IAFMM RESEARCH REPORT 1991-3

IAFMM

international association of fish meal manufacturers

Hoval House, Mutton Lane, Potters Bar,
Hertfordshire EN6 3AR. U.K.

Telephone: (Potters Bar) 0707 42343
Fax: 0707 45489
Telex: 8401 [illegible] (IAFM G)

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Our ref. SMB/RE/1991/11/SCWGSAF+FO

Your ref.

Date 11th January 1991

TO: ALL MEMBERS OF THE SCIENTIFIC WORKING GROUPS ON SAFETY AND FISH OIL

STRICTLY CONFIDENTIAL

Dear Sirs

DIOXINS AND FURANS IN SAMPLES OF CRUDE FISH OIL

* I have pleasure in enclosing four reports for your careful assessment, namely:

- Facts about dioxins and furans
- A letter from Dr. Duthie dated 2nd January 1991
- A report to Dr. Duthie from the University of Umea, Sweden
- A letter to Dr. Duthie from AEA Environment and Energy dated 28th September 1990.

In summary, two crude fish oils were selected from fishing grounds most likely to be contaminated with dioxins and furans. These samples were submitted to two laboratories capable of undertaking these expensive analyses. Discrepancy in the results from laboratories were noted. Taking the results from probably the most reliable laboratory and adopting some European action levels, one could calculate that the results suggest that the maximum intake of this fish oil by humans should be 2.8g-6.2g per day.

The average daily intake of partially hydrogenated fish oil in the U.K. is calculated at about 7g per day, and in Norway at 22g per day. In addition, members wish to increase refined fish oil in human diets in order to increase the EPA and DHA levels. Levels of fish oil contributed by specially prepared margarines would be an additional 5g per day.

/Cont'd.....

Dr. Duthie's advice to the Association, in view of these results, is that it would be prudent for members to have information on the dioxin and furan content of representative samples of their unrefined fish body oil, and for there to be information on the behaviour of these compounds during the standard refining procedure.

This matter will be discussed during the April Scientific Committee meeting in Scotland. However, if members have any preliminary comments to make it would be helpful to receive them in advance in writing.

Yours faithfully
International Association of Fish Meal Manufacturers

S.M. Barlow
Director General

Encls.

FACTS ABOUT DIOXINS AND FURANS.

by

Kristin Hafskjold and Svein Slungaard.

* Introduction
* Milk cartons
* Bleaching of pulp for paper and carton manufacturing
* Dioxins in the environment
* Toxicity to human beings
* Dioxins - What is it ?
* Calculation of toxicity
* Picogram and ppt as units for dioxin content
* Maximum daily dioxin intake
* Dioxin analysis

INTRODUCTION.

Even though dioxins have been known for about one hundred years, the interest for these chemical compounds has exploded during the last few years. The knowledge about dioxins is still limited, and as a result, their toxicities to human beings are still not known.

Dioxins are spread in the environment through wood fires, fly ash, car exhaust and smoke from municipal incineration plants, but they are also formed as a by-product from several industrial chemical processes. The dioxins and furans and then spread through products, through waste water and through air. Due to this, there exist a certain back ground level of dioxins in the environment.

Dioxins are very persistent towards decomposition and they are therefore widely spread and accumulated in the environment. The existence of these compounds in the nature has lead to detectable dioxin levels in our food and food products, and also in the raw milk of cows.

MILK CARTONS.

Over the last few years, it has been established that bleaching of pulp with pure chlorine generates dioxins and furans. The dioxins and furans can enter the environment through water effluent and through products manufactured by this pulp, for instance raw material for milk cartons. However, dioxins have also been detected in products made from unbleached pulp.

The introduction of new bleaching processes during the last year has resulted in a dramatic decrease in the dioxin content in milk board, which has reached the level of less than 1 ppt. This level was originally set by BGA (Bundes Gesuntheits-Amt, Germany) in May 1989 and was to be reached by May 1990.

Analyses results indicates that with a dioxin content less than 1 ppt in milk cartons, no migration of dioxins and furans to milk is taking place.

When the traditional chlorine bleaching were used, the dioxin content in the cartons were higher than today, and some migration took place. However, migration of dioxins from milk to carton were also found.

The migration consisted almost solely of 2,3,7,8-TCDF, a compound which is formed in particular during the bleaching process when pure chlorine is used as the bleaching agent.

BLEACHING OF PULP FOR PAPER AND CARTON MANUFACTURING.

Bleaching agents are used to remove the lignin from the unbleached pulp. Lignin is the chemical compound natural to the trees, which has the function to bind the fibers together. Lignin gives the characteristic brown color to the unbleached pulp, which is removed in the bleaching process, but in addition, the bleaching causes removal of a lot of other compounds, natural to the tree, which are unwanted in the milk carton, mainly because of off-taste.

Improvements in the bleaching processes have resulted in a change of bleaching agents using dioxide, oxygen, peroxide, enzymes and ozone instead of the traditional bleaching agent; Pure chlorine.

DIOXINS IN THE ENVIRONMENT.

Dioxins and furans are not only formed during chlorine bleaching of pulp. They can also be formed as a by product from different industrial chemical processes such as magnesium / nickel production and scrap metal melting. The dioxins and furans are also spread in the environment by fly ash, car exhaust and by smoke from municipal incineration.

Dioxins are very persistent against decomposition in the environment, which makes them exist in the environment for a long time (some years). After they are formed and released to air, they can be transported, bounded to particles, with the winds over a very long distance.

Since dioxins are practically not soluble in water, they are bounded to humus and particles, and collected in the soil or on the bottom of lakes and rivers. However, they tend to accumulate in fat, which results in high dioxin levels in water living animals with high fat content, for instance in herring, in mackerel and in crab.

The raw milk of cows contains dioxins which are solved in the milk fat, and due to this, dioxin content in cream is expected to be higher then the content in skimmed milk.

TOXICITY TO HUMAN BEINGS.

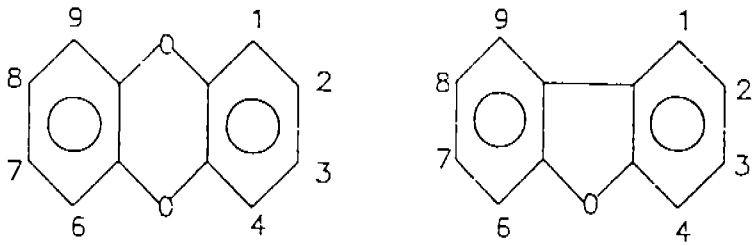
Some of the dioxins are very toxic to certain animals. The toxicity to human beings is not known exactly, but neither carcinogenic effects nor any other chronic effects have been documented, not even after heavy long-term occupational or accidental exposure.

One example of the latter is an accident at a factory in Seveso, Italy, in 1976, were 37.000 people of all ages where exposed to relatively large quantities of dioxins. A few got chloracne (a skin disease), headaches and digestive upsets, but no long term effects such as birth defects and chromosomal damages have been identified.

DIOXINS - WHAT IS IT ?

Dioxins is in a wide sense, a large chemical group containing chemical compounds with similar structures. However , dioxins are also widely used as a common term of the approximately 12 chemical compounds ("The dirty dozen") which have shown toxic effects to certain animals, and which belongs to the two chemical groups, the dioxins and the furans .

All these toxic dioxins and furans are chlorinated compounds which can be derived from the two chemical formulas, dibenzo-p-dioxin and dibenzo-furan, as shown in figure 1.



dibenzo-p-dioxin dibenzo-furan

Figure 1. Structures of dibenzo-p-dioxins and dibenzofurans.

Every position in the molecules can be substituted with one or several chlorine atoms, forming 135 different chlorinated furans and 75 different chlorinated dioxins, see table 1.

Table 1. PCDDs (Poly-chlorinated-dibenzo-p-dioxins) and PCDFs (Poly-chlorinated-dibenzofurans).

Number of Chlorine atoms	Prefix	Short term	Number of PCDDs	of PCDFs
1	mono	M	2	4
2	di	D	10	16
3	tri	Tr	14	28
4	tetra	T	22	38
5	penta	Pe	14	28
6	hexa	Hx	10	16
7	hepta	Hp	2	4
8	octa	O	1	1
Sum			75	135

CALCULATIONS OF TOXICITY.

Laboratory tests with certain animals have shown that 2,3,7,8-TCDD is the most toxic of the dioxins and furans. As a result, the toxicity of the other compounds are expressed relatively to this, using a factor, which is commonly referred to as the "equivalent factor":

A compound which is half as toxic as 2,3,7,8-TCDD has the factor 0.5, and one which is one tenth as toxic has the factor 0.1 etc.

Equivalent factors are used to calculate the toxicity of a sample, for instance the toxicity of milk, due to dioxins and furans. The contents of every dioxin and furan in the milk are then analyzed and multiplied with the equivalent factor corresponding to the compounds (table 2), giving a series of numbers, which are called TCDD-equivalents.

The sum of these TCDD-equivalents is "the toxicity", also referred to as "the dioxin content".

A set of equivalent factors are called an "equivalent model". Several equivalent models exists, and the difference between them consists of

variations in the numeric values of the factors. As a result, toxicity calculated using different models might give different results.

Comparison of common used equivalent models is shown in table 2.

Table 2. Equivalent factors of PCDDs and PCDFs.

Equivalent model:	Eadon 86	EPA	UBA	Nordic
2,3,7,8-TCDD	1	1	1	1
not 2,3,7,8-TCDD	-	0.01	0.01	-
2,3,7,8-PeCDD*	1	0.5	0.1	0.5
not 2,3,7,8-PeCDD	-	0.005	0.01	-
2,3,7,8-HxCDD*	0.033	0.04	0.1	0.1
not 2,3,7,8-HxCDD	-	0.0004	0.01	-
2,3,7,8-HpCDD*	-	0.001	0.01	0.01
not 2,3,7,8-HpCDD	-	0.00001	0.001	-
OCDD	-	0	0.001	0.001
2,3,7,8-TCDF	0.33	0.1	0.1	0.1
not 2,3,7,8-TCDF	-	0.001	0.01	-
2,3,7,8-PeCDF*	0.33	0.1	0.1	0.01
2,3,4,7,8-PeCDF				0.5
not 2,3,7,8-PeCDF	-	0.001	0.01	-
2,3,7,8-HxCDF*	0.021	0.01	0.1	0.1
not 2,3,7,8-HxCDF	-	0.0001	0.01	-
2,3,7,8-HpCDF*	-	0.01	0.01	0.01
not 2,3,7,8-HpCDF	-	0.00001	0.001	-
OCDF	-	0	0.001	0.001

Eadon 86 : Eadon et al 1986 (USA)
EPA : Environmental Protecting Agency (USA)
UBA : Umwelt Bundes Amt (West Germany)
Nordic : (Nordic countries)

* isomers having chlorine in positions 2,3,7,8 and elsewhere

As earlier mentioned, dioxins and furans tend to accumulate in fat. Dioxin content in for instance milk, are therefore often reported as "pg/g fat" instead of "pg/g milk".

PICOGRAM AND PPT AS UNITS FOR DIOXIN CONTENT.

The Total amount of dioxins and furans is given in picograms or fentograms. These units compared to more usual and well known units are shown in table 3.

Table 3. Picogram and fentogram compared to other mass units.

Unit	Short term	ant. grams	
Kilogram	kg	1000	10^{3}
Gram	g	1	
Milligram	mg	0.001	10^{-3}
Microgram	ug	0.000001	10^{-6}
Picogram	pg	0.000000000001	10^{-12}
Fentogram	fg	0.000000000000001	10^{-15}

(In popular terms, 1 ppt can be compared to 1 grain of barley in 100.000 tons of wheat!)

The Concentrations of dioxins and furans are given in ppt (parts per trillion), which equals to pg/g, and in ppq (parts per quadrillion) which equals to fg/g or pg/kg.

MAXIMUM DAILY DIOXIN INTAKE.

As earlier mentioned, laboratory tests have shown that some dioxins are toxic to certain animals. Based on these test results, a maximum dioxin intake for humans has been estimated as TCDD-equivalents per kg body-weight and day.

These maximum values varies from country to country, not only because of the use of different equivalent models. Different methods and estimations have been used while establishing these values.

As indicated in figure 2, there exists a wide spread in the recommended maximum daily dose, due to the fact that the dose are estimated, because of lack of toxicity documentation to human beings.

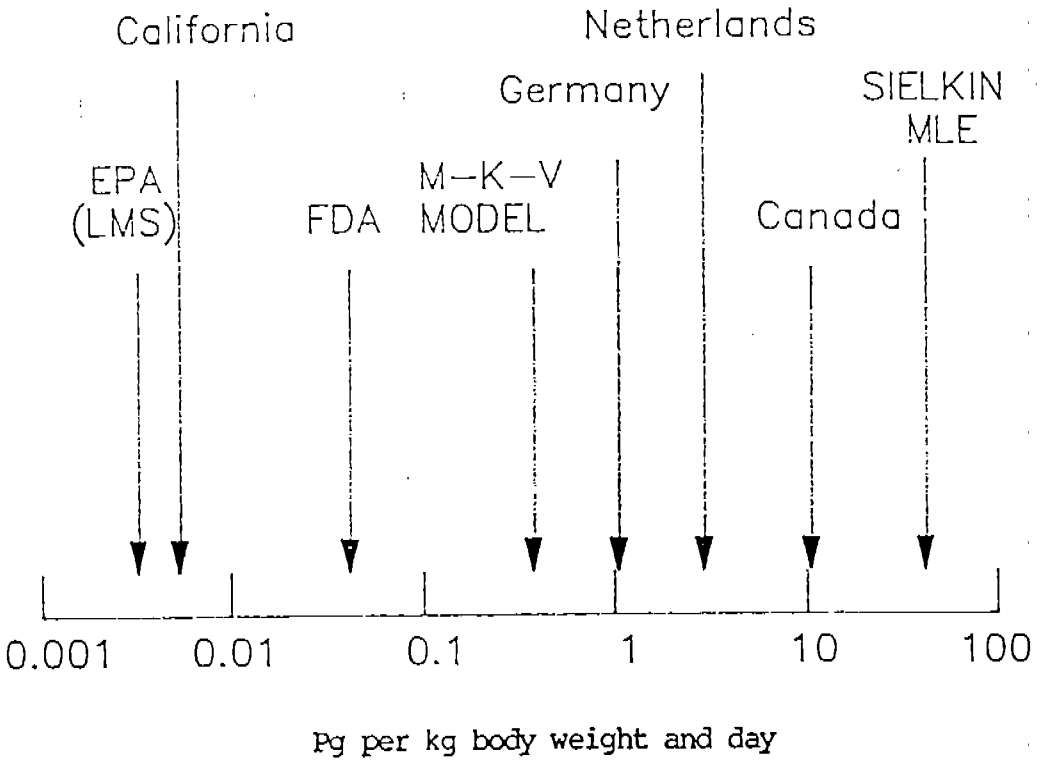


Figure 2. Variations in recommended maximum daily dioxin intake.

DIOXIN ANALYSIS.

To analyze dioxin contents at ppt and ppq levels, a HRGC (high resolution gas chromatograph) and a HRMS (high resolution mass spectrograph) are needed. The analyses are very time consuming, and besides just a few institutes are capable of doing this analysis properly, which makes the price of an analysis very high, ca. 12.500 NKr (3.500 DM, 1.800 USD) pr analysis.

In addition, also the fat content is analyzed, to allow for the results to be reported as "pg/g fat" instead of "pg/g sample".

There is no standard procedure for dioxin analyses, and as a consequence, analyses done by different institutes might give different results.

Norwegian Institute for Air Research has worked out a quality assurance list which should be followed when analyzing milk samples to get comparable and reliable results (available upon request to mr. Michael Oehme tlf. + 47 6 81 41 70).

IAIN F. DUTHIE

PH.D., B.SC. (BIOCHEM),
B.V.M.S., M.R.C.V.S.

INTERNATIONAL CONSULTANCY TO THE
FOOD, ANIMAL PRODUCTION AND
PHARMACEUTICAL INDUSTRIES

LITTLE THATCH
THE CHASE
KNOTT PARK
OXSHOTT
SURREY KT22 0HR, U.K.

TELEPHONE: (0)372 842972
FAX: (0)3727 20623

CONFIDENTIAL

2nd January 1991

Dr S.M. Barlow
IAFMM, Hoval House
Orchard Parade
Mutton Lane
Potters Bar
Herts EN6 3AR

Dear Stuart,

Analysis of dioxins and furans in fish oil: view of pilot study results

The report on the philosophy, decisions and design for the pilot study, given to the Scientific Committee at the Reykjavic meeting in September last year is to be included as an Appendix to the Minutes. I can't recall what I passed to you in the way of photocopies of data received from the labs, but for good order I enclose copies of the Umea fax of 5th Sept. 1990 and a letter from Harwell of 28th Sep. 1990.

Taken together, the results from our reference lab. Umea, Sweden, and from the lower cost candidate lab. Harwell, U.K., are a bit of a porridge. So it seems best, in the first instance, to make an overview of the findings, with a view to determining what further information might be required from the labs and/or the best way of reporting the pilot effort.

While Harwell gives a reasonable account of the clean-up methodology employed, Umea depends on citation of two references. Likewise, GC/MS conditions cannot readily be compared at present. Umea quoted the range of ^{13}C-labeled isomers added as internal standards with recovery information, but Harwell did not. Umea reported mostly levels for main individual isomers in parts per trillion (ppt), but Harwell reported a few individual isomers and mainly isomer groups in nanograms per kilogram (ng/kg). The various ways of expression of levels in this field are hopelessly confusing, but I think these should be equivalent (i.e. 1 part in 10^{12}).

Overall, I suppose the conclusion could be drawn that both labs found a range of PCDD and PCDF in both samples of unrefined fish body oil, so these compounds can be present and labs can detect them.

But because of the way the individual labs reported results, only five direct comparisons can be made as shown in the attached table. Of these, I believe we could focus on the 2,3,7,8- TCDD and TCDF congeners as being those regarded as of most concern from the toxicity point of view. The order of detection for these was similar, agreement being good for 2,3,7,8-TCDF, but Harwell found 3.1 to 2.4 times more 2,3,7,8-TCDD than Umea did. For the other three comparisons the differences are rather great,

apart for OCDD in Sample 2.

As far as rating the overall toxicity of the samples for TCDD/TCDF is concerned, Umea presented Nordic model TCDD equivalents of 21 and 9.7 ppt for Samples 1 and 2, respectively, while Harwell used NATO/CCMS International Toxic Equivalent Factors to give International Toxic Equivalent (I-TEC) ratings of 57 and 30 ng/Kg, respectively. I have comparisons of "Eadon 86", USA EPA, West Germany UBA and Nordic factors, but not a comparison of any of those with NATO/CCMS factors.

Thus, assuming that the Umea findings are reliable, for comparative purposes quite a few aspects could be taken up with Harwell. When we discussed this matter recently, I believe you had obtained hints that Harwell performance might not be reliable. Certainly it was said that new, supposedly, expensive equipment would be used for the work, and software problems encountered delayed the work. So I wonder, since you are primarily interested in economy of effort these days, if you would feel in a strong enough position to recommend to the Association, that apart from confirming presence of PCDD and PCDF in samples of unrefined fish oil selected as most likely to contain these compounds, Harwell might not be considered suitable for further work, and that even with likely higher costs it might be unwise not to use Umea for any further work. On how you view this question, would depend the questions which might be asked of Harwell. As far as Umea is concerned, I should prefer to have a more explicit account of sample clean-up and GC/MS analysis. Also, a Umea comment was that the PCDD/PCDF pattern was "quite different" from that normally seen for fish oil.

If for present purposes we take the Umea data as meaningful, then a range of tetra- to octa- CDD and CDF are present in both samples. There is a wide spread in recommendations for maximum daily dioxin intake from country to country. The U.K. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment* identified a guideline level for human exposure of 1* picogram (pg)/kg/body-weight per day of 2,3,7,8-TCDD or TCDD Equivalents, or 60 pg/d for the average person of 60 kg body-weight, as a trigger for investigation and appropriate measures to reduce levels. On the face of it, therefore, intakes of only 2.8 g* and 6.2 g* per day of Samples 1 and 2 by the average person would supply 60 pg/d according to the Umea results for TCDD equivalents.

× 10

Bearing in mind that the samples were selected as coming from fishing grounds most likely to be contaminated with PCDD/PCDF and presented in the unrefined state, the results cannot be taken as indicative of fish body oils generally or their possible PCDD/PCDF contents in a refined state. But my advice to the Association from the results obtained and the general public health attitude to PCDD/PCDF is that it would be as well for members to have information on the PCDD/PCDF content of representative samples of their unrefined fish body oils, and for there to be information on the behaviour of PCDD/PCDF during the standard refining procedure.

Perhaps you would let me know if you think further enquiries should be made of the labs, and if more formal reporting is required.

Yours sincerely,

Iain

Dr Iain F. Duthie

Encs:

* COT completed a 3rd review in 1995 and did not alter the Tolerable Daily Intake (TDI) of 10 pg/Kg bw/day

Dr Iain F. Duthie, Little Thatch, The Chase, Knott Park, Oxshott, Surrey KT22 0HR, U.K.

Concentrations of PCDD and PCDF reported by two laboratories for two fish oils samples

PCDD/PCDF (ppt*)	Sample 1		Sample 2	
	Lab 1	Lab 2	Lab 1	Lab2
2378-TCDD	2.6	8.0	3.4	8.0
2378-TCDF	28.0	29.0	13.0	13.0
23478-PeCDF	23.0	84.0	3.3	34.0
OCDD	5.2	<20.0	85.0	87.0
OCDF	0.96	<70.0	1.0	<30.0

* ~~ng/g~~ pg/kg

Dr Iain F. Duthie, Little Thatch, The Chase, Knott Park, Oxshott, Surrey KT22 0HR, U.K.

Sture Bergek
Institute of Environmental Chemistry
University of Umeå
S-901 87 Umeå
Sweden

Umeå 90-09-05

To
Dr Iain F. Duthie

Results from analysis of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in Fish oil.

Sture Bergek
Maria Hjelt
Christoffer Rappe
Institute of Environmental Chemistry
University of Umeå
S-901 87 Umeå
SWEDEN

Results from analysis of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in Fish oil.

The purpose of the analysis

The purpose of the analysis is to determine levels of poly-chlorinated dibenzo-p-dioxins (PCDDs) and dibenzo-furans (PCDFs) per gram oil in two fish oil samples.

Description of sample

The samples arrived at Umeå University, Institute of Environmental Chemistry , on the 13th of aug.1990.In our continous sample registration they got the number MPR1184:1-2.

Clean up and analysis

This is a summary of references 1-2.

The samples were kept in a refrigerator (+6°C) until extraction. The oil ,exact amount is stated in the table,was warmed to about 35°C and diluted with 200mL 50% cyclohexane in methylenechloride. Before the extraction, the following ^{13}C-labeled isomers were added as internal standards:

^{13}C-2,3,7,8-TCDF
^{13}C-2,3,7,8-TCDD
^{13}C-2,3,4,7,8-PeCDF
^{13}C-1,2,3,7,8-PeCDD
^{13}C-1,2,3,4,7,8-HxCDF
^{13}C-1,2,3,7,8,9-HxCDF
^{13}C-1,2,3,6,7,8-HxCDD
^{13}C-1,2,3,4,7,8,9-HpCDF
^{13}C-1,2,3,4,6,7,8-HpCDD
^{13}C-OCDF
^{13}C-OCDD

The ^{13}C-labeled compounds are used to correct losses in the clean up and variation in the mass spectrometer analysis. Recovery in % are used to recalculate the results at the end.
All solvents used during the analyses are of highest quality (glass distilled, Burdick & Jackson). The clean up is done in a multi step procedure including 2 LC-column systems.

I Sodium sulfate /potassium silicate/silica gel/potassium silicate/silica gel/ sodium sulfate /glass fiber filter/carbon column.

II Double column system with sodium sulfate/sulfuric acid silica gel/cesium silica gel//sodium sulfate/acid alumina/cesium silica gel.

After the last step 40µL tetradecane was added as a carrier to prevent losses during evaporation.

Analysis

For the analysis a high resolution gas chromatograph and a high resolution mass spectrometer (HRGC/HRMS) were used.

HRGC: HP-5890 provided with a 60 m Rt2330 column.

HRMS: VG 70-250S operating at a resolution of 9000.

Control of the mass spectrometer is made by using two recovery spikes:

^{13}C-1,2,3,7,8-PeCDF
^{13}C-1,2,3,4,6,7,8-HpCDF

The quantification is made by relating peak areas from the sample with peak areas of a standard, which contains specific amounts of all toxic isomers, all internal standards and recovery spikes.

Explanation of the table

The results are corrected for losses in the clean up.

Abbreviations:

T- = Tetra
Pe- = Penta
Hx- = Hexa
Hp- = Hepta
O- = Octa

-CDF = chlorinated dibenzofuran
-CDD = chlorinated dibenzo-p-dioxin

Calculation of TCDD-equivalents.

The amount of the different toxic-isomeres is multiplied with a factor and those values are added.The sum is called the "TCDD-equivalent". There are different methods to calculate the "TCDD-equivalent".In Sweden to day we use the Nordic model.
In the calculation of "TCDD-equvivalents" ND-values are divided by two.

	Nordic model (1988)	Eadon (1983)
2378-TCDF	0.1	0.333
2378-TCDD	1	1
12348/12378-PeCDF	0.01	0.333
23478-PeCDF	0.5	0.333
12378-PeCDD	0.5	1
123478/123479-HxCDF	0.1	0.01
123678-HxCDF	0.1	0.01
123789-HxCDF	0.1	0.01
234678-HxCDF	0.1	0.01
123478-HxCDD	0.1	0.033
123678-HxCDD	0.1	0.033
123789-HxCDD	0.1	0.033
1234678-HpCDF	0.01	-
1234789-HpCDF	0.01	-
1234678-HpCDD	0.01	-
OCDF	0.001	-
OCDD	0.001	-

References.

1. SMITH L. M.,STALLING D. L. and JOHNSON J. L. (1984), Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Analytical Chemistry, 56, p1830-1842.

2. BUSER H.-R., RAPPE C. and BERGQVIST P.-A. (1985), Analysis of polychlorinated dibenzofurans, dioxins and related compounds in environmental samples. Environmental Health Perspectives, 60, p293-302.

3. Proceedings of the third Finnish-Swedish seminar on the Gulf of Bothnia, presented i Pori, Finland 20-21 august 1984, National board of waters and environment, Finland, Helsinki 1986.

4. ÖSTEN ANDERSSON, CARL-ERIC LINDER and REGGIE VAZ. (1984), Levels of organochlorine pesticides, PCB and certain other organohalogen compounds in fishery products in Sweden, 1976-1982. Vår Föda, vol. 36, suppl. 1. The National Food Administration, Box 622, S-751 26 Uppsala, Sweden.

Sture Bergek
Institute of Environmental Chemistry
University of Umeå

Umeå 90-09-05

Analysis of fish oils for PCDDs and PCDFs (ppt).

Our number	11841	11842
Your number	No.1	No.2
Clean up (g)	20.2	20.3
2378-TCDF	28	13
2378-TCDD	2.6	3.4
12348/12378-PeCDF	7.3	3.0
23478-PeCDF	23	3.3
12378-PeCDD	5.4	4.1
123478/123479-HxCDF	3.4	1.8
123678-HxCDF	2.9	0.80
123789-HxCDF	ND 0.42	ND 0.31
234678-HxCDF	3.4	0.33
123478-HxCDD	1.0	1.3
123678-HxCDD	4.0	4.2
123789-HxCDD	0.92	2.4
1234678-HpCDF	1.5	1.6
1234789-HpCDF	0.35	0.24
1234678-HpCDD	2.0	13
OCDF	0.96	1.0
OCDD	5.2	85
TCDD EQUIVALENT	21	9.7
RECOVERY TCDF (%)	83	84
RECOVERY TCDD (%)	82	86
RECOVERY PeCDF (%)	97	91
RECOVERY PeCDD (%)	110	89
RECOVERY HxCDF Grp1 (%)	100	92
RECOVERY HxCDF Grp2 (%)	78	91
RECOVERY HxCDD (%)	100	90
RECOVERY HpCDF (%)	91	74
RECOVERY HpCDD (%)	90	71
RECOVERY OCDF (%)	76	60
RECOVERY OCDD (%)	80	66

ND 0.1 = Not detected at 0.1 ppt.
Values are calculated on fresh weight and corrected for recovery.
Values are corrected for internal standard (352) and (420).
No correction for blanks.
TCDD-equivalents according to Nordic equivalents.

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AEA TECHNOLOGY

Analytical Sciences Centre
B551 Harwell Laboratory
PO Box 34
Didcot
Oxfordshire OX11 0RE
Tel. 0235 821111 Ext. 4065
Direct Dial: 0235 434420 (Manager)
0235 434151 (Business Office)
Fax: 0235 434917
Telex: 83135 ATOMHA G

Our Ref: Q949D10/0

28 September 1990

Dr IF Duthie
Little Thatch
The Chase
Knott Park
Oxshott
Surrey KT22 OHR

Dear Dr Duthie

The two samples of fish oil you sent to Harwell Laboratory have been analysed for the polychlorinated dibenzo-p-dioxins (PCDDs) and the polychlorinated dibenzo furans (PCDFs) using capillary gas chromatography/high resolution mass spectrometry. The sample preparation methods and the analytical procedure are outlined below:-

About 20gm of the fish oil sample, as received, was weighed into a round bottom flask and 50μl of a solution of $^{13}C_{12}$- labelled 2,3,7,8 - containing PCDD and PCDF standards, one for each congener group, was added. The oil was then taken up in 25ml 50/50 (^{V}/v) n-hexane/dichloromethane and extracted with 2 x 20ml aliquots of dimethyl sulphoxide. The DMSO fractions were combined and an equal volume of deionized water added. The resulting solution was then back extracted using 2 x 20ml aliquots of n-hexane. The n-hexane fractions were combined, freeze-dried to a volume of about 10ml and then placed onto a prewashed column containing, in order, anhydrous solium sulphate, 50/50 (^{W}/w) concentrated sulphuric acid/Celite 545, 9/1 (^{W}/w) anhydrous sodium sulphate/sodium hydrogen carbonate, 60-120 mesh silica gel and finally anhydrous sodium sulphate. The column was washed with 90mls of n-hexane to ensure complete elution of the PCDDs and PCDDFs. The 100ml extract volume obtained was reduced to about 2ml by freeze-drying and was then transferred onto a chromatography basic grade alumina (Woelm) column. The column was washed with about 10ml of n-hexane containing 2% dichloromethane and the washings discarded. A subsequent wash, using a 50/50 n-hexane/dichloromethane mixture (10ml) was used to elute the PCDDs and PCDFs. This fraction was collected and reduced in volume to 100μl using n-dodecane as the final solvent. The n-dodecane used contains a known amount of $^{37}Cl_4$ - labelled TCDD as an internal chromatographic standard. This extract clean-up is based on the method of A. di Domenico et al. [Anal. Chem., 51, 735 (1979)] and has proved effective in removing most substances which interfere with the subsequent gas chromatography/mass spectrometry analysis.

The analysis of the concentrated extract from the samples for PCDDs and PCDFs was carried out using a BP5 50m fused silica capillary column (SGE Ltd) interfaced to a VG Analytical Autospec high resolution mass spectrometer. The mass spectrometer was operated at 10,000 resolution for the analysis. Analysis of the samples for each congener group, tetra- through to octa-, was carried out sequentially in the multiple ion detection mode using group switching. The mass spectrometer method for analysis of 2,3,7,8 and total TCDD is described below. This method is equally applicable to any of the tetra- to octa- dioxin and furan congener groups.

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A 2μl aliquot of the sample was injected onto the gc column using a split/splitless injector in the splitless mode. The column was temperature programmed from 180°C to 220°C at 15°C/min and then from 220°C to 280°C at 4°C/min. The ions monitored in Group 1 for TCDD were the 320 and 322 ions (corresponding to M^+ and $(M+2)^+$) and for the ^{13}C-labelled TCDD the ions at 332 and 334 were used, in both cases using the masses of the ions correct to 4 decimal places. Group 1 also contained the ions for TCDF and ^{13}C-labelled TCDF. Once the tetra-dioxin and furan congeners had eluted, the instrument was switched to Group II ions to monitor the penta-congeners and so on until the analysis was complete.

Calibration was carried out by injection of a solution containing known amounts of 2,3,7,8 TCDD and $^{13}C_{12}$ 2,3,7,8 TCDD to determine the response factors by ratioing the measured areas under the peaks in the mass chromatograms. Injection of this standard solution also established retention time data for the 2,3,7,8 containing isomer. The 2,3,7,8 isomer specific analysis was carried out by measuring the area, in the mass chromatogram of the sample, of the peak eluting at the same time as the $^{13}C_{12}$ 2,3,7,8 TCDD added standard. From the known amount of the $^{13}C_{12}$ standard added and the calculated response factor, the 2,3,7,8 containing isomer concentration was determined. Similarly the concentration of all tetra isomers was determined by measuring the total area under the peaks in the mass chromatogram and comparing this to the area under the peak due to the added $^{13}C_{12}$ standard, assuming an identical response for each isomer. In this way both the 2,3,7,8 TCDD and total TCDD contents of the sample were determined whilst, by use of the $^{13}C_{12}$ 2,3,7,8 TCDD added standard, compensating for the efficiency of recovery of these species from the sample.

The use of more than one mass for monitoring both TCDD and $^{13}C_{12}$ 2,3,7,8 TCDD meant that in addition to mass specificity, intensity ratio measurements of the different ions could be made to detect any interference which may have been occurring due to the elution of other species at or close to the retention time of the species of interest. The criterion of similar gc retention time in the sample relative to the calibration standard was also used to aid identification and specificity. It was assumed for the 2,3,7,8 isomer specific analysis that the sample response at the retention time of the 2,3,7,8 $^{13}C_{12}$ TCDD was due only to 2,3,7,8 TCDD. Overlap with other isomers may have occurred at this retention time and so the 2,3,7,8 result may represent an upper limit.

The results obtained from the analysis of the samples are given in Table 1, together with the Toxicity Equivalent (TEQ) of the sample obtained using the NATO/CCMS International Toxic Equivalent Factors. The results and the I-TEQs are in units of ppt (ng/Kg). For those isomers where a "less than" figure for the PCDD/PCDF concentration has been reported, then the convention to use one half of this figure for the TEQ calculation has been adopted. These "less than" figures are derived from a measurement of the noise in the mass chromatogram in the area where that isomer would be expected to elute.

I trust this report contains all the information you require. If we can be of any further assistance to you please contact me.

Yours sincerely

P.F. Ambidge

PF Ambidge

Table 1

PCDD and PCDF Concentrations in Fish Oils

	Fish Oil 1	Fish Oil 2 (240)
2378 TCDD	8	8
Total TCDD	160	170
Isomer PeCDD	1	3
Total PeCDD	6	62
Isomer HxCDD	10	<4
Total HxCDD	<20	44
Isomer HpCDD	<4	19
Total HpCDD	<8	34
OCDD	<20	87
2378 TCDF	29	13
Total TCDF	950	1000
23478 PeCDF	84	34
12378 PeCDF	35	15
Total PeCDF	280	230
Isomer HxCDF	4	4
Total HxCDF	99	140
Isomer HpCDF	<7	<4
Total HpCDF	<15	60
OCDF	<70	<30
I-TEQ	57	30

All values in ppt (ng/Kg)

Isomer refers to 2,3,7,8 - containing isomers

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Analytical Sciences Centre
B551 Harwell Laboratory
PO Box 34
Didcot
Oxfordshire OX11 0RE
Tel. 0235 821111 Ext. 4065
Direct Dial: 0235 434120 (Manager)
0235 434151 (Business Office)
Fax: 0235 434917
Telex: 83135 ATOMHA G

Ref: Q926/D

23rd January 1991

Dr I F Duthie
Little Thatch
The Chase
Knott Park
Oxshott, Surrey
KT22 OHR

Dear Dr Duthie

In response to your letter of 14th January 1991, I enclose the results of the analysis of your fish oils samples with the Toxicity Equivalent calculated using the Nordic Toxic Equivalent Factors. As you can see there is little difference in the calculated TEQ's, to be expected since the Nordic and International models are very similar. In the table "isomer" refers to 2,3,7,8-containing isomers, eg for HxCDD, then the 1,2,3,4,7,8-, 1,2,3,6,7,8- and 1,2,3,7,8,9- isomer concentrations are included under this heading, and similarly "isomer" HpCDF refers to both the 1,2,3,4,6,7,8- and 1,2,3,4,7,8,9- HpCDFs. I use this grouping of the various 2,3,7,8-containing isomers in the table since it makes calculation of the TEQ easy because the various specific isomers under this heading have the same toxic equivalent factors. The only situation where this is not the case is for the two 2,3,7,8-containing isomers of PeCDF, and as you can see from the table these are quoted individually.

I trust this answers your questions and if I can be of any further assistance please contact me.

Yours sincerely

P. F. Ambidge

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Isomer HxCDF	4	4
Total HxCDF	99	140
Isomer HpCDF	<7	<4
Total HpCDF	<15	60
OCDF	<70	<30
NORDIC TEQ	55	29

All values in ppt (ng/Kg)

Isomer refers to 2,3,7,8 - containing isomers