AR226-1810

Title

Adipate (T-7211) Metabolite Identification in Rats

Final Report
Date: 2-24-04

3M Medical Department Study Number: T-7211.1
3M Strategic Toxicology Laboratory Protocol Study No. ST-37
3M Environmental Laboratory Report No.: FACT Tox-141

Sponsor: 3M Specialty Chemicals Division (PCDP)
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Summary
The objective was to provide liver and sera from rats dosed with the Adipate for method development and qualitative metabolite identification. A single five mg/kg dose was administered via oral gavage to two male and two female Sprague Dawley rats. One male and one female rat were euthanized on day 1 and day 4 post dose. The fluorochemicals quantified in liver were perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamido acetate (M556), N-methyl perfluorooctanesulfonamido acetate (M570), and MeFOSE-Epi-1-alcohol. The apparent cumulative amount of these monomers present in the liver exceeded the cumulative amount of these residual monomers present in the dose in both the liver and in the serum. For example, the percent of residuals that were present in the dose that were present in the liver ranged from 128% on day one post-dose to 825% on day 4 post dose in males. Thus these data suggest that the Adipate polymer was apparently metabolized to some extent by the rat following a single oral dose. However, this study was designed to provide qualitative data only, therefore, a definitive conclusion cannot be made based on these data.

***Test Material*: Name**: The "Adipate". (Lot Number: LB-I000-1546-8; L-15468, 118103-594 ("Adipate"); R-11483-9, Lot 3, Stripped. Originator: M. Burleigh , 2/16/98. 3M Environmental Lab: TN-A-2432. 3M Medical Department: T-7211). **Structure**: The adipate is synthesized by the addition of 2 moles epichlorohydrin (epi) to N-MeFOSE and subsequent esterification with adipic acid. The ideal structure of the product should be:
[C8F17SO2N-(CH3)-CH2-CH2-O-(CH2-CH-(CH2Cl)-O)2-COCH2CH2-]2
However other major N-MeFOSE-epi-adipate products exist consisting of at least three primary ester types. These structures are elucidated in an NMR Analysis of the Adipate report completed by Richard A. Newmark, 3M Corporate Analytical Tech Center (Request C147746, 4/5/99).
Purity: The overall purity of the Adipate as identified by NMR (Newmark R. M. Request C147746), and was reported to be:

Table 1: NMR analysis of the Adipate, L-15468, Lot 3, Stripped.

Component	mole %	wt%
All N-MeFOSE-epi-adipate products	89.5%	95.1%
MIBK	9.6%	4.4%
Toluene	0.050%	0.024%
Probable triethyl ammonium salt	0.8%	0.5%

Analytical characterization of this same lot of the FC-Adipate for residual FC alcohols was conducted by HPLC Analysis by Joel W. Miller of the 3M SMMD

Analytical (Request: 58175, 4/ 22/1999). These levels are based on the weight percent analyte in the sample as received (Table 2). .

Table II. Residual FC alcohol levels in the Adipate as determined by HPLC

Sample Prep	Wt % N-MeFOSE	Wt % N-MeFOSE-EPI
1	0.0445%	0.269%
2	0.0446%	0.267%
3	0.0438%	0.273%
Average±SD	0.0443 ±0.0004%	0.269%± 0.003%

As described in the method, the N-MeFOSE-EPI level is the total concentration of all the N-MeFOSE-EPI oligomers in the sample.

Further analysis was performed by LC/MS revealed that there was 0.0029% perfluorooctanesulfonate (PFOS) in this sample of Adipate (SMMD Analytical Report 58175, In FACT Tox 141).

Procedures:

Dose and Dosing Procedures:
A single dose of adipate, five mg/kg body weight, was administered via oral gavage to two male and two female Sprague Dawley rats from Harlan Laboratories, Inc., 6-8 weeks old, weighing approximately 310 - 480 g. The dose was be prepared by forming a 5 mg/ml solution of the Adipate in acetone. The adipate/acetone solution was then be suspended in corn oil to a final concentration of 1 mg Adipate per ml of 20% acetone/80% corn oil. A volume of 5 ml/kg of the suspension was be administered to all rats. One male and one female rat were euthanized on day 1 and day 4 post dose according to the schedule in Table 3.

Table 3 Schedule

Group	Dose	N	Sacrifice Day
1	5 mg/kg Adipate	2 (1M, 1F)	Day 1 post dose
2	5 mg/kg Adipate	2 (1M, 1F)	Day 4 post dose

Sample Collection:
During necropsy, systemic blood (approximately 6 ml) was collected via heart puncture and transferred to blood collection tubes without anticoagulant. All blood samples were allowed to clot for a period of 15 to 30 minutes at room temperature, then centrifuged at 1100 x g for 5 minutes. The serum was transferred to labeled 1.5 ml microfuge tubes and centrifuged again at 2000 x g to remove any remaining red blood cells. The serum was transferred to labeled polypropylene microfuge tubes and frozen on dry ice. Livers were removed,

placed separately into labeled tubes and frozen on dry ice. Samples (liver and serum) were stored in a freezer set to maintain -60 to -80°C then packed in dry ice and shipped to: Kris Hansen, 3M Environmental Technology and Safety Services
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Analysis:
Liver and sera samples were analyzed by the 3M Environmental for the parent compound, methyl FOSE and metabolites all N-MeFOSE-epi-adipate products. An aliquot of the dosing solution of 1 mg/ml adipate suspended in acetone/corn oil (20:80) is included for analytical verification of dosing material (Amended report FACT Tox-141).

Results and Discussion

Body weight, liver weight and dose amount are shown in Table 4. No effects of treatment were noted during the in-life phase of the study or by gross observation of internal organs at necropsy. The residual fractions (Wt/Wt) and amount (μg) of PFOS, N-MeFOSE, N-MeFOSE Epi-1 Alcohol and the Total residuals in dose are shown in Table 5.

The analytical results for fluorochemical concentrations in the liver are shown in Table 6, and the fluorochemical concentrations in the serum are shown in Table 7. The fluorochemicals quantified in liver were perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamido acetate ($C_8F_{17}S0_2N(H)CH_2CH_2OH$; M556), N-methyl perfluorooctanesulfonamido acetate ($C_8F_{17}S0_2N(CH_3)CH_2CH_2OH$; M570), and MeFOSE-Epi-1-alcohol ($C_8F_{17}S0_2N(CH_3)CH_2CH_2O(CH_2C(H)(CH_2Cl)O)H$; Epi-1-OH). The cumulative amount of these monomers present in the liver exceeded the cumulative amount of these residual monomers present in the dose in both the liver and in the serum. For example, the percent of residuals that were present in the dose that were present in the liver ranged from 128% to 825%. These analyses support the conclusion stated in the 3M Environmental Lab report (FACT TOX 141) that " the levels of (MeFOSE-OH metabolites) detected (in the liver) greatly exceeded theoretical levels predicted based on MeFOSE-OH residuals in the dose material" . However, one should use caution not to over interpret the quantitative aspect of this study as it was not designed as a definitive study to quantify the metabolites of Adipate. Thus, these data suggest that the Adipate polymer was apparently metabolized to some extent by the rat following a single oral dose. However, a definitive conclusion can not be made based on these data alone.

Signatures:

Andrew M. Seacat 2/24/04

Andrew M. Seacat — Date
Toxicology Specialist
Study Director

References

Altman PL and Dittmer DS, Blood and Other Body Fluids. Bethesda , Maryland. Federation of the American Societies for experimental Biology (FASEB), 1971, p5

Hansen K. Amended Analytical Lab Report. Data for metabolite identification of L-15468 Adipate (Reference number 118103-59A) in rat liver and sera samples – A pilot study. FACT TOX-141, LRN-W2430, 12/20/00

Tables

Table 4 Biological Parameters

ST-37; T-7211.1 Adipate Pilot Study in Rats - BW & Liver Wt Data								
Dose	**animal # 8R03-xxx**	**sex**	**necropsy date**	**Day 0 Body wt (g)**	**Adipate Dose (mg)**	**terminal Body wt (g)**	**serum volume/rat (ml)[1]**	**liver wt (g)**
5mg/kg adipate	823	male	dy 1 post dose	478	2.39	453	14.6	15.8
5mg/kg adipate	872	female	dy 1 post dose	315	1.57	313	10.1	8.7
5mg/kg adipate	824	male	dy 4 post dose	463	2.31	449	14.5	17.6
5mg/kg adipate	871	female	dy 4 post dose	311	1.55	314	10.1	10.4
1. There are 32.3 mL Plasma per Kg of rat.(Alman and Dittmer, Blood and Other Body Fluids. FASEB, 1971, p5)								

Table 5 Fluorochemicals in the Dose

ST-37; T-7211.1 Adipate Dose data							
Dose	**animal # 8R03-xxx**	**Adipate Dose (mg)**	**Residual fraction PFOS in dose (wt/wt)**	fraction of residual N-MeFOSE in dose (wt/wt)[1]	Fraction N-MeFOSE Epi-1 Alcohol residual in dose (wt/wt)	Total residual Fraction in dose (wt/wt)	Amount of residual (MeFOSE + Epi-1-OH) in dose (ug)
5mg/kg adipate	823	2.39	0.00003	0.00044	0.00269	0.00316	7.56
5mg/kg adipate	872	1.575	0.00003	0.00044	0.00269	0.00316	4.98
5mg/kg adipate	824	2.315	0.00003	0.00044	0.00269	0.00316	7.32
5mg/kg adipate	871	1.555	0.00003	0.00044	0.00269	0.00316	4.92
1) from Miller J. W. SMMD Analytical report Request # 5`75 , April 22, 1999 HPLC-UV analysis of FC- Adipate for residual FC alcohols							

Table 6: Liver Fluorochemical Concentration Amount and Percent of Dose.

ST-37; T-7211.1 Adipate Pilot Study in Rats - BW & Liver Wt Data															
Dose of Adipate (mg/Kg)	**animal # 8R03-xxx**	**Sex**	**Necropsy day**	PFOS in liver (ppm) (1)	PFOSA in liver (ppm) (1)	M556 in liver (ppm) (1)	M570 in liver (ppm) (1)	Epi-1-OH in liver (ppm) (1)	Total PFOS in liver (ug)	Total PFOSA in liver (ug)	Total M556 in liver (ug)	Total M570 in liver (ug)	Total Epi-1-OH in liver (ug)	Total residual in liver (ug)	Percent of residual dosed in liver (2)
5	**823**	**M**	**d1**	0.19	0.11	0.00	0.31	0.00	3.02	1.75	0.00	4.90	0.00	9.67	128%
5	**872**	**F**	**d1**	0.27	0.17	0.26	0.64	0.00	2.37	1.49	2.23	5.55	0.00	11.63	234%
5	**824**	**M**	**d4**	1.55	0.26	0.36	1.27	0.00	27.28	4.51	6.28	22.35	0.00	60.42	825%
5	**871**	**F**	**d4**	0.80	0.25	0.30	0.84	0.00	8.33	2.61	3.14	8.77	0.00	22.85	465%
1.) Analytical Laboratory Report on Data for Metabolite Identification of L-15468 Adipate. (3M Medical Department Number: T-7211.1; 3M Environmental Lab Number: FACT TOX 141).															
2.)Percent of residual dosed in liver = (Total PFOS residual in liver (ug))/(Amount of residual (MeFOSE + Epi-1-OH) in dose (ug))*100															

Table 6: Serum Fluorochemical Concentration Amount and Percent of Dose.

ST-37; T-7211.1 Adipate Pilot Study - Serum data															
Dose Adipate (mg/Kg)	**animal # 8R03-xxx**	**sex**	PFOS in serum (ppm) (1)	PFOSA in serum (ppm) (1)	M556 in serum (ppm) (1)	M570 in serum (ppm) (1)	Epi-1-OH in serum (ppm) (1)	Total PFOS in serum (ug)(2)	Total PFOSA in serum (ug)(2)	Total M556 in serum (ug)(2)	Total M570 in serum (ug)(2)	Total Epi-1-OH in serum (ug)(2)	Total metabolite in serum (ug) (3)	Percent of residual dosed in serum (4)	% of total dose in serum (5)
5	823	M	0.011	0.006	0.056	0.221	0.018	0.16	0.09	0.83	3.23	0.26	4.58	61%	0.19
5	872	F	0.105	0.021	0.214	0.574	0.040	1.06	0.22	2.16	5.80	0.40	9.65	194%	0.61
5	824	M	0.093	0.010	0.073	0.560	0.010	1.34	0.14	1.06	8.12	0.15	10.81	148%	0.47
5	871	F	0.393	0.026	0.251	0.665	0.000	3.99	0.27	2.55	6.74	0.00	13.54	275%	0.87
Note: There are 32.3 mL Plasma per Kg of rat.(Alman and Dittmer, Blood and Other Body Fluids. FASEB, 1971, p5)															
1.) Analytical Laboratory Report on Data for Metabolite Identification of L-15468 Adipate. (3M Medical Department Number: T-7211.1; 3M Environmental Lab Number: FACT TOX 141).															
2) PFOS in serm (ug) = Serum volume (ml) x(PFOS in serum (ppm)															
3.) Total residual in serum (ug) = sum of residuals serum (ppm) x serum volume (ml)															
4) Fraction of residual dosed in serum = (Total PFOS residual in serum (ug)) x (Amount of residual (MeFOSE + Epi-1-OH) in dose (ug))															
5)% of total dose in serum = (Total PFOS residual in serum (ug)/ residual in dose (ug))*100															