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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM (CONFIDENTIAL)

Subject: Analysis of Technical 2,4-D and Technical 2,4-D Isooctyl Ester for Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans. Response to DCI. DP Barcode D160163. I.D. Nos. 61272-3; 61272-1. MRID Nos. 417242-01, -02, -03. CBRS No.7532.

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Background

In response to a 06/87 DCI for analytical chemistry data on polychlorinated dibenzo-p-dioxins and dibenzofurans in technical 2,4-dichlorophenoxyacetic acid (2,4-D) and in technical 2-ethylhexyldichlorophenoxyacetate (2,4-D IOE) Nufarm USA, Inc. previously submitted analytical chemistry data. The data were reviewed, and it was concluded that the studies contained 13 deficiencies (05/24/90 Memorandum, S. Funk, DEB No. 6295). The registrant responded to the deficiencies with a commitment to provide data/information to remove the deficiencies and requested a 90 day extension to perform laboratory work (11/15/90 Memorandum, S. Funk, DEB No. 7211). The registrant has now submitted the requested data (received 12/12/90) in two volumes entitled

1 of 4

"Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in 2,4-D Acid by Method of Analysis 50288: Study No. 90-4: 11/30/90" and "Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in 2,4-D Isooctyl Ester by Method of Analysis 50288: Study Number 90-5: 11/30/90." The performing laboratory is Chemserv Industries Service Ges.m.b.H., Linz, Austria. The registrant provided a complete sample reanalysis package rather than addressing the specific deficiencies.

Discussion

The seven 2,4-D samples and the seven 2,4-D IOE samples were reextracted and reanalyzed (in duplicate). Results are summarized as follows:

2.15

Table 1: Concentration of Dioxin and Dibenzofuran in Technical 2,4-D and in 2,4-D IOE

Compound	DCI LOQ (ng/g)	2,4-D Acid Average (ng/g)	2,4-D Acid Range (ng/g)	2,4-D IOE Average[5] (ng/g)	2,4-D IOE Range (ng/g)
2,3,7,8-TCDD	0.1	0.06[1]	0.02 - 0.12[1]	0.04[1]	0.03 - 0.04[1]
1,2,3,7,8-PCDD	0.5	1.22; 1.42[1]	0.35 - 2.56; 0.42 - 3.39[1]	0.59; 0.66[1]	0.39 - 0.86; 0.42 - 0.93[1]
1,2,3,4,7,8-HxCDD	2.5	0.81[2,3]	< ? - 0.812[1]	0.09	< ? -0.66
1,2,3,6,7,8-HxCDD	2.5	0.58	0.43 - 0.77	0.40	0.17 - 0.63
1,2,3,7,8,9-HxCDD	2.5	0.32	0.23 - 0.54	0.25	0.11 - 0.40
1,2,3,4,6,7,8-HpCDD	100	0.06	0.02 - 1.10	0.58	0.24 - 1.0
2,3,7,8-TCDF	1	0.09[1]	0.04 - 0.12[1]	0.10[1]	<? - 0.22[6]
1,2,3,7,8-PCDF	5	0.62[2,3]	< ? - 0.62[2,3]	0.58	< ? - 1.4[5]
2,3,4,7,8-PCDF	5	<< 5[2,4]	< ?[2,4]	<< 5[2,4]	< ?[2,4]
1,2,3,4,7,8-HxCDF	25	0.63	0.14 - 1.37	0.67	0.15 - 1.3
1,2,3,6,7,8-HxCDF	25	0.19	0.11 - 0.29	0.11	< ? - 0.94[6]
2,3,4,6,7,8-HxCDF	25	0.26	0.15 - 0.36	0.27	0.12 - 0.48
1,2,3,7,8,9-HxCDF	25	0.25	0.08 - 0.61	0.10	< ? - 0.27[1]
1,2,3,4,6,7,8-HpCDF	1000	3.93	0.72 - 8.26	2.13	0.82 - 4.0
1,2,3,4,7,8,9-HpCDF	1000	0.16	0.07 - 0.27	0.23	0.09 - 0.41

[1] Quantitated from the confirmatory column (DB-225).
[2] Registrant did not provide limits of detection/quantitation or adequate raw data to calculate. [3] Six of seven samples were ND. [4] Seven of seven samples were ND. The < 5 value is based on the 5 ng/g $^{13}C_{12}$-2,3,4,7,8-PCDF spiking level. [5] Two of seven samples were ND. [6] One of seven samples was ND.

Each sample was prepared and analyzed in duplicate. Recoveries of the $^{13}C_{12}$-isomers in each sample were calculated relative to the

recovery standards. Recoveries of the target analytes spiked (at or below the EPA LOQ's) into samples 2955088, 2955090, 2953093, 2955095, 2955097, and 2955100 were also calculated. Recovery data are summarized in Table 2 and in Table 3.

Table 2: Recovery of Internal Standards from All Samples.

Analyte	2,4-D Recovery Average $^{13}C_{12}$ (%)	2,4-D Recovery Range $^{13}C_{12}$ (%)	2,4-D IOE Recovery Average $^{13}C_{12}$ (%)	2,4-D IOE Recovery Range $^{13}C_{12}$ (%)
2378-TCDD	99[1]	93 - 112[1]	102[1]	98 - 105[1]
12378-PCDD	125; 102[1]	81 - 147; 87 - 113[1]	115; 108[1]	91 - 145; 99 - 119[1]
123478-HxCDD	104	84 - 117	90	74 - 110
123678-HxCDD	120	83 - 145	115	89 - 132
123789-HxCDD	116	87 - 129	99	87 - 116
1234678-HpCDD	109	80 - 137	100	91 - 119
2378-TCDF	96[1]	80 - 107[1]	104[1]	95 - 120[1]
12378-PCDF	139	117 - 155[2]	117	97 - 143
23478-PCDF	139	122 - 160[3]	119	102 - 137
123478-HxCDF	100	87 - 112	99	88 - 115
123678-HxCDF	113	96 - 144	118	98 - 146
123789-HxCDF	106	97 - 132	94	86 - 113
234678-HxCDF	106	97 - 132	107	92 - 122
1234678-HpCDF	102	92 - 114	103	92 - 108
1234789-HpCDF	100	93 - 105	95	82 - 122

[1] Analysis on confirmatory column (DB-225). [2] One recovery in one of two sample replicates >150%. [3] Three recoveries >150% (152% in 2955090, 151% and 160% in 2955091).

Table 3: Recovery of Analytes from Fortified Samples

2,4-D Acid

Analyte	Analyte Spike Level (ng/g)	2953088 Average Recovery (%)	2953088 Average Recovery /RPD (%) $^{13}C_{12}$	2953090 Average Recovery (%)	2953090 Average Recovery /RPD (%) $^{13}C_{12}$	2953093 Average Recovery (%)	2953093 Aver. Recov./ RPD (%) $^{13}C_{12}$
2378-TCDD	0.1	90[1]	101/2.0[1]	90[1]	104/1.9[1]	85[1]	99/8.1[1]
12378-PCDD	0.5	150; 164[1,2]	126/4.0; 98/11.3[1]	134; 127[1]	125/3.2 110/0.9[1]	103; 58[1]	137/15; 104/1[1]
123478-HxCDD	2.5	94	105/5.7	93	110/0.9	110	106/6.6
123678-HxCDD	2.5	93	120/4.1	92	116/5.2	91	140/6.4
123789-HxCDD	2.5	94	116/2.6	94	117/3.4	94	128/0.8
1234678-HpCDD	5	92	110/0.9	102	109/1.8	81	134/3.7
2378-TCDF	1	104[1]	96/0.0[1]	124[1]	88/12.4[1]	108[1]	101/12[1]
12378-PCDF	5	141	136/6.6	140	144/2.1	101	140/14
23478-PCDF	5	104	135/0.7	112	142/4.9	92	134/17
123478-HxCDF	5	102	96/3.1	102	100/1.0	79	111/1.8
123678-HxCDF	5	96	108/0.9	100	107/0.9	84	142/3.5
123789-HxCDF	5	97	101/1.0	100	100/2.0	79	114/0.0
234678-HxCDF	5	98	103/1.0	100	103/0.0	81	128/5.4
1234678-HpCDF	5	101	106/0/9	99	105/0.0	103	106/2.8
1234789-HpCDF	5	91	106/0.9	100	105/0.0	102	99/2.0

2,4-D IOE

Analyte	Analyte Spike Level (ng/g)	2955095 Average Recovery (%)	2955095 Average Recovery/ RPD (%) $^{13}C_{12}$	2955097 Average Recovery (%)	2955097 Average Recovery/ RPD (%) $^{13}C_{12}$	2955100 Average Recovery (%)	2955100 Average Recovery /RPD (%) $^{13}C_{12}$
2378-TCDD	0.1	100[1]	103/0.0[1]	85[1]	104/1.0[1]	90[1]	102/3.9[1]
12378-PCDD	0.5	59; 71[1]	102/21[2]; 104/1.9[1]	55; 89[1]	138/11; 108/12[1]	85; 115[1]	110/21[2]; 110/12[1]
123478-HxCDD	2.5	104	90/7.7	108	98/23[2]	104	92/7.7
123678-HxCDD	2.5	95	100/22[2]	92	130/1.5	100	118/5.9
123789-HxCDD	2.5	100	91/0.0	94	108/18	99	104/0.0
1234678-HpCDD	5	96	93/4.3	95	98/15	100	113/11
2378-TCDF	1	112[1]	100/1.0[1]	104[1]	100/7.0[1]	104[1]	104/1.9[1]

12378-PCDF	5	80	110/23[2]	96	138/8.0	109	115/17
23478-PCDF	5	95	112/9.8	91	136/1.5	99	119/13
123478-HxCDF	5	83	100/1.0	87	107/15	94	97/0.0
123678-HxCDF	5	101	108/18	100	139/10	102	114/14
123789-HxCDF	5	96	93/4.3	98	100/25[2]	97	95/8.4
234678-HxCDF	5	94	104/6.8	93	120/3.3	92	106/4.7
1234678-HpCDF	5	82	102/1.0	81	100/1.0	91	107/1.9
1234789-HpCDF	5	96	91/15	91	88/14	91	110/23[2]

[1] Quantitated from the confirmatory column (DB-225). [2] Outlier. Limits are: recovery 50% - 150% for spiked analytes and for $^{13}C_{12}$-internal standards; RPD $\leq$ 20% for $^{13}C_{12}$-internal standards in duplicate sample analyses.

Other aspects of the analytical report (such as method blanks and calibration) are addressed under the deficiency review that follows.

Deficiency No. 1:

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

Nufarm USA, Inc. Submission:

No response. This deficiency is not resolved.

Deficiency No. 2:

Reextraction/reanalysis of 2,4-D IOE sample 2955097 (Lot MD29) must be performed, because 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.

Nufarm USA, Inc. Submission:

The sample was reextracted/prepared in duplicate on 09/05/90 and reanalyzed on 09/13/90. The surrogate $^{13}C_{12}$-2,3,7,8-TCDD recoveries were 98% and 92%. All other surrogate recoveries were acceptable (50% - 150%), except $^{13}C_{12}$-2,3,7,8-TCDF, 253% and 283% on the primary column. The $^{13}C_{12}$-2,3,7,8-TCDD recoveries were acceptable (104%, 97%) on the confirmatory column. No 2,3,7,8-TCDD (< 0.1 ng/g) was found in the sample. The sample did contain 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 0.6 ng/g. The EPA LOQ is 0.5 ng/g. Sample 2955097 was also spiked with the target analytes at or below the EPA LOQ levels and was prepared and analyzed. All recoveries were acceptable (50% - 150%), except 2,3,7,8-TCDF on the primary column, -638%. Recovery was acceptable on the confirmatory column (104%).

With the reanalysis of sample 2955097, seven lots have now been successfully analyzed for 2,3,7,8-TCDD. The result for 2,3,7,8-TCDD in 2,4-D IOE (< 0.1 ng/g) is considered valid. It is noted that all seven samples were reprepared and reanalyzed.

Deficiency No. 3:

Validation of the initial response factors and/or calibration curves (11/08/89) must be performed on each day of samples analyses (11/09, 11/10, 11/13/89). No daily calibration data were submitted.

Nufarm USA, Inc. Submission:

The 2,4-D IOE samples were reanalyzed on 09/12 and 09/13/90. The 2,4-D samples were reanalyzed on 09/13, 09/14, 09/15, and 09/17/90. Initial five-point calibration curves were established on 09/12/90. The calibrations were verified on 09/13, 09/14, 09/15, and 09/17/90 by analysis of the lowest concentration standard. The initial and continuing response factor data (09/13 and 09/17) are summarized in Table 4.

Table 4: Calibration Data (DB-5).

Analyte or Internal Standard	Calibration Range (ng/g)	Mean RRF[1] (09/13)	% RSD	% Difference (09/13)	% Difference (09/17)
$^{13}C_{12}$-2,3,7,8-TCDF	1.0	0.75	13.5	1	-3
2,3,7,8-TCDF	1.0 - 10.	1.1	11.2	-5	-11
$^{13}C_{12}$-2,3,7,8-TCDD	0.5	0.98	2.90	0	2
2,3,7,8-TCDD	0.1 - 1.0	1.1	1.88	-21	22
$^{13}C_{12}$-1,2,3,7,8-PCDF	5.0	0.59	8.12	44	24
1,2,3,7,8-PCDF	5.0 - 50	1.1	5.15	22	10
$^{13}C_{12}$-2,3,4,7,8-PCDF	5.0	0.65	10.6	40	29
2,3,4,7,8-PCDF	5 - 50	1.0	2.08	15	2
$^{13}C_{12}$-1,2,3,7,8-PCDD	0.5	0.44	11.0	25	-14
1,2,3,7,8-PCDD	0.5 - 2.5	1.1	3.72	5	14
$^{12}C_{12}$-1,2,3,4,7,8-HxCDF	5.0	1.3	4.66	2	-1
1,2,3,4,7,8-HxCDF	5.0 - 50.	1.00	4.30	-1	1
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	5.0	1.4	7.68	10	8
1,2,3,6,7,8-HxCDF	5.0 - 25	1.1	2.08	-6	-9

Table 4: Calibration Data (DB-5).

Analyte or Internal Standard	Calibration Range (ng/g)	Mean RRF[1] (09/13)	% RSD	% Difference (09/13)	% Difference (09/17)
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	5.0	1.3	5.05	2	0
2,3,4,6,7,8-HxCDF	5.0 - 25	0.90	2.53	-2	-1
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	5.0	0.87	4.25	1	-1
1,2,3,7,8,9-HxCDF	5.0 - 50	1.0	3.49	1	-2
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	2.5	0.64	4.46	6	-12
1,2,3,4,7,8-HxCDD	2.5 - 25	1.1	4.26	-8	6
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	2.5	0.73	12.6	20	-10
1,2,3,6,7,8-HxCDD	2.5 - 25	1.00	2.49	0	15
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	2.5	0.67	6.26	10	-6
1,2,3,7,8,9-HxCDD	2.5 - 25	1.0	3.15	-2	4
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	5.0	0.63	4.47	5	6
1,2,3,4,6,7,8-HpCDF	5.0 - 25	1.4	3.40	-2	0
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	5.0	0.46	5.75	0	2
1,2,3,4,7,8,9-HpCDF	5.0 - 25	1.5	3.64	-6	-5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	5.0	0.46	5.96	6	-2
1,2,3,4,6,7,8-HpCDD	5.0 - 25	1.2	0.775	-5	0

[1] Analyte RRF's are relative to the labeled analog (located immediately before in the table). $^{13}C_{12}$-labeled analog RRF's are relative to the recovery standards 1,2,3,4-$^{13}C_{12}$-TCDD (0.5 ppb) or 1,2,3,4,6,7,8-$^{13}C_6$-HpCDF (2.5 ppb). Calibrations were also made for 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 1,2,3,7,8-PCDD on the DB-225 column.

The per cent relative standard deviation for all initial calibration response factors was < 15%. The DCI and the Guidelines for the Determination of Halogenate Dibenzo-p-Dioxins and Dibenzofurans in Commercial Products (EPA-560/5-87/007) do not specify initial calibration criteria. EPA Method 1613 specifies a coefficient of variation of < 20% for the relative response over a five-point calibration range. The present calibrations meet/exceed this criterion. The Guidelines specify a maximum difference of 30% for the continuing response factor and the initial mean relative response factor. All analytes meet this requirement, but two of the internal standards fail ($^{13}C_{12}$-1,2,3,7,8-PCDF and $^{13}C_{12}$-2,3,4,7,8-PCDF) on 09/13/90.

The registrant has demonstrated an acceptable initial calibration for each target analyte and successful continuing calibrations for each analyte on each day of sample analyses. Acceptable initial

calibration and continuing calibration are also presented for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, and 2,3,7,8-TCDF on the DB-225 confirmatory column.

This deficiency is resolved.

Deficiency No. 4:

Chromatograms for 2,4-D and for 2,4-D IOE indicated a peak about 2 seconds outside the 2,3,7,8-TCDF retention time window with the correct ion ratio for TCDF. Because the amount appears substantial (50 - 300 ppb), the compound must be identified and quantitated.

Nufarm USA, Inc. submission:

All samples were reanalyzed, and the unknown peak corresponded to 2,3,7,8-TCDF on the primary column (DB-5). The peak had the same (± 1 sec.) retention time as the labeled analog, and no other peak with the correct ion mass ratios was found in the retention time region (± 20 seconds). Based on the primary column only, 2,3,7,8-TCDF concentration ranged from 25 to 56 ng/g in 2,4-D IOE and from 38 to 50 ng/g in 2,4-D. Analysis of the samples on the DB-225 secondary column revealed that the peak in question was not 2,3,7,8-TCDF (< 0.22 ng/g). $^{13}C_{12}$-2,3,7,8-TCDF eluted at 21:33; the peak in question eluted at 21:59. The unknown was not further delineated.

The registrant has shown that the unknown is not a target analyte and has, therefore, met the DCI requirements. The issue is resolved.

Deficiency No. 5:

An acceptable recovery was not achieved for 2,3,7,8-TCDD spiked at 0.1 ng/g in four different 2,4-D 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.

Nufarm USA, Inc. submission:

Acceptable recovery (50% - 150%) was demonstrated for 2,3,7,8-TCDD in three samples of 2,4-D acid and in three samples of 2,4-D IOE, based on data from the confirmatory column (DB-225). Results are summarized in Table 5.

Table 5: Recovery of 2,3,7,8-TCDD (0.1 ng/g) from 2,4-D and 2,4-D IOE			
Sample	Concentration Found in Unspiked Sample[1] (ng/g)	Concentration Found in Spiked Sample[1] (ng/g)	Recovery[1] (%)
2955095 2,4-D IOE	0.10; 0.04	0.15; 0.14	50; 100
2955097 2,4-D IOE	0.11; 0.04	0.17; 0.12	60; 85
2955100 2,4-D IOE	0.11; 0.03	0.11; 0.12	0[2]; 90
2953088 2,4-D	0.09; 0.08	0.12; 0.17	30[2]; 90
2953090 2,4-D	0.08; 0.03	0.21; 0.12	135; 90
2953093 2,4-D	0.15; 0.12	0.14; 0.20	-10[2]; 85
[1] First entry is primary column. Second entry is confirmatory column. [2] Recovery unacceptable.			

This deficiency is resolved.

Deficiency No. 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 initial calibration standard runs (average) as a control, 18 of 36 analyses failed. The following analyses, where one or both recovery standards failed in both replicates, must be repeated: 2955087; 2955094; 2955095; 2955097; 2955100.

Nufarm USA, Inc. submission:

All samples (lots) were reanalyzed for both 2,4-D and 2,4-D IOE. The lots in question passed the performance criteria, except sample 2955087 (2,4-D), where $^{13}C_{12}$-1,2,3,4-TCDD failed in both replicates. Results are summarized in Table 6.

Table 6: Recovery Standard Response Variation

Standards 09/12/90

$^{13}C_6$-1,2,3,4,6,7,8-HpCDF Response (12.5 ng)	$^{13}C_{12}$-1,2,3,4-TCDD Response (2.50 ng)
13,036,000	1,324,600
11,380,000	876,300
12,018,000	804,900
9,755,000	759,300
10,672,000	729,700
Mean ± 1 s.d.: 11,372,000 ± 1,254,000 (11%)	899,000 ± 244,300 (27%)
Limits (-50% to +100%): 5,686,000 - 22,744,000	449,500 - 1,798,0000

Sample	Date Analyzed	$^{13}C_6$-1,2,3,4,6,7,8-HpCDF Response	$^{13}C_{12}$-1,2,3,4-TCDD Response
2955087	09/13/90	16,373,000 18,455,000	2,195,700FAIL 2,387,300FAIL
2955094	09/12/90	14,388,000 7,733,000	1,439,000 675,400
2955095	09/12/90	8,192,000 15,098,000	624,500 1,644,000
2955097	09/13/90	13,083,000 13,034,000	1,645,000 1,686,000
2955100	09/13/90	15,591,000 16,485,000	1,456,000 1,468,000

The registrant has demonstrated adequate stability of the recovery standards in five of the samples for the $^{13}C_{12}$-HpCDF and in four of five samples for the $^{13}C_{12}$-TCDD. The deficiency is resolved.

Deficiency No. 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.

Nufarm USA, Inc. submission:

The registrant reextracted and reanalyzed all samples in duplicate and submitted both calculation sheets and chromatograms for all samples. Potential identifications, such as 2,3,7,8-TCDD, were confirmed on a DB-225 column. The registrant now reports the presence of 2,3,7,8-TCDD in all samples, but at levels below the 0.1 ng/g LOQ. For 2,4-D acid, quantitative agreement between the

two columns is generally good (± 20%). The ion ratio is within 20% on the DB-225 column, but not on the DB-5 column. An overlapping peak occurs on the DB-5, and this no doubt adversely effects the ion mass ratio. Registrant calculations were checked and confirmed. For the 2,4-D acid samples, concentrations ranged from 0.02 ng/g to 0.12 ng/g. For the 2,4-D IOE samples, concentrations ranged from 0.03 to 0.04 ppb on the DB-225 column, and ion ratios were within the 20% of theoretical acceptance range on both columns. However, there was poor quantitative agreement between the two columns. Values ranged from 0.04 to 0.15 ng/g on the DB-5 column.

The registrant has successfully identified the "no peak" response as 2,3,7,8-TCDD. The deficiency is resolved.

Deficiency No. 8:

Submit a detailed outline of standards and spiking solution preparations and analyses (dilution ,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.

Nufarm USA, Inc. submission:

None of the issues were addressed directly. Analysis Method 50288 (revision of previous Method 40288) and the reanalysis package for all samples were submitted. The revised method clearly explains the procedure for standard preparation. The recovery standard $^{13}C_6$-1,2,3,4,6,7,8-HpCDF concentration is 12.5 ng/ml, or 2.5 ng/g based on the addition of 1 ml to 5 g of sample. The "pg/5g" units on the calculation spreadsheets have been changed to "ng/g." The apparent discrepancy factor between standards and samples is real, but does not alter the validity of results. An isotope dilution calculation is used for the target analytes; analyte and standard undergo the same manipulations (concentrations, dilutions, etc.) in each sample. The standard calibration curves are used to obtain an average response factor only. Two minor errors are noted in Method 50288: 2,3,7,8-TCDF concentration in the spiking mix described on page 5 is 5.0 ng/ml, not 1.0 ng/ml; the "Q_{is}" in the numerator of the internal standard percent recovery equation on page 12 should be "Q_n."

The deficiency is resolved.

Deficiency No. 9:

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

12
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Nufarm USA, Inc. submission:

The analyses were repeated, new method blanks were prepared and analyzed. The internal standards and recovery standards were not added to the blanks. No spreadsheets (raw data) were provided, and no recoveries were presented. For both the 2,4-D acid method blank (analyzed 09/14/90) and the 2,4-D IOE method blank (analyzed 09/13/90), analyte peaks are present for 2,3,7,8-TCDF; 1,2,3,7,8-PCDD; 1,2,3,4,7,8-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 1,2,3,4,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF; and 1,2,3,4,6,7,8-HpCDD. From a comparison of the method blank peak heights to the peak heights of the internal standards in a sample extract it can be estimated that each analyte detected in the blank is less than 10% of the corresponding internal standard concentration and therefore substantially below the EPA LOQ.

This deficiency is resolved.

Deficiency No. 10:

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

Nufarm USA, Inc. submission:

Tune conditions (accelerating voltage, electron energy, trap current, source slit, collector slit, resolution, detector voltage, source temperature, and interface temperature) were submitted for the analytical period 09/12 - 09/21/90. Conditions show little or no variation over the period. Chromatograms supporting the claimed 10000 resolution were not supplied.

This deficiency is resolved.

Deficiency No. 11:

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

Nufarm USA, Inc. submission:

Method 50288 more clearly defines the terms of the equations than did method 40288. No example calculations were supplied. Random checks indicate that calculations are correct.

The deficiency is resolved.

Deficiency No. 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.

Method 50288 agrees with the analytical report. The recovery standard discrepancy has been removed. The deficiency is resolved.

Deficiency No. 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.

Nufarm USA, Inc. submission:

No CSF was submitted for either 2,4-D acid or 2,4-D IOE. A "Certificate of Analysis" was submitted for each sample. This deficiency is not resolved.

Conclusions

The following deficiencies cited in the 05/24/90 Memorandum have not been resolved and must be addressed by the registrant:

1. Sampling must be documented. Information must be supplied on the dates and times of sample collection, the total number of lots produced during the sampling period, and the handling (storage) of samples prior to workup. This information is needed to evaluate the randomness of the sample selection process, the adequacy of the population from which the samples were acquired, and the lack of sample deterioration/manipulation prior to analysis.

2. A Confidential Statement of Formula (CSF) must be submitted for technical 2,4-D Acid (61272-3) and for technical 2,4-D IOE (61272-1). The CSF's are to incorporate the results of the analyses reported for polyhalogenated dibenzo-p-dioxins and dibenzofurans. Only values greater than the respective EPA LOQ values need be listed.

All other deficiencies have been satisfied by the current submission. The outstanding items will not entail any additional laboratory analytical effort.

Based on the reanalyses presented, it may be concluded that fourteen of the fifteen 2,3,7,8-tetra- to 2,3,7,8-hepta-chlorinated dibenzo-p-dioxins and dibenzofurans are detected in technical 2,4-D Acid and in 2,4-D isooctyl ester. However, only

two of the compounds are present in 2,4-D Acid at or above the EPA LOQ limits, and only one of the compounds is present in 2,4-D IOE at or above the EPA LOQ limit, as follows:

2,4-D Acid

Compound	Maximum Concentration (ng/g)	LOQ (ng/g)
2,3,7,8-TCDD	0.12	0.1
1,2,3,7,8-PCDD	3.4	0.5

2,4-D IOE

Compound	Maximum Concentration (ng/g)	LOQ (ng/g)
1,2,3,7,8-PCDD	0.93	0.5

These values are in agreement with the results reported for the original analyses (05/24/90 Memorandum, S. Funk, DEB No. 6295). Outstanding deficiency no. 1 (above) may alter the validity or sufficiency of these results. Values lower than those found would not be anticipated.

Assuming the ratio of dioxin/dibenzofuran to active ingredient remains constant from the technical through the end-use product, through plant application, and through animal/human consumption of the treated rac's, the amount of contaminant expected on the various commodities can be calculated. The ratios of CDD's to 2,4-D and to 2,4-D IOE are as follows:

<u>2,4-D</u>

2,3,7,8-TCDD: 0.12×10^{-9} g / 0.98 g = 0.12×10^{-9}.
1,2,3,7,8-PCDD: 3.4×10^{-9} g / 0.98 g = 3.5×10^{-9}.

<u>2,4-D IOE</u>

1,2,3,7,8-PCDD: 0.93×10^{-9} g / 0.98 g = 0.95×10^{-9}.

These ratios may be applied to the established tolerances (40 CFR 180.142) to arrive at maximum anticipated TCDD and PCDD levels in the commodities. These values reflect worst case levels when the 2,4-D or the 2,4-D IOE is used according to label specifications. CBRS anticipates actual levels, if any, of TCDD and PCDD in commodities to be significantly lower. Anticipated TCDD and PCDD concentrations are summarized in Tables 7 and 8.

WRONG UNIT ppb NOT but ppm

Table 7: Anticipated TCDD and PCDD Residues From the Use of Nufarm 2,4-D

Commodity	Tolerance (ppb)	2,3,7,8-TCDD (ppt)[1]	1,2,3,7,8-PCDD (ppt)[2]
Eggs	0.05	0.6×10^{-6}	18×10^{-6}
Fruits	5.	60×10^{-6}	1800×10^{-6}
Grains	0.5	6×10^{-6}	180×10^{-6}
Kidney	2.	24×10^{-6}	700×10^{-6}
Meat	0.2	2.4×10^{-6}	70×10^{-6}
Milk	0.1	1.2×10^{-6}	35×10^{-6}
Nuts	0.2	2.4×10^{-6}	70×10^{-6}
Poultry	0.05	0.6×10^{-6}	18×10^{-6}
Sugarcane	2.	24×10^{-6}	700×10^{-6}

[1] Concentration TCDD (parts per trillion) = [Tolerance (ppb)] X [0.12×10^{-6} g TCDD / 1 g technical] X [100 g technical / 98 g a.i.] X [1000 ppt / 1 ppb].
[2] Concentration PCDD (parts per trillion) = [Tolerance (ppb)] X [3.5×10^{-6} g PCDD / 1 g technical] X [100 g technical / 98 g a.i.] X [1000 ppt/ 1 ppb].

Table 8: Anticipated PCDD Residues From the Use of Nufarm 2,4-D IOE

Commodity	Tolerance (ppb)	1,2,3,7,8-PCDD (ppt)[1]
Eggs	0.05	4.8×10^{-6}
Fruits	5.	480×10^{-6}
Grains	0.5	48×10^{-6}
Kidney	2.	190×10^{-6}
Meat	0.2	19×10^{-6}
Milk	0.1	9.5×10^{-6}
Nuts	0.2	19×10^{-6}
Poultry	0.05	4.8×10^{-6}
Sugarcane	2.	190×10^{-6}

[1] Concentration PCDD (parts per trillion) = [Tolerance (ppb)] X [0.93×10^{-6} g PCDD / 1 g technical] X [100 g technical / 98 g a.i.] X [1000 ppt/ 1 ppb].

Should be 10^{-5}.

J Rowland Tox, HED 4/27/92

CBRS defers to HED Toxicology Branch 1 as to the significance of the chlorinated dibenzo-p-dioxin and dibenzofuran contaminant levels anticipated in the rac's.

Recommendation

CBRS recommends that Nufarm USA, Inc. be requested to supply expeditiously the information noted in deficiency nos. 1 and 2 of the Conclusions.

CBRS defers to Toxicology Branch 1 to determine the significance of the levels of polyhalogenated dibenzo-p-dioxin and dibenzofuran found.

cc: RF, Dioxin SF, , 2,4-D Registration Standard File, S. Funk, C. Furlow/J. Burrell (PIB, FOD), K. Baetcke/A. Clevenger (Tox. Branch 1/HED), 2,4-D Subject File, P. Deschamp (Update File).

RDI: A. Rathman:11/25/91: D. Edwards:11/25/91: E. Zager:11/25/91:H7509C:CBRS:S.Funk:557-1430:CM#2:RM803-A:SF(DIOX.120):11/21/91.