through a 4 cm long column (6 mm I.D. Pasteur pipette) of 40% H_2SO_4 on silica gel and 5 ml of hexane was collected. The volume was reduced to 1 ml using the KD condenser.

Final cleanup and separation of the dioxins and furans from PCB's was accomplished using Florisil. The Florisil was activated by heating at 500°C for 2 h, packed in 4 cm long columns (Pasteur pipettes), and kept overnight in an oven at 130°C until use. The columns were prerinsed with 5 ml dichloromethane (DCM) followed by 5 ml hexane, then the 1 ml extract was placed on the column. The column was eluted with 10 ml hexane (most of the PCB's are present in this fraction), then with 20 ml DCM (the dioxins and furans are present in this fraction). The dioxins and furans were transferred to hexane by adding 10 ml hexane to the DCM eluent, and evaporating to 1 ml. This procedure provided extracts sufficiently clean for µg/kg quantitation, but not clean enough for determinations of environmental samples in the ng/kg range.

Concentrations were determined using a Varian 4600 gas chromatograph equipped with electron capture detectors. The extracts were run on two 30 meter fused-silica columns (SE54 and OV1), and agreement between columns was satisfactory. Helium was the carrier gas. The chromatographic conditions were: injector, 280°C; program, 50-280°C at 4°C per min; final hold, 15 min;

detectors, 350°C. Recovery studies of the method (n=6) by spiking uncontaminated trout tissue with dioxin and furan standards indicated efficiences which ranged from 60-80% depending on compound with a reproducibility of ±20%. Detection limits, based on the 15 g sample extract, for the chemicals at an instrument signal to noise ratio of 5:1 were 200 ng/kg for 2,7-TCDD and 3,6-DCDF; 70 ng/kg for 2,3-DCDD; 40 ng/kg for 1,2,4-TCDD and OCDD; and 20 ng/kg for 1,2,3,4-TCDD and OCDF.

Half-life was calculated using the relationship $Y = A_e^{bt}$ where the clearance coefficient (b) was based on the body burden (Y) of each compound following the days exposed (t) to account for declining concentrations attributable to growth dilution {17}. Body burden was calculated by multiplying chemical concentration by body weight.

RESULTS

Fish weight averaged 970±275 g (mean±SD), 960±205 g, 1060±705 g, and 1110±210 g for the first group, and 920±85 g, 1170±190 g, 960±90 g, and 1280±205 g for the second group of exposed fish at the successive sample intervals. The control fish averaged 860±260 g, 865±210 g, and 975±105 g. Residue levels among the respective compounds in control fish did not differ significantly among the three sample intervals which averaged 4.2±0.9 µg/kg for 2,7-DCDD, 0.5±0.1 µg/kg for 2,3-DCDD, 2.7±1.3 µg/kg for 1,2,4-TCDD, 0.3±0.1 µg/kg for 1,2,3,4-TCDD, 0.2±0.1 µg/kg for OCDD, 0.8±0.1 µg/kg for 3,6-DCDF, and 0.1±0.1 µg/kg for OCDF. It is probable that the hexane extract from these fish contained substances such as PCB's which coeluted with the dioxin and furan standards although their influence would be minimal because of the low concentrations. These concentrations were used to adjust the values reported in exposed fish for residual levels present in fish and any that may have been acquired through the food during the study. No mortalities or anomalous behavior were observed among

the control and exposed fish during the study.

The residue levels of each compound was estimated from the concentration and body weight for each fish at the successive sample intervals. Body burden rapidly declined among all the compounds, reaching near detection limit levels by the end of the study (Table 1). In view of this, the regression analysis to estimate half-life for each compound was based on the values from two to four sample intervals which showed successive declines in residue levels. The biological half-lives of the five dioxins ranged from 2-43 days, and 7-12 days for the two furans (Table 2).

Absorption efficiency of each compound was estimated using the amount fed, and that present in fish at the beginning of the study. The values ranged from 2% for 1,2,3,4-TCDD to great than 30% for 2,7-TCDD (Table 2). It was not possible to calculate an exact absorption efficiency for 2,7-DCDD because the only measurable levels occurred at the first sample interval (Table 1). It is noteworthy that this compound has a structure that is related to 2,3,7,8-TCDD although it contains two less chlorines.

DISCUSSION

The biochemical behavior of dioxins and furans have been compared to those of PCB's because these planar halogenated hydrocarbon groups contain a large number of isomers and congeners whose activities are influenced by structural similarities although relative toxicities vary within and among groups {18,19}. Members of these groups also have physico-chemical properties such as high octanol-water partition coefficients which have been used to predict the bioaccumulative behavior of similar priority chemicals in the aquatic environment {20,21}.

The biological half-lives and absorption efficiences of dioxins and furans observed in this study were markedly different from those reported for PCB's in

trout and carp {17,22}. Half-lives of di- to decachlorobiphenyl isomers generally increased from a few days to no apparent elimination as the degree of chlorination increased, and absorption efficiences ranged from 70-90%. In view of the short half-lives and low absorption efficiences observed in this study, it cannot be established if the behavior of one factor influenced the response of the other. The low absorption efficiences resulted in residue concentrations that were lower than expected, therefore the regression analyses did not include the entire data set for those substances whose levels did not change during subsequent sampling intervals (Table 2).

The cumulative information on dioxins and furans would suggest their half-lives within isomeric groups can vary, and are generally shorter than those of comparable PCB isomers in fish. Limited data suggests 2,3,7,8-TCDD is one of the more persistent tetra- isomers with a half-life of 58 days, compared to 43 days for 1,2,3,4-TCDD (this study) and 4 days for 1,3,6,8-TCDD in trout {14,16}. The estimates of 12 days for OCDF and 18 days for OCDD reported in this study were much shorter than those reported for octa- to decachlorobiphenyls which exceeded 200 days {17}. Influence of the degree of chlorination on half-life was suggested among the di-, tri, and tetra-CDDs although this trend was not supported by the results for OCDD, DCDF, and OCDF. If this relationship exists for dioxins and furans, it is not as well defined as that for PCB's in fish.

Absorption efficiences of 2 to > 30% estimated for trout among the compounds reported in this study are lower than the 65-90% values reported for 2,3,7,8-TCDD and 2,3,7,8-TCDF for rats {23,24}. It could be inferred from this and other food exposure studies that absorption efficiences of dioxins and furans in fish are low. Only trace amounts or no detectable levels of five dioxin and furan congeners were reported in fish fed diets containing mg/kg quantities for extended periods {12,13}. A study on aquatic insects using a sediment-water exposure system also reported poor absorption efficiences for OCDD, and the half-lives reported to OCDD and 1,3,6,8-TCDD averaged 2 and 9 days

respectively {25}.

The results of this and other laboratory studies are consistent with an analysis of the residue levels reported in environmental samples. Residue levels in fish indicate 2,3,7,8-TCDD and 2,3,7,8-TCDF are often present at the highest concentrations and frequency in samples among the tetra isomers {6}. A comparative analysis of furan levels between sediment and fish indicate tetra- and penta-CDF isomers occur at ratios at least 70-210 times greater in fish than sediment {26}. Conversely, hepta- and octa-CDF isomer levels are at least 50-180 times greater in sediment than fish {26}. This observation is consistent with the qualitative analyses of furans in fish which indicated isomers of tri- and penta-CDF were present in fish but not hexa- or hepta-CDF {8}. Others have reported tetra- to octa-CDD and tetra- to hepta-CDF were present in fish collected from polluted areas {27}. The last observation is particularly important because while dioxins and furans appear to be poorly absorbed and have relatively short half-lives in fish, elevated levels may be attained through bioaccumulation under certain environmental conditions.

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able 1. Amount of each compound fed, and retained by trout after 5-70 days. ach value represents the mean±SD of six fish.

ompound	Amount fed, µgs	µgs per fish			
		5 d	15 d	35 d	70 d
2,7-DCDD	82	23.2±15.8	<0.2	<0.2	<0.2
2,3-DCDD	37	2.3±0.7	0.9±0.6	0.1±0.1	0.1±0.1
1.2,4-TCDD	38	11.4±7.4	2.6±2.8	1.6±1.1	0.1±0.1
,3,4-TCDD	30	0.6±0.3	0.4±0.1	0.3±0.2	0.2±0.1
OCDD	30	3.4±3.3	1.0±0.4	0.6±0.3	0.6±0.3
3,6-DCDF	115	14.5±2.6	6.3±4.9	1.9±0.7	0.4±0.4
OCDF	15	0.6±0.5	0.2±0.1	0.2±0.1	0.1±0.1

Table 2. Relationship between µgs of each compound (Y) retained by trout and days after exposure (t). All regression equations are statistically significant at $P \leq 0.05$. The biological half-life (mean±95% confidence interval) and absorption efficiency are shown for each compound.

Compound	Equation	r^2	F-value	n	Half-life, days	% Absorption efficiency
2,7-DCDD	$Y = 192_e^{-0.462t}$	0.82	45.22**	12[1]	2±1	>30
2,3-DCDD	$Y = 3.5_e^{-0.097t}$	0.87	108.09**	18	7±1	9
1,2,4-TCDD	$Y = 6.1_e^{-0.058t}$	0.56	28.51**	24	12±2	16
1,2,3,4-TCDD	$Y = 0.5_e^{-0.016t}$	0.52	24.10**	24	43±8	2
OCDD	$Y = 2.4_e^{-0.046t}$	0.43	11.91**	18	15±4	8
3,6-DCDF	$Y = 13.8_e^{-0.056t}$	0.80	86.11**	24	12±1	12
OCDF	$Y = 0.7_e^{-0.012t}$	0.43	7.65*	12	7±2	5

[1]A value of 0.1 µgs 2,7-DCDD was used at the 15 day sample interval for calculation purposes.