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GENERAL ELECTRIC

CORPORATE ENVIRONMENTAL PROGRAMS
GENERAL ELECTRIC COMPANY • FAIRFIELD, CONNECTICUT 06431

October 11, 1984

TO: J.F. Brown
J.C. Carnahan
W.V. Ligon

Attached is a copy of the preliminary report from the dioxin and furan round robins conducted by EPA and NIEHS.

I would appreciate your comments on same.

S.B. Hamilton

SBH:cas

Attachment

cc: B.I. MacDonald

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GENP 010474

NON-OBJECTIVES OF THIS STUDY

(Any attempt to treat this study as though it had any of the following objectives, or to draw conclusions about these issues, will be discredited through publications.)

1. To provide an opportunity to advertise commercially ones analytical skills.
2. To provide a training exercise.
3. To perform a quality check on contractors.
4. To provide an opportunity to discredit competitors.
5. To determine the lower limits of detection of different techniques for these analytes.
6. To determine precision or replicability.
7. To determine whether it is physically possible to make a sample that can not be accurately analyzed for these analytes.

REAL OBJECTIVES OF THIS STUDY

1. To determine which of the various analytical approaches taken by different laboratories are able to obtain quantitatively accurate measurements of the levels of toxic dioxins and furans in the ppt range, when the "right answer" is not based on statistical evaluation of analytical data presumed to be reliable, but on amounts spiked. (Note: In order to accomplish this, the levels of analytes in the unspiked sample found by a given laboratory must be considered to be correct, for that laboratory!)
2. To do so (1. above) using a natural and relevant matrix.
3. To determine, for at least some of the analytes, whether or not they are likely to be accurately determined in spite of the presence of selected interferences. Similarly, to attempt to detect which methodologies are more susceptable to interferences.
4. Since "real" samples are usually so limited in size that averaging replicate determinations is often impossible, and since the cost of these analyses is so great that replication is usually not done even when possible, to obtain at least an impression of the likelyhood of obtaining a reasonable approximation of an accurate result without replication and determination of precision.

5. Most importantly, to identify aspects of this general analytical problem that need further examination, validation, method development, or whatever. To if possible design specific experiments to recommend be supported/performed. This may even extend to considerations arising out of the findings on the unspiked sample.

6. Approximately nine sets of objectives that the participants hoped to accomplish.

ESTIMATED LIMITS OF QUANTIFICATION IN 5 g SAMPLE PRIOR TO RECEIVING THESE SAMPLES, PPT

	Columbia	Dow	Monsanto	U.S.E.P.A.	N.Y.D.O.H.	Wright St.
2378-TCDD	5-6	4-12	1	1-50	5	4-5
2378-TCDF	5-6	4-12	1	1-50	5	4-5
12378-PCDD	5-6	4-12	1	20-120	10	8-10
23478-PCDF	5-6	4-12	1	20-120	10	8-10
123478-HCDD	5-6	20-28	1.4	40-140	18	8-10
123789-HCDF	5-6	20-28	1.4	40-140	18	8-10
OCDD	5-6	20-28	2	110-400	25	12-15

LABORATORIES REPORTING AT LEAST SOME INSTRUMENT OUTAGE/DOWN TIME DURING THIS STUDY:

Dow
Univ. Nebraska
N.Y.D.O.H.
Columbia NFRL

SOME NONUNIFORMITIES

A. Methods of reporting results.

1. Stalling/Smith: Two numbers for sample #1. First value used by me in the comparison tables.
2. Rappe: Two complete data sets. Both treated separately.
3. Gross/Weerasinghe: Concentrations for $>Cl_4$ calculated two different ways, giving very different results. One or the other way must be chosen.
4. Harless: Values for $>Cl_4$ reported as "Total PCDD", etc.

B. Starting level of experience/claims:

Not all the labs have experience at determination of isomers other than 2,3,7,8-, or higher chlorologues. Not all the labs use methods considered by themselves to be validated or suitable for all dioxins and furans. Not all labs consider their techniques to be isomer specific. Not all labs have extensive experience with furans.

SAMPLES REPORTED AS DAMAGED OR POTENTIALLY DAMAGED IN SHIPMENT

For certain sample numbers a "spare" was available (no problems at my end.) For others, no spare was available (problem with ampule sealing, breakage, etc. at my end.) The lipid content corresponding to 5 g of adipose was known independently. The volume content of each ampule was not known because $CHCl_3$ evaporation was not identical for all. A radioactive marker at extremely low level was present in known amount in each ampule. Thus in most cases it was possible to deal with minor leaks or evaporation if it occurred.

(1) Not reporting any loss or damage during shipment:
Columbia NFRL; N.Y.D.O.H.; E.P.A.; Wright State.

(2) Reporting possible leak based on comparative volumes, later judged no significant leak had occurred (various criteria):
Univ. Nebraska - #1
Monsanto - #1

(3) Ampule broken, leak certain, replacement able to be provided:
Umeå Univ. (#3, #8).

(4) Leak, amount lost able to be determined by independent means at two sites, data corrected accordingly:
Dow (#1)

(5) Major leak, not replacable, not accurately measurable, data either not included or must be considered questionable:
Dow (#3)
Univ. Nebraska (#9)
Monsanto (#5)

DATA ON SPECIFIC SAMPLES OR COMPONENTS NOT PROVIDED

E.P.A. data on penta- and hexachloro isomers not known to be isomer-specific, so reported as "total PCDD", "total PCDF", "total HCDD", "total HCDF".

Instrument breakdowns prevented N.Y.D.O.H. from completing measurements; HCDF and OCDD levels not reported.

TIME FACTORS IN THE STUDY

A. Original deadline for receipt of data: 1 July, 1984.
Extended deadline (C.J.) : 8 July, 1984.
Ultimate deadline after begging and pleading: Aug. 31, 1984.

B. Dates data received by P.A. :

Columbia NFRL	16 June 1984
Dow	29 June 1984
Monsanto	2 July 1984
E.P.A.	5 July 1984
Tunney's Pasture	9 Aug 1984
Univ. of Nebraska	20 Aug 1984
N.Y.D.O.H.	10 Sept 1984
Umeå Univ.	17 Sept 1984
Wright State	

C. Time elapsed between shipping samples and receiving data:

Columbia NFRL	48 days
Dow	61 days
Monsanto	64 days
E.P.A.	67 days
Tunney's Pasture	101 days
Univ. of Nebraska	112 days
N.Y.D.O.H.	133 days
Umeå Univ.	140 days
Wright State	

D. Number of worker-days spent on workup/analysis as reported by participants;

Columbia NFRL	5 workup, GC-MS not given.
Dow	26 total
Monsanto	6 total
Others	Not reported.
Umeå	(given as 36-48 hr.per sample)

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GENP 010480

SOURCES OF STANDARDS

2,3,7,8-TCDD	P. Albro, N.I.E.H.S.
2,3,7,8-TCDF	Bell/Carnahan, General Electric
1,2,3,7,8-PCDD	P. O'Keefe, N.Y.D.O.H.
2,3,4,7,8-PCDF	General Electric
1,2,3,4,7,8-HCDD	Cambridge Isotope Laboratories, Inc.
1,2,3,7,8,9-HCDF	General Electric
OCDD	J. Ryan, Tunney's Pasture
Other CDF's	General Electric

All standards were donated for this study at no charge. All standards were received as solids with the weights recorded on the containers. The listed weights were taken to be correct whether they were or not. All disolution and dilution of standards were made using PG toluene, on the day they were used for spiking. All standard containers were stored, opened, and eventually disposed of, in a different building from where the adipose was processed. Preparation and storage (very short-term) of spiking solutions was not done in the same laboratory where the adipose was spiked. All glassware was washed with Micro®, DD water, PG acetone and PG methylene chloride, with a final toluene rinse just before use. Spiking solutions were prepared in volumetric flasks with glass stoppers, sealed with Teflon® tape.

All participating laboratories noticed that the HCDD level in the reference standard solution was about 10X higher than the other components. The same would apply to the spiked fat.

Standards were received along with evidence of identity and whatever information was available on purity. That none of the standards contained potentially troublesome levels of any of the other analytes was checked by GC-ECD using a 25 meter column of SP 2100. (Since what was being checked was absence of any peak at specific elution positions, ability to resolve all possible isomers was not necessary.) None of the standards contained interfering (>1%) levels of other analytes, although some extraneous contaminants were sometimes seen.

EXTRACTION OF THE ADIPOSE TISSUE

The tissue was thawed and weighed. Most of the available tissue, a 600g portion, was combined with 4800g of anhydrous, Pesticide Grade sodium sulfate in a Sears food grinder purchased new for this project.(The grinder was washed with Micro, water, and methylene chloride before being used.) The "dry" blend was reprocessed three times for uniformity, and had acquired a granular state at the end.

The blend was packed into two Pyrex columns, 20" by 4"diam., and elutriated first with a total of three liters of ethyl acetate containing 0.001% BHT antioxidant. This was followed by 6 liters of methylene chloride. The extracts were combined and concentrated by rotary evaporation in vacuo at 37°. The residue was taken up in chloroform and made to 900 ml. The weight of lipid from 600 g of human adipose tissue was 531.9 g. There was no visible residue insoluble in the chloroform. Pilot studies indicated that this extraction procedure gives a yield of lipid not statistically significantly different from that obtained by Soxlett-extraction for 16 hours.

BLIND SPIKING PROCEDURE

Each of ten flasks containing a Teflon stirring bar and glass stopper was weighed, loaded with 1/10 of the lipid extract, and weighed again. One at a time, each flask was spiked with 1 ml of a blind spiking solution provided (also one-at-a-time) by the person who made it (different from the person doing the spiking). Each flask was numbered to correspond with the number on the spiking solution. Those samples numbered 3,5,7,9 and 10 also received 100 µl of a solution labeled "general interference solution". Sample #9 additionally received 100 µl of a solution labeled "Furan Interference Solution"; the "interference" solutions also were of compositions unknown to the person doing the spiking.

NOTE: There was no "#1" spiking solution. Nothing was added to flask #1, except the lipid.

Each flask was again weighed, after which the glass stopper was sealed in place with Teflon tape. The flask contents were stirred magnetically until, based on previous experience, they should have been thoroughly mixed. They were then let stand with the stirrer off for 5 minutes. Aliquots (100 µl) were sampled from the top, bottom, and middle of the flask and radioassayed for ^{14}C, Since the spiking solutions had all contained a ^{14}C-labeled hexachlorobiphenyl (2,2',3,3',6,6'), uniformity of ^{14}C concentration was taken to indicate uniformity of dispersal of the spiking solution throughout the flask. Analysis of variance indicated that none of the samples showed a CV greater than 3.37% of the mean (predetermined criterion being 5%).

Each flask was again weighed, and, again one at a time, aliquots were transferred on a weight basis to ampules. Each ampule received sample equivalent to 5 g of original adipose tissue. That is, the 900 ml of lipid in chloroform represented 600 g of adipose. Each flask received 1/10 of this, or 60 g eq. Aliquots removed for radioassay averaged 0.20 g of adipose equiv. per flask, leaving 59.8 g equiv. in X g total weight. Then (5/59.8)X grams of solution was transferred to each ampule.

The ampules corresponding to a given sample number were labeled directly, taped, and loaded into a vacuum desiccator (a separate desiccator for each sample). Each desiccator was connected through a water-backup trap and Drierite trap to its own aspirator. Chloroform was reduced under very slight vacuum (to avoid the possibility of bumping) somewhat, but it was found that this process would have taken intollerably long to go to completion and it was decided to leave most of the chloroform in the samples. The samples were imbedded in Dry Ice (solid CO_2) and the ampules sealed with a methane/oxygen torch. Since the samples had sat in the desiccators for about 24 hours prior to sealing, and since care had been taken to deposit the solutions into the bottom of the ampules originally, there was no detectable residue in the heated area that might have charred.

Sets of samples (#s 1-10) were then packaged for shipment and delivered to Bob Harless of the U.S.E.P.A. who handled the shipping. Each package also contained the reference standard solution and a descriptive cover sheet.

SPECIFIC INTERFERENCES

In addition to the analytes listed, samples 3,5,7,9,and 10 were also spiked with:

(1) A mixture of polychlorinated diphenyl ethers, 4-8 chlorines, at 200 ppt total.

(2) Two different tetrachloro methoxy biphenyls, at 50 ppt each.

(3) Aroclor 1016, at 10 ppb. This Aroclor chosen for its negligible furan contamination.

(4) 1,2,3,4-TCDD at 50 ppt.

(5) o,p- and p,p-DDE at 500 ppt each.

In addition to the above, sample 9 was also spiked with:

(1) 2,3,4,8-TCDF at 10.6 ppt.

(2) 1,2,4,8,9-PCDF at 7.9 ppt.

(3) 1,2,3,4,6,9-HCDF at 26.8 ppt.

PREPARATION OF SPIKING SOLUTIONS AND REFERENCE STANDARD

The ampules containing dry dioxin and furan standards were opened one at a time for processing. The contents of each ampule was repeatedly leached into small portions of toluene, the "extracts" being transferred to a volumetric flask. Eventually each stock solution became 50 ml in toluene, a different concentration for each standard in accordance with the recorded weight. In this way the stock solutions became of defined concentrations which subsequently were assumed to be correct, and it was not necessary to assume that no material remained unextracted from the original containers.

Calculated aliquots of each stock solution to give a final concentration of 1.0 µg/ml in 50 ml final volume were combined in a 50 ml volumetric flask and mixed thoroughly through 40 inversions after making to volume with toluene. The pipettes used for this were guaranteed ± 1%, traceable to NBS.*

Other aliquots of the stock solutions were combined such that, after making to volume, 1 ml would contain 60 X the desired ppt concentration in spiked fat extract (since 1 ml would be added to 60 g eq. adipose extract in each case). Before making to volume, each spiking solution also received 261000 dpm of ^{14}C-labeled hexachlorobiphenyl per ml.

INTERFERENCE SOLUTIONS

The "Furan Interference Solution" was made as above. The "General Interference Solution" was made on a direct weight basis for stock solutions, which were combined and diluted as above. These solutions did not contain ^{14}C, but were in toluene, were spiked into the lipid extracts at the same time as the spiking (analyte) solutions, and received identical mixing.

* Note: Each participant received 1 ml of this single reference solution, which was only prepared once.

ADDITIONAL CONSIDERATIONS RELATED TO SPIKING

Each spiking solution was carried to the lab where the spiking was done only after the previous spiking solution had been used, the flask sealed, and the residual spiking solution removed from that lab and taken to storage. Thus there was only one spiking solution in the "spiking lab" at any given time, and all previously spiked flasks of lipid were already sealed and stirring. This semi-paranoid procedure made it essentially impossible to spike the same sample twice or mislabel the flasks. No flask was opened for radioassay until the previously opened flask had passed the radioassay criterion and been resealed. Cross contamination after spiking should have been as close to impossible as we could manage .

One aspect of the blind spiking was that, at the end of the process, no one in the world except the person who made the spiking solutions (me), and who was alone in the lab while making them, could know what or how much was spiked. This made subsequent security consistant with the former standards of the U.S.Army Biological Warfare Labs, which ought to be good enough for this study.

LABORATORY CONTAMINATION

Extraction of the adipose was done in a laboratory newly set up for sample workup, in a building not previously used for making dioxin solutions or performing dioxin/furan analysis. However, it is not possible to be absolutely certain that contamination was completely absent from the total relevant environment. The procedures used should have ensured that if contamination did occur, it would appear as "background" in all samples. It may be worth noting that we were able to process liver samples in a similar manner in these laboratories

right after finishing the human fat work. Blank liver samples did not show 2,3,7,8-TCDD at a 0.7 ppt detection limit relative to ^{13}C-TCDD internal standard. Unfortunately, those samples were not analyzed for other dioxins or furans.

CLEANUP: BASIC APPROACHES USED.

A. Columbia National Fisheries Research Laboratory

1. Add internal standards (^{13}C-TCDF & ^{37}Cl-TCDF).
2. Sample in CH/CH_2Cl_2 through two K Silicate/silica gel columns, then through charcoal trap. Wash with two solvent mixtures.
3. Back-elute with toluene.
4. Concentrate, 55°, vacuum.
5. Through H_2SO_4/silica gel in hexane.
6. Dry at room temp. with N_2, dissolve in 5 ul o-xylene.

(Note: composit sample later treated by alumina chrom. to remove chlorinated naphthalenes not removed by above.)

B. University of Nebraska

1. Evap. $CHCl_3$ with hot water bath, weigh lipid.
2. Add internal standard (^{13}C-TCDD).
3. Samples #1-#5, saponify in refluxing wet ethanolic KOH, 60 min.
4. Dilute, extract with hexane 4X, wash extract with water.
5. Samples #6-#10, reflux 2 hr. in hexane.
6. Partition 4X against conc. H_2SO_4.
7. Wash with water, dry under N_2 to 1 ml.
8. "Silica" column, eluted with 20% benzene. Exchange into hexane.
9. Alumina column. CCl_4, 10% CH_2Cl_2, 25% CH_2Cl_2. Last fraction saved, conc. under N_2 into hexane.

c. Monsanto

1. Add standards (^{13}C-TCDD, ^{13}C-PCDD, ^{13}C-HCDD, ^{37}Cl-TCDF, ^{37}Cl-PCDF, ^{37}Cl-HCDF).
2. Digest in conc. HCl, 1 hour.
3. Extract 2X hexane, dry with Na_2SO_4.
4. Wash through H_2SO_4 on silica with hexane. Wash eluate with water, dry, conc.
5. Alumina column; 5% and 50% CH_2Cl_2. Sec. fraction to 1 ml.
6. Carbopack C/Celite, prewashed. Sequence of solvents, saving toluene fraction. Concentrate to 10 ul with dodecane keeper.

D. Dow

1. Add standards (^{13}C-TCDD, ^{13}C-TCDF, ^{13}C-HCDD, ^{13}C-OCDD.)
2. Partition hexane soln. against conc. H_2SO_4, 13-54 hr.
3. Organic phase through silica, NaOH on silica, silica, H_2SO_4 on silica, silica; washed through with 5% benzene in hexane.
4. Concentrate, dry, redissolve in hexane.
5. $AgNO_3$ on silica column, hexane eluant, then basic alumina. 80% CCl_4, 75% CH_2Cl_2. Evap. under N_2, dissolve in $CHCl_3$.
6. Reverse Phase HPLC, fraction 6 to GC-MS; fr. 1-5 to:
7. Silica HPLC.

E. E.P.A.

1. Add internal standards (^{13}C-TCDD, ^{13}C-OCDD).
2. Evaporate chloroform with N_2.
3. Add ethanol: 45%KOH aq., 20:40. Stir at room temp. for 16 hours.
4. Partition into hexane using more ethanol. Combine two hexane washes of aq. phase.
5. Wash hexane extract with 2N KOH aq.
6. Partition hexane phase against conc. H_2SO_4 three times. Wash final hexane phase with water. Dry extract, concentrate to 1-2 ml. (D-K).
7. Neutral Alumina column. CCl_4 (discard) and CH_2Cl_2 (save). Exchange solvent to hexane.
8. PX-21/silica column. Prewash, load, wash with hexane, CH_2Cl_2, and benzene: CH_2Cl_2, 1:1.
9. Back elute with toluene. Concentrate just to dryness under N_2 at 60°.

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F. Umeå University

(1) Add internal standards (^{13}C-TCDD, ^{13}C-TCDF, ^{13}C-OCDD).

(2) Dissolve in CH_2Cl_2/cyclohexane, pass through silica/K silicate/Na_2SO_4/silicate/silica/carbon.

(3) Wash with CH_2Cl_2/cyclohexane and with CH_2Cl_2/CH_3OH/benzene, 75:20:5.

(4) Back elute with toluene.

(5) Concentrate at 35°.

(6) Pass through silicate/ H_2SO_4 silica with hexane, onto acidic alumina.

(7) Elute with hexane, 2% CH_2Cl_2 in hexane, and 1:1 CH_2Cl_2/cyclohexane. Last fraction saved for analysis.

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G. N.Y.D.O.H.

(1) Add internal standards (^{13}C-TCDD, ^{37}Cl-TCDF, ^{37}Cl-OCDD).
(2) Dilute with CH_2Cl_2, partition against H_2SO_4, neutralize through Na_2CO_3 and KOH/silica. Concentrate to 10 ml with boiling water bath. Exchange through cyclohexane into hexane.
(3) Semi-automated cleanup. First onto acidic alumina, wash with 3% CH_2Cl_2; elute with 50% CH_2Cl_2 onto Charcoal/Celite.
(4) Wash with 10% benzene in hexane; back elute with 50% xylene in hexane onto neutral alumina.
(5) Wash with 3% CH_2Cl_2; elute with 50% CH_2Cl_2 in hexane.
(6) Clean sample loop with benzene:hexane, 1:1; flush rest of system with hexane before running next sample.

H. H.P.B. Canada

(1) Add internal standards (^{13}C-TCDD, ^{37}Cl-TCDF, ^{13}C-OCDD).
(2) Partition repeatedly between hexane and H_2SO_4.
(3) Wash with 1% KOH, water; dry with Na_2SO_4.
(4) Chromatograph on Florisil. Discard 2% CH_2Cl_2 fraction, save 100% CH_2Cl_2 fraction. Evaporate, redissolve in toluene or isooctane.

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GENP 010491

INTERNAL STANDARDS USED

(1) Columbia NFRL: 50 ppt each, ^{13}C-TCDF, ^{37}Cl-TCDF, ^{13}C-OCDD.

(2) Dow: 3 ng ^{13}C-TCDD, 200 pg ^{13}C-TCDF, 1 ng ^{13}C-HCDD, 5 ng ^{13}C-OCDD.

(3) Monsanto: Unspecified amounts, ^{13}C-TCDD, ^{13}C-PCDD, ^{13}C-HCDD, ^{37}Cl-TCDF, ^{37}Cl-PCDF, ^{37}Cl-HCDF, ^{37}Cl-OCDD.

(4) U.S.E.P.A.: 5 ng ^{13}C-TCDD, 20 ng ^{13}C-OCDD.

(5) U. Nebraska: 90 pg ^{13}C-TCDD.

(6) Umeå Univ.: 0.5 ng each, ^{13}C-TCDD, ^{13}C-TCDF, ^{13}C-OCDD.

(7) Canada: 250 pg each, ^{13}-TCDD, ^{37}Cl-TCDF; ^{13}C-OCDD(1 ng).

(8) N.Y.D.O.H: 377 pg ^{13}C-TCDD, 246 pg ^{37}Cl-TCDF, 1 ng ^{37}Cl-OCDD.

GLC COLUMNS USED

(1) Columbia NFRL: 30Mx0.25mm DB-5

(2) Dow: 30Mx0.25mm DB-5, and 60Mx0.25mm SE-54 (for HCDD)

(3) Monsanto: 60Mx ? mm SP-2330 (Cl_4 - Cl_6); 30Mx ? mm DB-5 (OCDD)

(4) U.S.E.P.A.: 60M SP-2330, 30M SP-2340, and 10M OV-101 WCOT.

(5) U. Nebraska: 30Mx0.32mm SP-2340 and 60Mx0.25mm SP-2330.

(6) Umeå Univ.: SP-2330.

(7) Canada: 15Mx0.32mm DB-5; 30M DB-5.

(8) N.Y.D.O.H: 60Mx0.25mm SP2330

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GENP 010492

IONIZATION TECHNIQUE AND MS RESOLUTION USED

(1) Columbia NFRL: 55 eV E.I.; (Resoln. not given but quadrupole).

(2) Dow: E.I., resoln. not given.

(3) Monsanto: E.I.; 1000.

(4) U.S.E.P.A.: E.I.; 8000.

(5) U. Nebraska: 70 eV E.I.; 7500.

(6) Umeå Univ.: E.I.; 5000/2000; and MNCI, 1000.

(7) Canada: Air CI; CID for M-Cl; confirm. EI; 10000.

(8) N.Y.D.O.H: E.I.: Resoln. not given.

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GENP 010493

KEY TO THE DATA TABLES

1= Columbia National Fisheries Research Laboratory

2= Dow Chemical

3= Monsanto

4= U.S. Environmental Protection Agency

5= University of Nebraska

6= Health Protection Branch, Canada

7= N.Y. State Department of Health

8= Umeå University, Sweden

9= Wright State University

BACKGROUND LEVELS REPORTED

	CNFRL 1	Dow 2*	Monsanto 3	EPA 4	NEA 5	Canada 6	NYSDH 7	Kuppe 8
2,3,7,8-TCDD	17;18	12,16	14	20	35	12	18	31,31
2,3,7,8-TCDF	5,10	2,2	<2	2[a]	30	5	6	5,4
1,2,3,7,8-PCDD	17,24	17,23	16	29[a]	<7	14	<7	5,40
2,3,4,7,8-PCDF	21,26	20,26	22	38[a]	10/35[b]	15	<13	35,24
1,2,3,4,7,8-HCDD	0,9	4,5	2	14[a]	3	2	<1.6	5,4
1,2,3,7,8,9-HCDF	<7	<2	<3	<19[a]	<8	<1	---	5,18
OCDD	1790, 1900	2800, 3700	2830	2430	55	1584	---	6450, 7181

*First No. = level found; second number = corrected for sample leakage.

[a]Reported as Total TCDF, Total PCDD, Total PCDF, Total HCDD, Total HCDF respectively.

[b]Level calculated two different ways, gave two different results.

Note: All entries beginning with "<" represent "none detected" at the stated detection limits.

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GENP 010495

OBSERVATIONS ON DATA FOR BACKGROUND SAMPLE #1

(1) The levels of OCDD were so out of range of the others that it would be meaningless to attempt to evaluate performance on the spiked samples (whose spiking level did not exceed 500 ppt) for this compound. However, the agreement among laboratories was very poor for this high level of OCDD in #1. Some approaches obviously result in low recoveries of OCDD, while extremely high levels reported might either involve laboratory contamination or precipitation of this highly insoluble compound out of the standard solution during its storage in those labs.

(2) Two labs reported levels of 2,3,7,8-TCDD in #1 much higher than levels seen in other labs. Only one of the two labs uses reflux saponification, but both use multiple exposure to strong base on columns. Could this be the cause, considering the high background of OCDD, HpCDD and what appears to be 1,2,3,6,7,8-HCDD? If this possibility can not be ruled out, even determination of 2,3,7,8-TCDD, which is at least 90% resistant to alkaline dechlorination under these conditions, may be compromised when there is a high background of higher chlorinated species in fat.

(3) As will be seen subsequently, levels of several analytes, as reported by various labs, were higher in #1 than in some of their other samples. Is this simply variability of recovery of other analytes relative to the internal standards, or can the other components, "interferences", etc. have a significant effect either on selective recoveries (when the non-CDD/CDF has a higher affinity for some adsorbant than some of the analytes but not others), or on mass spec. response (matrix effect)? Is this a problem?

LEVELS REPORTED FOR 2,3,7,8-TCDD, WITH THE LEVEL FOUND IN #1 SUBTRACTED

Sample No.	CVFRL 1	DOW 2	MONS 3	4	5	Canada 6	NJ 7	Chem 8	Spiked
2	0	0[a]	0	0[a]	0[a]	0	7	0[a]	0
3	35	39	46	44	45	36	42	45*	50
4	5	6	6	15	30	3	1	12	5
5	5	11	ND*	19	20	1	27	17	10
6	3	1	0	0	0[a]	0	0	0[a]	0
7	0[a]	6	4	5	10	2	12	5	5
8	0[a]	0	2	0	0	3	52	0[a]	0
9	24	58	44	48	--*	40	54	62	50
10	0[a]	0	0[a]	0[a]	0[a]	2	2	0[a]	0

* Sample leaked in shipment, data not reliably adjustable.

[a] Data as reported actually less than level reported for sample #1.

All data rounded to integers. Corrected values below zero reported as zero.

783308

GENP 01047

OBSERVATIONS ON REPORTED 2,3,7,8-TCDD, SAMPLES 2-10

(1) Labs reporting high levels for samples 5 and 7, which contained tetrachloro methoxybiphenyls and 1,2,3,4-TCDD, did not report high values for sample 10, which also contained these possible interferences. Moreover, these same labs reported high levels for sample 4 and, in one case, 8, which did not contain these interferences. One lab had moderately low levels for all the samples, suggesting that their value for sample #1 was probably too high for some reason. Yet that lab had the lowest reported level for sample #1 of any of the labs. It seems more likely that there was a problem with the ^{13}C internal standard in that lab.

(2) The other lab showing at least several low values for 2,3,7,8-TCDD did so only for the samples containing the interferences. This might reflect an effect of the interferences on recovery of this isomer, since this was also the only lab that did not use a ^{13}C-TCDD internal standard.

LEVELS REPORTED FOR 2,3,7,8-TCDF, WITH THE LEVEL FOUND IN #1 SUBTRACTED

Sample No.	1	2	3	4[b]	5	6	7	8	Spiked
2	6	5	5	10	0[c]	0	12	8,9	5.4
3	2*	0[a]	0	2	5	3	0	15,12[a]	0
4	12	10	15	24	30	4	27*	17,16	10.9
5	19	20	17[a]	35	30	26	22	29,26	21.7
6	0.5	0	0	2	0	0	0	0,2	0
7	12*	0	0	3	0	0	0	0,2	0
8	5	0	0	0	0	2	0	0,2	0
9	12	10	11	20[d]	--[a]	19	15	12,15	10.9
10	4	0	0	2	0[c]	0	6	0,1	0

* Potential interference reported.

[a] Sample damaged in shipping.

[b] Reported as Total TCDF.

[c] Value measured was less than in Sample #1.

[d] True level of "total TCDF" spiked, including 2,3,4,8, was 21.5 ppt.

783310

COMMENTS ON VALUES REPORTED FOR 2,3,7,8-TCDF

(1) One lab reporting apparent interference identified the problem as due to chlorinated naphthalenes, which could be removed by an additional cleanup step using alumina.

(2) All of the values from one of the labs were almost exactly twice the right answers. This suggests either an error in calculation or a factor error in the assumed recovery.

(3) Several labs had difficulty with sample #4, which did not receive the interference mixtures. Other labs did not. This is difficult to account for.

(4) There was little background of 2,3,7,8-TCDF reported by most of the labs. In spite of that, false positives (amounts more than twice the reported background) were occasionally seen for unspiked samples, suggesting that "quantitative resolution", or ability to distinguish between twofold differences becomes very difficult in the 2 ppt range.

Dioxin

LEVELS REPORTED FOR 1,2,3,7,8-PCDD, WITH LEVELS IN SAMPLE #1 SUBTRACTED

Sample No.	1	2	3	4	5	6	7	8	Spiked
2	1*	0	2	2	0	0	0	0,2	0
3	31	21[a]	29	22	22/75[b]	14	0	56,25	24.8
4	18	13	18	38	0	1	0	35,12	10.3
5	12	1	0[a]	0	10/20[b]	2	16	27,0	0
6	31	20	21	19	0	7	16	61,42	16.5
7	13	15	14	3	0	1	22	53,36	16.5
8	1	1	4	0	0	14	0	29,0	0
9	0	0	0	0	---[a]	0	23*	30,2	0
10	8*	4	10	12	0	2	46	35,24	10.3

*—Potential Interference Reported.

[a] Sample damaged in shipping.

[b] Two different methods of calculating results gave two different answers.

783312

GENP 010501

Farm

LEVELS REPORTED FOR 2,3,4,7,8-PCDF, WITH LEVELS IN SAMPLE #1 SUBTRACTED

Sample No.	1	2	3	4	5	6	7	8	Spiked
2	8	0	0	5	0	0	0	0	0
3	56	37*	66	66	0	24	0	41,32*	23.9
4	24	20	23	34	5/15[b]	10	0	66,62	15.9
5	5	0	0*	0	10	15	26	22,19	0
6	4	0	2	2	5/15[b]	0	25	0,0	0
7	32	30	27	0	20/55[b]	10	54	50,37	31.9
8	16[a]	0	1	0	0	15	30	0,0	0
9	33	18	33	28[c]	---[b]	15	54	19,14	15.9
10	0	0	2	0	5/20[b]	0	19	0,0	0

* Sample damaged in shipment.

[a] Possible interference reported.

[b] Two methods of calculating results gave two different answers.

[c] True level of "total PCDF" spiked, including 1,2,4,8,9, = 23.8 ppt.

783313

GENP 010502

LEVELS OF 1,2,3,4,7,8-HCDD REPORTED, WITH SAMPLE #1 SUBTRACTED

Sample No.	1	2	3	4	5	6	7	8	Spiked
2	0,33	28	24	14	0	9	0	40,42	26.7
3	0	0*	0	1	0	0	0	0,0	0
4	1,32	30	28	48	0	12	0	44,56	33.3
5	0,3	0	0*	0	0	0	13	0,0	0
6	1,3	0	0	0	0	0	9	0,0	0
7	66[a]	53	51	23	0	24	65	84,88	53.3
8	0,28	26	21	11	0	17	43	45,33	26.7
9	0	0	0	0	---*	0	19	0,0	0
10	0,36	31	30	24	0	14	61	40,43	33.3

* Sample damaged in shipping.

[a] Interference reported.

783314

GENP 010503

Furan

LEVELS REPORTED FOR 1,2,3,7,8,9-HCDF, WITH LEVELS IN SAMPLE #1 SUBTRACTED

Sample No.	1	2	3	EPA 4	Nebr. 5	Can. 6	MgSO4 7**	8	Spiked
2	0	0	0	0	0	0		0	0
3	45	29[a]	25	58	0	12		46,24	33.2
4	0	0	0	0	0	4		0,0	0
5	19	9	<8[a]	32	<10	0		17,7	11.1
6	0	0	0	0	0	0		0,0	0
7	0	0	0	0	0	0		0,0	0
8	49	39	46	50	25	22		97,68	53.4
→ 9	0	0	19*	25(c)	--[a]	0		0,0	0
10	0	0	0	0	0	0		0,0	0

* Potential interference reported.

[a] Sample damaged in shipment.

(c) Actual level of "total HCDF" spiked, including 1,2,3,4,6,9, = 26.8

** Not measured by this lab.

783315

GENP 010504

COMMENTS ON LEVELS REPORTED FOR 1,2,3,7,8-PCDD

(1) The contents of the "general interference solution", which did not include any other PCDD's, did cause positive interference in some of the labs. However, it appears to have caused a strong negative interference in some cases. The effect of variability in the apparent level in sample #1 made the two sets of data from one of the labs inconsistant with each other. Averaging the data would not have improved this problem.
(2) "False Positives" occurred quite a few times, and even on occassion in samples that did not receive extraneous interferences.

COMMENTS ON LEVELS REPORTED FOR 2,3,4,7,8-PCDF

(1) Several labs experienced positive interference in the determination of this compound. However, there were four cases of "false negatives" reported also. There are only about 13 entries in the table (out of 36 possible) that are quite close to the "right answers" for the spiked samples. This was apparently one of the more challenging analytes. None of the participating labs lack at least one out-of-range answer, and most have several.

COMMENTS ON LEVELS REPORTED FOR 1,2,3,4,7,8-HCDD

(1) It must be kept in mind that the true level of this compound in the spiked samples is about 10X that indicated, because of the mislabeling of the standard.
(2) This compound was apparently also destroyed by the reflux saponification procedure, and partially destroyed even by room temperature alkaline hydrolysis. Variability in the measured levels in sample #1 produced incompatible pairs of corrected results from one lab.
(3) False positives occurred in only one lab; false negatives were more common.
(4) The "general interference" mixture apparently did not cause any problems with this analyte.

COMMENTS ON LEVELS OF 1,2,3,7,8,9-HCDF REPORTED

(1) Recovery of this compound was low in two labs, suggesting the possibility of alkaline destruction (partial).

(2) The contents of the "general interference" solution caused positive interference in two labs. The 1,2,3,4,6,9-HCDF also present in sample #9 only prevented correct measurement of 1,2,3,7,8,9 HCDF in one lab, and it was detected as an interference in that one also.

(3) One lab had a calibration problem with this compound.

(4) In general, this analyte offered less problems than some, but its determination can experience positive interference from non-CDF's with some of the techniques.

SOME GENERAL OBSERVATIONS

(1) All of the labs except one had to decide how to quantitate the analytes for which they did not have an isotopic internal standard. Several different approaches were used. Comparing peak areas to those of external standards in separate injections could not compensate for partial and variable recoveries. Assuming that recoveries for a given sample would be the same for all analytes as was determined for 2,3,7,8-TCDD was generally not a valid assumption. When data for recoveries of several isotopic internal standards from the same sample were reported, it was obvious that different compounds showed significantly different recoveries, even in a given sample. Recoveries of CDF's as a class usually differed, sometimes considerably, from recoveries of CDD's as a class. It was possible to get very good numbers without having all 7 isotopic internal standards, but it was clear that better numbers resulted from the use of both CDD and CDF internal standards than from only one class. Replicability appeared to be somewhat better using EI than using CI, but data to evaluate this was minimal and this question should be tested in some lab that has experience with and capability of doing both.

(2) Mass spectral resolution did not seem to matter much for 2,3,7,8-TCDD. What out-of-range high values were reported occurred in samples not given the interferences, and there is no obvious reason to assume they were due to insufficient MS resolution. This conclusion would not necessarilly apply to all of the analytes, however. The lab reporting use of EI at the lowest reported MS resolution appeared to have some non-CDF interference with the PCDF.

(3) The issue of "false positives" could not be addressed in this study for most of the analytes, because of the background in the human fat. This issue should be addressed in another study using a synthetic matrix that simulates human fat extract but lacks the background.

(4) Some techniques are clearly incompatible with determination of both unsymmetrical TCDX's and higher chlorinated species. In particular, strong alkaline saponification and even multiple exposure to strongly basic adsorbants cause losses to varying extents when a human fat matrix is processed.

783318

(5) There was no indication in this study, looking at the limited data where replicates were reported, that the mean of two determinations was more likely to be close to the right answer than one or the other of the individual measurements. This may be misleading, if some of the participants reported individual results that were in fact the result of averaging replicate determinations. If that is not the case, then this study would suggest that increasing the cost of analysis by making multiple determinations on a single cleaned-up preparation is not likely to yield significantly improved numbers.
(6) There were no sample/analyte combinations in this study for which no lab was able to generate a number very close to the right answer. Sample #3/PCDF came the closest to this of any (only one lab came very close to the right answer), and additional effort to improve reliability for this analyte might be desirable.
(7) No lab in this study generated exclusively "right answers" throughout. Nothing worth doing can be done perfectly.
(8) There was no one method conspicuously "better" than all other methods. This study would not support the conclusion that there is only one right way to approach this analytical problem.
(8) Any future studies that take an approach similar to this one must find a better way to ship samples to avoid what appeared to be unpressurized storage compartment-related leakage problems. Also, "spares" must be available for all of the samples. In particular, the "background" sample should be replicated at least three times in every set (unless it is truely blank). None of these improvements were unrealized at the start of this experiment; they were merely physically impossible.
(9) Although the "performance" on OCDD could not be evaluated in the same way as that for the other analytes, it is disturbing that levels reported for sample #1 were so very different between labs when the level was in the ppb range. Have we specialized in ppt level analysis to such an extent that higher levels can no longer be handled? Perhaps this question also should be addressed in some future study.