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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Analysis Results for Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans in 2,4-D and 2,4-D 2-Ethylhexyl Ester. I. D. Nos. 61272-3, 61272-1. Record No. 259031. MRID/Accession Nos. 41349001, 41349002. DEB No. 6295.

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Background

In response to a 06/87 Data Call-In (DCI) for analytical chemistry data on polychlorinated dibenzo-p-dioxins (CDD's) and dibenzofurans (CDF's) in 2,4-dichlorophenoxyacetic acid (2,4-D) and in 2,4-D salts and esters, Nufarm, USA, Inc., St. Louis, MO submitted (received 01/10/90) analysis reports for 2,4-D (98.0% technical) and for 2-ethylhexyl-(2,4-dichlorophenoxy)acetate (2,4-D IOE, 98.0% technical). Volume II (01/05/90) is entitled "Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in 2,4-D Acid, EPA Reg. No. 61272-3," and Volume III (01/05/90) is entitled "Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in 2,4-D Isooctyl Ester Technical, EPA Reg. No. 61272-1." Both consist of the results of analyses of seven samples, including all raw data, a discussion of the results, and three appendices. Appendix I describes the GC/MS method of analysis. The analyses were conducted by Chemserv Industries Service Ges.m.b.H., Linz, Austria, using Method 40288. This method was reviewed previously

58

in detail (R. Loranger, 09/20/88 Memo, RCB No. 4138) and was approved with the recommendation that an example calculation of analyte concentration and recovery be included in the final report for at least one sample. Appendix II presents the calibrations (spread sheets, chromatograms, calibration curves), and Appendix III details the sampling protocol. The sampling protocol was reviewed previously (M. Flood, 12/30/88 Memo, DEB Nos. 4695 and 4696) and was found acceptable with the stipulation that pesticide grade solvents (as opposed to ACS reagent grade) be used for container cleaning.

Discussion

Seven 2,4-D samples were taken at the production facility in Victoria, Australia, and seven 2,4-D IOE samples were taken at the production facility in Glenwood, Illinois. All were analyzed by Chemserv Industries Service Ges.m.b.H., Linz, Austria. No sample documentation was provided, e.g., lot numbers, dates and times of sampling, and COC copies. Each sample was prepared and analyzed in duplicate and four 2,4-D and four 2,4-D IOE samples were each spiked with all DCI natural abundance isomers at or below the LOQ's. The 2,4-D samples were extracted and cleaned-up on July 11 -12, 1989, and the 2,4-D IOE samples were extracted and cleaned-up on July 12, 1989. The 2,4-D extracts were analyzed on November 9 - 10, 1989, and the 2,4-D IOE extracts were analyzed on November 10 and 13, 1989. Results are summarized below. Results are <u>not</u> corrected for surrogate recovery, and values below the method limits were not used in calculating average values.

Compound	DCI LOQ (ng/g)	2,4-D Average (ng/g)	Range	2,4-D IOE Average (ng/g)	Range
2378-TCDD	0.1	0.1[1]	0.03-0.1	<0.1[4]	<0.1
12378-PeCDD	0.5	1.7[2]	0.2-4.0	0.6[5]	0.2-0.6
123478-HxCDD	2.5	<2.5	<2.5	<2.5	<2.5
123678-HxCDD	2.5	<2.5	<2.5	<2.5	<2.5
123789-HxCDD	2.5	<2.5	<2.5	<2.5	<2.5
1234678-HpCDD	100	<5.0	<5.0	<5.0	<5.0
2378-TCDF	1	<1.0	<1.0	<1.0	<1.0
12378-PeCDF	5	<5.0	<5.0	<5.0	<5.0
23478-PeCDF	5	<5.0	<5.0	<5.0	<5.0
123478-HxCDF	25	<5.0	<5.0	<5.0	<5.0
123678-HxCDF	25	<5.0	<5.0	<5.0	<5.0
123789-HxCDF	25	<5.0	<5.0	<5.0	<5.0
234678-HxCDF	25	<5.0	<5.0	<5.0	<5.0
1234678-HpCDF	1000	6.4[3]	2.0-8.0	<5.0[6]	2.0-<5.0
1234789-HpCDF	1000	<5.0	<5.0	<5.0	<5.0

[1] Quantitated in 1 sample, below the method limit (0.1 ng/g), but detected, in 2 samples.

[2] Quantitated in 6 samples, below the method limit (0.5 ppb), but detected, in one sample.

[3] Quantitated in 3 samples, below the method limit (5.0 ppb), but detected, in 4 samples.

[4] Six valid samples. No surrogate recovery and no recovery standard recovery in one sample (both replicates).

[5] Quantitated in 4 samples, below the method limit (0.5 ppb), but detected, in 3 samples.

[6] Below the method limit (5.0 ppb), but detected, in one sample.

Only 1,2,3,7,8-PeCDD had concentration levels in excess of the DCI LOQ in both 2,4-D and 2,4-D IOE. Individual values in the 2,4-D were 1.8, 2.0, 0.8, 0.8, 0.8, <0.5, and 4.0 ng/g. Corrected for surrogate recovery, the values are 1.8, 2.0, 0.6, 0.6, 0.6, <0.5, and 4.2 ng/g, average 1.36 ng/g. Individual values in the 2,4-D IOE were <0.5, 0.6, 0.5, 0.5, 0.6, <0.5, and <0.5 ng/g. Corrected for surrogate recovery, the values are <0.7, 0.8, 0.6, 0.6, 0.8, <0.5, and <0.6 ng/g, average 0.7 ng/g. The registrant indicates that verification of these particular results are in progress and claims that the polychlorinated dioxins/dibenzofurans are carryover from pentachlorophenol manufacture, a process performed in the same equipment used to produce 2,4-D. The registrant also maintains that the lower 1,2,3,7,8-PeCDD values in the 2,4-D IOE substantiate the theory that dioxins/furans are not formed during the esterification. The PeCDD presence is a dilution of that found in the starting 2,4-D.

In 2,4-D, 2,3,7,8-TCDD was present at the DCI LOQ (0.1 ng/g). It was found in one sample (2955093, A and B) at 0.13 ng/g and 0.14 ng/g, respectively. Corrected for surrogate recovery, the values are 0.11 ng/g and 0.12 ng/g, average 0.12 ng/g. For the remaining six sample, 2,3,7,8-TCDD was found in one of two replicates for sample 2955088 at 0.12 ng/g (0.10 ng/g corrected) and in one of two replicates for sample 2955092 at 0.04 ng/g (0.03 ng/g corrected). The other replicate in each case had no 2,3,7,8-TCDD response.

The registrant included all chromatograms (with exceptions noted) and all raw data (height response, retention time, isomer ratio) for samples and standards. However, no example calculations were provided to relate raw data to reported concentrations or recoveries, and deviations from the approved method protocol were not explained. According to the protocol, standards of DCI natural abundance isomers and corresponding $^{13}C_{12}$-congeners were analyzed (in one solution) at concentrations corresponding to the $^{13}C_{12}$-internal standard sample spiking levels (see calibration range below). Also added to the solution and analyzed was a recovery standard solution consisting of $^{13}C_{12}$-1,2,3,4-TCDD at a concentration of 0.5 ng/ml and $^{13}C_6$-1,2,3,4,6,7,8-HpCDF at a concentration of 12.5 ng/ml. Both are added to sample extracts before analysis. The amount added corresponds to 0.1 ng/g and 2.5 ng/g, respectively,

3 6e

referenced to the original sample (5 g). Additional concentration levels of natural abundance isomers were analyzed, but the $^{13}C_{12}$ and recovery standard concentrations were held at the initial (spike) values. Concentrations 1, 2.5, 5, 7.5, and 10 times the original spike level concentration were analyzed on 11/08/89, one day before the first set of sample analyses. The response factors for the analytes were calculated relative to the corresponding $^{13}C_{12}$-congeners (except 2,3,4,6,7,8-HxCDF, calculated relative to $^{13}C_{12}$-1,2,3,6,7,8-HxCDF), and the response factors for the $^{13}C_{12}$-isomers were calculated relative to $^{13}C_{12}$-1,2,3,4-TCDD for the $^{13}C_{12}$-2,3,7,8-TCDD and relative to $^{13}C_6$-1,2,3,4,6,7,8-HpCDF for the $^{13}C_{12}$-furans and the remaining 5 halogenated dibenzo-p-dioxins. The $^{13}C_{12}$-TCDD was substituted for the $^{37}Cl_4$-1,2,3,4-TCDD of the protocol. This substitution was not mentioned by the registrant. Appropriate equations for relative response factor calculations are:

$$RRF_{12C} = (H_{12C} \times pg_{13C}) / (H_{13C} \times pg_{12C})$$

$$RRF_{13C} = (H_{13C} \times pg_R) / (H_R \times pg_{13C})$$

where RRF = relative response factor of a specific ^{12}C or ^{13}C CDD or CDF.

H_{12C} = peak height of a specific native CDD or CDF.

H_{13C} = peak height of a specific $^{13}C_{12}$ standard.

H_R = peak height of a specific recovery standard.

pg_{12C} = mass of a specific native CDD or CDF.

pg_{13C} = mass of a specific CDD or CDF $^{13}C_{12}$ standard.

pg_R = mass of specific recovery standard.

Peak heights for $^{13}C_6$-1,2,3,4,6,7,8-HpCDF and for $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF must be corrected for overlapping masses.

$$H_{13C6\text{-}HpCDF} = H_{m/z\ 414} - (0.178)H_{m/z\ 408}.$$

$$H_{13C6\text{-}HpCDF} = H_{m/z\ 416} - (0.036)H_{m/z\ 408}.$$

HpCDF contributes 17.8% of m/z 408 to m/z 414 and 3.6% m/z 408 to m/z 416.

$$H_{13C12\text{-}1234678HpCDF} = H_{m/z\ 420} - (0.178)H_{m/z\ 414}.$$

$$H_{13C12\text{-}1234678HpCDF} = H_{m/z422} - (0.036)H_{m/z\ 414}.$$

4 6t

$^{13}C_6$-HpCDF contributes 17.8% of m/z 414 to m/z 420 and 3.6% of m/z 414 to m/z 422.

The registrant constructed five-point calibration charts for the target analytes, plotting response factor versus amount of standard (pg), where response factor is defined as:

$RF = (H_{12C} \times pg_{13C}) / (H_{13C})$.

The following results were obtained:

Analyte	Calibration Range (ng/g)	Corr. Coefficient	Low Aver. RRF[1]	Mean RRF	S.D. RRF
2378-TCDF	1 - 10	0.99898	1.06	1.386	0.327
2378-TCDD	0.1- 1	0.99611	0.81	0.961	0.160
12378-PeCDF	5 - 50	0.99997	1.41	1.546	0.151
23478-PeCDF	5 - 50	0.99999	1.16	1.251	0.111
12378-PeCDD	0.5- 5	0.99829	1.31	1.729	0.408
123478-HxCDF	5 - 50	0.99971	1.16	1.324	0.168
123678-HxCDF	5 - 50	0.99951	1.22	1.367	0.178
234678-HxCDF	5 - 50	0.99984	0.96	1.112	0.184
123789-HxCDF	5 - 50	0.99987	1.25	1.459	0.228
123478-HxCDD	2.5- 25	0.99876	1.15	1.487	0.345
123678-HxCDD	2.5- 25	0.99646	1.14	1.539	0.402
123789-HxCDD	2.5- 25	0.99808	1.05	1.370	0.321
1234678-HpCDF	5 - 50	0.99855	1.22	1.478	0.271
1234789-HpCDF	5 - 50	0.99919	1.28	1.634	0.361
1234678-HpCDD	5 - 50	0.99795	1.36	1.748	0.403
$^{13}C_6$-1234678-HpCDF	2.5			1.00	
$^{13}C_{12}$-1234-TCDD	0.1			1.00	
$^{13}C_{12}$-2378-TCDF	1.0		2.81	2.302	0.515
$^{13}C_{12}$-2378-TCDD	0.1		0.95	0.921	0.078
$^{13}C_{12}$-12378-PeCDF	5.0		2.08	1.846	0.245
$^{13}C_{12}$-23478-PeCDF	5.0		2.92	2.597	0.343
$^{13}C_{12}$-12378PeCDD	0.5		0.63	0.529	0.092
$^{13}C_{12}$-123478-HxCDF	5.0		1.83	1.566	0.263
$^{13}C_{12}$-123678-HxCDF	5.0		1.62	1.412	0.209
$^{13}C_{12}$-123789-HxCD	5.0		1.10	0.966	0.126
$^{13}C_{12}$-123478-HxCDD	2.5		0.68	0.571	0.103
$^{13}C_{12}$-123678-HxCDD	2.5		0.64	0.559	0.078
$^{13}C_{12}$-123789-HxCDD	2.5		0.82	0.706	0.102
$^{13}C_{12}$-1234678-HpCDF	5.0		1.01	0.858	0.147
$^{13}C_{12}$-123478-HpCDF	5.0		0.64	0.545	0.094
$^{13}C_{12}$-1234678-HpCDD	5.0		0.44	0.380	0.058
$^{13}C_{12}$-234678-HxCDF	NOT ANALYZED				

[1] For analytes, average of two lowest concentration RRF's. For surrogates ($^{13}C_{12}$-), average of first two RRF's determined. See text below.

5 62

The protocol requires that each concentration level be analyzed three times in establishing RRF's or calibration curves. Data were submitted for one analysis only at each level. The mean RRF was not used in calculating target analyte concentrations in the samples. Rather, the registrant averaged the two lowest concentration RRF's (Low Aver. RRF) and used that average in all calculations. The Guidelines for the Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in Commercial Products permit the use of the spiking level (LOQ) RF if analyte values are within 2 orders of magnitude of the spike level. The mean RRF would have generally yielded somewhat lower values (10% - 30% less). Adequate linearity of response was demonstrated for the calibration ranges indicated above for all compounds. For the 2,4-D samples, the 1,2,3,7,8-PeCDD found at an average value of 1.7 ppb falls at 3.4 times the spiking concentration (0.5 ng/g) and on a demonstrated linear range (0.5 - 5 ng/g). The 1,2,3,4,6,7,8-HpCDF found at an average value of 6.4 ppb falls at 1.3 times the spiking value and in a demonstrated linear range (5 - 25 ng/g). The 2,3,7,8-TCDD falls at the DCI LOQ (0.1 ng/g) and at the demonstrated method limit (0.1 ng/g).

The registrant calculated the $^{13}C_{12}$-congener concentrations using the response factors for the congeners relative to the recovery standards. The recovery standards were added after preparation and before analysis and would not, therefore, be subject to the inefficiencies of extraction/clean-up/concentration. Again, the registrant did not use an average response factor for the five standard analyses (all at the same concentration). The average of the first two determinations was used. Recoveries of the $^{13}C_{12}$-congeners were calculated for every analysis and are summarized as follows:

2,4-D

$^{13}C_{12}$-Compound	$^{13}C_{12}$-Conc. (ng/g)	Unspiked Recovery (%) Average	Range	Spiked Recovery (%) Average	Range
2378-TCDF	1.0	102	79-127	74	67-80
2378-TCDD	0.1	30	130-154[1]	124	114-128
12378-PeCDF	5.0	95	74-116	71	67-75
23478-PeCDF	5.0	98	71-134	72	68-75
12378-PeCDD	0.5	112	87-152[1]	84	82-87
123789-HxCDF	5.0	93	84-106	74	70-77
123478-HxCDF	5.0	104	76-121	86	80-91
123678-HxCDF	5.0	116	101-135	95	89-97
123478-HxCDD	2.5	127	108-140	98	97-99
123678-HxCDD	2.5	141	122-165[2]	113	112-119

123789-HxCDD	2.5	135	120-152[3]	103	99-106
1234678-HpCDF	5.0	99	88-110	91	89-95
1234789-HpCDF	5.0	130	118-143	120	118-122
1234678-HpCDD	5.0	152[4]	140-167	133	128-143

[1] One value out-of-control.
[2] Four values out-of-control, including both in one sample (2955090).
[3] Two values out-of-control.
[4] Out-of-control analysis. Ten values out-of-control, including both results for four samples (2955090, 2955091, 2955092, 2955093).

2,4-D IOE

$^{13}C_{12}$-Compound	$^{13}C_{12}$-Conc. (ng/g)	Unspiked Recovery (%) Average	Range	Spiked Recovery (%) Average	Range
2378-TCDF	1.0	65	56-89	66	55-96
2378-TCDD	0.1	119	95-142[1]	105	76-134
12378-PeCDF	5.0	68	59-82	71	53-106
23478-PeCDF	5.0	69	50-112	52	34-84[2]
12378-PeCDD	0.5	87	71-162[3]	92	65-141
123789-HxCDF	5.0	84	71-98	85	66-98
123478-HxCDF	5.0	88	77-109	78	70-86
123678-HxCDF	5.0	99	92-106	98	84-107
123478-HxCDD	2.5	121	96-153[4]	131	108-144
123678-HxCDD	2.5	134	112-183[3]	121	114-131
123789-HxCDD	2.5	116	103-148	117	104-129
1234678-HpCDF	5.0	94	93-98	89	79-95
1234789-HpCDF	5.0	120	104-129	126	106-137
1234678-HpCDD	5.0	140	129-156[3]	146	125-163[4]

[1] No recoveries could be calculated for 2955097 or its duplicate; no response for $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-2,3,7,8-TCDD.
[2] Three values out-of-control.
[3] One value out-of-control.
[4] Two values out-of-control.

With three exceptions, at least one analysis for each 2,4-D and each 2,4-D IOE sample had all $^{13}C_{12}$-congener recoveries within the 50 - 150% limits. For $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD in the 2,4-D samples, recoveries were unacceptable (>150%) for four of the seven samples. For $^{13}C_{12}$-1,2,3,6,7,8-HxCDD, one 2,4-D sample out of seven failed (>150%). For the HpCDD, the spike level of 5.0 ng/g was far below the LOQ of 100 ng/g, and recovery was out-of-control on the high side. Any significant HpCDD level would have been detected. For the HxCDD, the out-of-control recovery was marginal (152%, 160%), and no 1,2,3,6,7,8-HxCDD was detected in the remaining six samples with in-control surrogate recoveries. For 2,4-D IOE, one

64

sample gave no recovery for $^{13}C_{12}$-2,3,7,8-TCDD in both replicates.

The DCI required <u>one</u> sample to be spiked in duplicate with the $^{13}C_{12}$-congeners at the LOQ's and to be analyzed after extraction and work-up. Recoveries are to be 50 -150%, and the replicate $^{13}C_{12}$-isomer values are to agree to within ±20%. One 2,4-D and one 2,4-D IOE sample were selected and the relative per cent differences calculated. Adequate accuracy and precision have been demonstrated, as the following results indicate:

<u>2,4-D Sample 2955087.</u>

$^{13}C_{12}$-Surrogate	Spike Level (ng/g)	LOQ (ng/g)	A Recovery (%)	B Recovery (%)	RPD (%)
2378-TCDF	1.0	1.0	96	89	7.6
2378-TCDD	0.1	0.1	140	142	1.4
12378-PeCDF	5.0	5	86	74	10.
23478-PeCDF	5.0	5	80	71	13.
12378-PeCDD	0.5	0.5	99	92	7.3
123478-HxCDF	5.0	25	85	84	1.2
123678-HxCDF	5.0	25	90	79	13.
123789-HxCDF	5.0	25	102	101	1.0
123478-HxCDD	2.5	2.5	125	129	3.1
123678-HxCDD	2.5	2.5	133	126	5.4
123789-HxCDD	2.5	2.5	132	133	0.8
1234678-HpCDF	5.0	1000	90	88	2.2
1234789-HpCDF	5.0	1000	118	121	2.5
1234678-HpCDD	5.0	100	140	145	3.5

<u>2,4-D IOE Sample 2955095.</u>

$^{13}C_{12}$-Surrogate	Spike Level (ng/g)	LOQ (ng/g)	A Recovery (%)	B Recovery (%)	RPD (%)
2378-TCDF	1.0	1.0	61	61	0
2378-TCDD	0.1	0.1	133	118	12
12378-PeCDF	5.0	5	67	67	0
23478-PeCDF	5.0	5	66	67	1.5
12378-PeCDD	0.5	0.5	80	78	2.5
123478-HxCDF	5.0	25	79	78	1.3
123678-HxCDF	5.0	25	89	91	2.3
123789-HxCDF	5.0	25	99	100	1.0
123478-HxCDD	2.5	2.5	111	109	1.8
123678-HxCDD	2.5	2.5	132	134	1.5
123789-HxCDD	2.5	2.5	110	108	1.8
1234678-HpCDF	5.0	1000	93	93	0
1234789-HpCDF	5.0	1000	119	116	2.6
1234678-HpCDD	5.0	100	134	135	0.74

For both 2,4-D and 2,4-D IOE, four samples were spiked with the natural abundance congeners at or below the DCI LOQ's. The Guidelines for the Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in commercial Products specify a recovery of 50 - 150% for each spike component. The following recoveries, corrected for amounts found in the unspiked samples (at or above the lowest concentration standard), were calculated, based upon the raw data supplied by the registrant:

2,4-D

Analyte	Spike Conc. (ng/g)	Sample 2955088S Recovery (%)	Sample 2955089S Recovery (%)	Sample 2955091S Recovery (%)	Sample 2955093S Recovery (%)
2378-TCDF	1.0	29000[1]	14000[1]	18000[1]	16000[1]
2378-TCDD	0.1	196	180	176	156
12378-PeCDF	5.0	115	130	135	103
23478-PeCDF	5.0	80	82	85	84
12378-PeCDD	5.0	110	180	195	83
123789-HxCDF	5.0	71	79	78	76
123478-HxCDF	5.0	79	87	96	85
123678-HxCDF	5.0	78	78	91	79
123478-HxCDD	2.5	79	132	134	126
123678-HxCDD	2.5	95	112	111	119
123789-HxCDD	2.5	84	93	96	99
1234678-HpCDF	5.0	121	105	178	109
1234789-HpCDF	5.0	75	78	80	79
1234678-HpCDD	5.0	77	98	96	81
234678-HxCDF	5.0	80	86	91	85

[1] See text. Probably not 2378-TCDF. Also present in unspiked samples.

2,4-D IOE

Analyte	Spike Conc. (ng/g)	Sample 2955095S Recovery (%)	Sample 2955096S Recovery (%)	Sample 2955098S Recovery (%)	Sample 2955100S Recovery (%)
2378-TCDF	1.0	10200[1]	10800[1]	8400[1]	6800[1]
2378-TCDD	0.1	152	190	182	148
12378-PeCDF	5.0	100	107	95	107
23478-PeCDF	5.0	73	80	82	88
12378-PeCDD	5.0	145	139	152	255
123789-HxCDF	5.0	70	76	74	80
123478-HxCDF	5.0	82	83	81	87
123678-HxCDF	5.0	71	82	78	99
123478-HxCDD	2.5	119	137	--	139

9
66

123678-HxCDD	2.5	86	97	91	96
123789-HxCDD	2.5	75	87	87	88
1234678-HpCDF	5.0	140	118	90	110
1234789-HpCDF	5.0	69	77	77	81
1234678-HpCDD	5.0	82	89	82	88
234678-HxCDF	5.0	88	105	95	99

[1] See text. Probably not 2378-TCDF. Also present in unspiked samples.

Both the 2,4-D and the 2,4-D IOE spiked samples showed responses corresponding to 2,3,7,8-TCDF, with recoveries of 7000 - 29000%, i.e., unspiked concentrations of 70 - 290 ppb 2,3,7,8-TCDF. The same responses were found in the sample analyses. Retention times were not within the ± 1 second window. Isomer ratios were acceptable (±20 % theoretical). Retention times of the suspect peak normally differed by 2 or 3 seconds from the corresponding $^{13}C_{12}$-peak's retention time. This is a rejection criterion, according to the protocol (±1 second). The registrant's protocol notes that 2,3,7,8-TCDF will coelute with other TCDF isomers. A different column, such as a DB-225 or SP-2330 or DB-Dioxin, is needed to identify the TCDF. No additional effort was reported to identify the peak. Given its substantial concentration, it ought to be characterized.

For 2,4-D, all samples had out-of-control high recoveries for 2,3,7,8-TCDD. This could be an indication of 2,3,7,8-TCDD presence in the samples. The definite presence in sample 2955093 at 0.12 ng/g substantiates this probability. The high out-of-controls for 1,2,3,7,8-PeCDD in 2,4-D samples 2955089 and 2955091 suggest that 2.0 ng/g, the value measured for (unspiked) sample 2955088, may be a more realistic value for 1,2,3,7,8-PeCDD concentration in the unspiked samples than 0.8 ng/g, the value found for samples 2955089 and 2955091.

For 2,4-D IOE, three of four samples had high out-of-control values for the 2,3,7,8-TCDD, and the remaining sample's value was just inside the limits (148%). This suggests the presence of 2,3,7,8-TCDD in the unspiked samples. The 1,2,3,7,8-PeCDD values were high out-of-control for two samples. Particularly significant was the 255% recovery for sample 2955100. The corresponding unspiked sample was found to contain <0.1 ppb 1,2,3,7,8-PeCDD. Recoveries were in-control or close (152%) to in-control for samples with 0.5 - 0.6 ppb levels found (unspiked).

The recovery standards, in addition to providing an internal standard for calculating $^{13}C_{12}$-congener concentrations (recoveries), provide a means of tracking GC/MS performance. Assuming the conditions of the analytical protocol were followed, i.e., all extracts were brought to the same (110 ul) volume and equal injections (5 ul) were used, the recovery standards' responses

measure the variability of the GC/MS response. Using typical -50% to +100% of the standards' average responses for recovery standards as control criteria, four samples had 2,4-D analyses that were out-of-control. Of particular concern is sample 2955087, where both replicates' recovery standard responses exceeded the upper response bound. For 2,4-D IOE, five samples and two spiked samples had analyses that were out-of-control. For samples 2955094, 2955095, and 2955100 both replicates for the $^{13}C_6$-1,2,3,4,6,7,8-HpCDF recovery standard exceeded the control limits. For sample 2955097, both replicates had no recovery for the $^{13}C_{12}$-1,2,3,4-TCDD recovery standard.

Sample	Date Analyzed	$^{13}C_{12}$-1234678-HpCDF Response (10.00 pg)	$^{13}C_{12}$-1234 TCDD Response (0.50 pg)
Standards	11/08	799,203	53,762
		844,767	45,551
		965,147	42,667
		1,244,815	48,389
		1,008,672	39,415
	Mean ± 1 s.d.	972,527 ± 156,129 (16%)	45,957 ± 4912 (11%)
	Control	1,945,054 - 486,264	91,914 - 22,978
2,4-D			
2955087	11/09	2,559,589 FAIL	136,465 FAIL
		3,662,835 FAIL	169,558 FAIL
2955088	11/09	3,690,683 FAIL	202,200 FAIL
		1,272,957	80,051
2955089	11/09	579,611	43,442
		360,874 FAIL	36,749
2955090	11/09	643,336	54,166
		527,223	38,893
2955091	11/09	355,596 FAIL	38,932
		530,024	38,781
2955092	11/10	635,866	42,771
		896,982	52,663
2955093	11/10	745,248	50,851
		982,784	53,231

68

2955088S	11/10	762,468		40,215	
2955089S	11/10	1,469,572		81,776	
2955091S	11/10	1,539,177		76,669	
2955093S	11/10	1,513,655		73,062	
2,4-D IOE					
2955094	11/10	2,659,066	FAIL	85,945	
		2,392,940	FAIL	89,214	
2955095	11/10	2,124,230	FAIL	74,272	
		2,024,136	FAIL	81,429	
2955096	11/10	1,770,189		70,310	
		2,121,301	FAIL	88,127	
2955097	11/10	1,706,333		-------	FAIL
		1,196,346		-------	FAIL
2955098	11/13	1,734,288		75,134	
		116,872	FAIL	14,224	FAIL
2955099	11/13	1,681,357		89,722	
		1,479,092		77,574	
2955100	11/13	2,119,510	FAIL	86,001	
		2,037,062	FAIL	89,787	
2955095S	11/13	1,077,169		98,055	FAIL
2955096S	11/13	1,737,919		82,276	
2955096S	11/13	2,166,503	FAIL	91,570	
2955100S	11/13	5,503,906	FAIL	357,343	FAIL

The seriousness of these fluctuations is mitigated by:

1. Responses are out-of-control on the high side, i.e., increased sensitivity (except 2955098 replicate).
2. Internal $^{13}C_{12}$-congener standards were utilized for the analytes.

The registrant handled unit designations in a careless fashion in this submission. The summary "Certificate of Analysis" sheets contain no units. The calibration curves' x-axes indicate

69 12

"Amount." The corresponding calibration spreadsheets indicate "pg." This could be pg on-column (5 ul) or pg/ul or pg adjusted for some dilution. The Dioxin/Furan Spreadsheets are labeled "pg/5g." The correct label is "ng/5g." Labeling all tables, graphs, and summary sheets with units is critical, especially when example calculations are not provided.

Several errors or omissions were noted. The "Laboratory Sample Tracking Form" has incorrect analysis dates. For 2,4-D, sample 2955087 is missing TetraCDD chromatograms for the A replicate. In several instances, the spreadsheets indicate "no peak" for 2,3,7,8-TCDD when a small peak is apparent on the corresponding chromatograms. For example, 2955092A yields "no peak," while 2955092B reports an area. The two chromatograms are similar. This may be related to some area rejection value in the data system. The RRF for 1,2,3,4,7,8-HxCDD is 1.16, not 0.67 as listed on the spreadsheets. This has no effect, as 1,2,3,4,7,8-HxCDD was not detected in the samples. The sample spreadsheets indicate a $^{13}C_6$-1,2,3,4,6,7,8-HpCDF concentration of 2.0 ng/g. The protocol specifies 2.5 ng/g. The calibration sheets show 2.0 and 2.5 ng/g at different points. A value of 2.0 ng/g was used for calculations.

The analytical protocol appears to be in error with regards to calibration standard preparation. All calibration, spiking, and $^{13}C_{12}$-surrogate solutions have identical initial concentrations for the respective compounds. For example, the $^{13}C_{12}$-surrogate solution contains $^{13}C_{12}$-2,3,7,8-TCDD at a concentration of 0.5 ng/ml. The spiking and calibration solutions also contain 2,3,7,8-TCDD at 0.5 ng/ml. Samples (5 g) are eventually extracted into 50 ul, 10 ul of which is diluted to 110 ul for analysis via 5 ul injection. The lowest concentration level standards are diluted 1 ml to 11 ml, and 5 ul injections are made. (Note that actual concentrations of the standards are 5/11 of the stated concentrations.) The process, as detailed in the protocol, does not account for the difference between samples with "X" ng/50 ul and standards with "X" ng/1 ml, that is, a factor of 20. This factor must, in fact, be eliminated via an unmentioned standard adjustment (dilution). Otherwise, $^{13}C_{12}$-surrogate recoveries would be reported high by a factor of 20. A clarification is required.

No data were reported on the blank analyses for either 2,4-D or 2,4-D IOE. The results and data for daily verification of analyte response factors and/or calibration curves were not provided. Daily GC/MS tune conditions were not supplied.

Conclusions

Nufarm, USA, Inc. conducted analyses on seven lots of 2,4-D and seven lots of 2,4-D IOE for the six tetra- to hepta- chlorinated dibenzo-p-dioxins and nine tetra- to hepta- chlorinated

13
70

dibenzofurans specified in the 06/87 DCI. The following compounds were found at or above the LOQ's:

2,4-D

Compound	Maximum Concentration (ng/g)	LOQ (ng/g)
2,3,7,8-TCDD	0.12	0.1
1,2,3,7,8-PeCDD	4.2	0.5

2,4-D IOE

Compound	Maximum Concentration (ng/g)	LOQ (ng/g)
1,2,3,7,8-PeCDD	0.8	0.5

None of the remaining target analytes were found at or above the LOQ's in any of the seven 2,4-D samples or in any of the seven 2,4-D IOE samples. The registrant believes that these impurities result from pentachlorophenol production performed in the same equipment. Regardless of the source, the impurities are present.

Adequate accuracy and precision at or below the DCI LOQ's were demonstrated via $^{13}C_{12}$-compound recoveries from replicate samples.

The above conclusions are regarded as tentative and subject to change, pending resolution of the following problems:

1. Documentation on sampling must be provided: lot numbers, dates and times of sampling, chain of custody (COC) copies.

2. Reextraction/reanalysis of 2,4-D IOE sample 2955097 (Lot MD29) must be performed. The surrogate $^{13}C_{12}$-2,3,7,8-TCDD and the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD were not recovered (0%) in both replicates. There are only six valid results for 2,3,7,8-TCDD. Seven different samples (lots) must be successfully analyzed.

3. Validation of the initial response factors and/or calibration curves (11/08/89) must be performed on each day of sample analyses (11/09, 11/10, 11/13/89). The daily RF may differ from the average RRF by <30%. No daily calibration validation data were submitted.

4. Chromatograms for 2,4-D and for 2,4-D IOE indicated a peak 2 - 3 seconds outside the 2,3,7,8-TCDF retention time window with the correct isomer ratio for a TCDF.

Because of its apparent substantial amount, 50 - 300 ppb, this compound must be identified and quantitated.

5. An acceptable recovery was not achieved for 2,3,7,8-TCDD (natural abundance) spiked at 0.1 ng/g in four different 2,4-D samples and in 3 of 4 2,4-D IOE samples. Recoveries for the 2,4-D samples ranged from 156% to 196%. A successful recovery must be demonstrated for 2,3,7,8-TCDD in 2,4-D. Because this may indicate 2,3,7,8-TCDD levels in the unspiked samples ranging from 0.01 - 0.09 ng/g, the registrant is advised to establish a 0.05 ng/g standard and repeat unspiked analyses and the spiking procedure/analysis with at least two 2,4-D samples. This may be combined with item no. 2 above.

6. Recovery standard areas varied substantially over the 3 days of analysis. Using the range of -50% to +100% of internal standard response in the standard runs (average) as a control, 18 of 36 analyses failed. This would lead to rejection of the data, were not 14 internal standards ($^{13}C_{12}$-congeners) present for the 15 compounds. The following analyses, where one or both recovery standards failed <u>in both replicates</u>, must be repeated:

   2955087
   2955094
   2955095
   2955097
   2955100

   The original extracts, if available and if properly stored, may, at the registrant's option, be utilized.

7. Check chromatograms for 2,3,7,8-TCDD. Several spreadsheets indicate "no peak," whereas a response is noted on the chromatogram. For example, see 2955092A.

8. Submit a detailed outline of standards and spiking solution preparations and analyses (dilutions, injection volume). There is an apparent discrepancy factor of 20 between standards and samples. Also, if the protocol were followed, standards would be 5/11 of the stated calibration curve concentrations. Explain what concentration of recovery standard $^{13}C_6$-1,2,3,4,6,7,8-HpCDF was used, 2.0 or 2.5 ng/g. Correct or explain the "pg/5g" units on the analyte calculation spreadsheets.

9. Submit chromatograms and spreadsheets for the sample blank(s).

10. Submit a summary of initial (calibration day) and daily MS tune conditions.

11. Sample calculations shall be included, showing each step of determining an analyte and a $^{13}C_{12}$-congener concentration in a given sample. Sample calculations shall be included, showing each step of determining a $^{13}C_{12}$-CDD and a $^{13}C_{12}$-CDF recovery. Sample calculations shall be included, showing each step of determining a natural abundance CDD and a natural abundance CDF spike recovery.

12. Detail any deviations from the approved protocol, for example, the substitution of recovery standard $^{13}C_{12}$-1,2,3,4-TCDD for $^{37}Cl_4$-1,2,3,4-TCDD.

13. A confidential statement of formula (CSF), per the DCI, is required with the final report. It should encompass the results of the CDD/CDF analyses.

Until item nos. 1 - 13 above are resolved, DEB can make no firm conclusion on the validity and significance of the analytical results.

The following deficiencies are noted in the analysis report, but no corrections will be required:

A. Calibration standards were analyzed only once in arriving at the curves and RRF's. Each concentration was to be analyzed 3 times, per the Guidelines and the Nufarm protocol.

B. The LOQ (or lowest) RRF's should have been used, not the average of the two lowest concentration RRF's.

C. Four 2,4-D lots yielded out-of-control analyses for 1,2,3,4,6,7,8-HpCDD. The $^{13}C_{12}$-spike level was much lower than the LOQ (5.0 ng/g versus a LOQ of 100ng/g), and the recoveries failed on the high side (>150%). Internal standard (recovery standard) responses were satisfactory (except one 2955091 replicate). The increased recovery, therefore, may be attributed to increased sensitivity for HpCDD. The chromatograms do not indicate interferences.

D. One 2,4-D sample (both replicates) had marginally unacceptable (152%, 160%) surrogate recoveries for $^{13}C_{12}$-1,2,3,6,7,8-HxCDD. Also, the remaining six in-control samples did not show any 1,2,3,6,7,8-HxCDD.

E. Unit designations must be entered on all tables, spreadsheets, and calibration curves.

Recommendation

DEB cannot render a decision on the significance and validity of the analytical chemistry data submitted by Nufarm, USA, Inc. until the additional data and clarifications (Conclusion item nos. 1 - 13) are made available by the registrant. This additional material will be reviewed and a decision made.

Also, after the additional information is found acceptable, DEB will defer to TOX on the significance of the dioxins detected.

cc: Toxicology Branch, RF, Dioxin SF, 2,4-D Registration Standard File, Circ., R. Schmitt (Branch Chief), S. Funk, C. Furlow (PIB/FOD).

RDI:A. Rathman:05/22/90:E. Zager:05/22/90:

H7509C:DEB:S. Funk:557-1439:CM#2:Rm803-A:SF(DIOX.42):03/30/90.