

STUDY TITLE

Determination of Residues of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP), and Dimethyl Dithiophosphate (DMDTP) in Human Urine

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PTL PROJECT ID

PTL119801

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B) or (C).

Company: Cheminova Agro A/S

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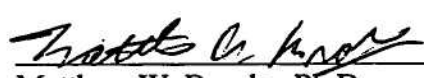
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the U.S. EPA Good Laboratory Practice Standards, (40 CFR 160).

 10/11/00

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PTL QUALITY ASSURANCE STATEMENT

PTL Quality Assurance Unit reviewed Study PTL119801, "Determination of Residues of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP), and Dimethyl Dithiophosphate (DMDTP) in Human Urine," for Cheminova Agro A/S, Lemvig, Denmark. The following inspections/audits were conducted on this study.

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PROJECT PERSONNEL

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Roselyn Billones	Medical Technologist
Adriana Martinez	Laboratory Technician
Michael Aragon	Head Accessioner

STORAGE LOCATION OF RAW DATA

Facility records are archived permanently at Pacific Toxicology Laboratories.

A copy of the final report and all raw data will be retained at Pacific Toxicology Laboratories, Woodland Hills, California.

The sponsor's copy of the final study report and all original supporting raw data will be archived at EPL Archives, Inc., 22900 Shaw Road, Unit 130, Sterling, Virginia 20166 at study completion.

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1.0 SUMMARY

Pacific Toxicology Laboratories (PTL) analyzed human urine samples to evaluate the magnitude of five metabolites of Malathion [Diethyl (dimethoxythiophosphorylthio)succinate]: malathion dicarboxylic acid (DCA) [Butanedioic acid, [(dimethoxyphosphinothioyl) thio-]]; malathion monocarboxylic acid (MCA) [Butanedioic acid, [(dimethoxyphosphinothioyl) thio]-, monoethyl ester]; dimethyl phosphate (DMP) [phosphoric acid, dimethyl ester]; dimethyl thiophosphate (DMTP) [phosphorothioic acid, O,O-dimethyl ester]; and dimethyl dithiophosphate (DMDTP) [phosphorodithioic acid, O,O-dimethyl ester]. Urine samples were received frozen from Inveresk Research, Tranent, Scotland, where the clinical portion of the study was conducted (ICR Study No: 013177). The samples were from groups of volunteers who were dosed with malathion at levels 0.5, 1.5, 5.0, 10.0, or 15.0 mg/kg as single oral doses. Additional subjects receiving placebos were also included in the study. Urine samples were collected from the volunteers from 12 hours before dosing to 48 hours after dosing at -12-0, 0-12, 12-24, and 24-48 hour intervals. The analyses of all human urine samples for malathion metabolites were conducted at Pacific Toxicology Laboratories (PTL) in Woodland Hills, California. Samples were analyzed for the five metabolites mentioned above, using validated analytical methods described herein.

Aliquots of urine samples were fortified with internal standards diazinon (for DCA and MCA analysis) and fenthion (for DMP, DMTP, and DMDTP analyses). For analysis of the carboxylic acid metabolites, urine samples were cleaned by passing through solid-phase columns, washed with acidified water, and eluted with ethyl acetate. The eluants were derivatized with diazomethane, concentrated, and analyzed by a gas chromatograph equipped with a flame photometric detector (FPD). For determination of phosphate metabolites, urine samples were freeze-dried, reconstituted in acetone and derivatized with benzyl-p-tolyltriazine. Samples were then partitioned into cyclohexane and analyzed by gas chromatography utilizing FPD detection.

The analyses indicated that the majority of malathion metabolites were excreted in urine within 12 hours of dosing, and essentially total metabolite clearance occurred within 24 hours of dosing. Although malathion monocarboxylic acid (MCA) constituted the primary urinary metabolite, total amounts of all metabolites were proportional to the dose levels. The results of the analysis are summarized in the following tables.

Summary of Malathion Dicarboxylic Acid, Malathion Monocarboxylic Acid, Dimethylphosphate, Dimethylthiophosphate, and Dimethyldithiophosphate Metabolites in Human Urine Samples

Sample Description ^a	DCA ^b Metabolite (ppm) ^c	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
SUBJ001,0.5,-12-0	N.D. ^d	N.D.	N.Q. ^e	N.D.	N.D.
SUBJ001,0.5,0-12	2.615	6.544	0.764	0.431	N.D.
SUBJ001,0.5,12-24	1.636	0.099	0.203	0.209	0.052
SUBJ001,0.5,24-48	N.Q.	N.D.	N.Q.	N.D.	N.D.
SUBJ002,0.5,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
SUBJ002,0.5,0-12	1.106	6.483	0.813	1.596	0.258
SUBJ002,0.5,12-24	0.859	0.070	0.089	0.113	0.053
SUBJ002,0.5,24-48	N.Q.	N.D.	N.Q.	N.D.	N.D.
SUBJ003,PL,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
SUBJ003,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ003,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ003,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ004,0.5,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
SUBJ004,0.5,0-12	2.779	9.314	1.458	3.548	0.721
SUBJ004,0.5,12-24	0.022	N.D.	0.190	0.196	0.039
SUBJ004,0.5,24-48	0.892	0.089	0.177	0.202	0.040
SUBJ005,1.5,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ005,1.5,0-12	2.314	28.753	7.086	15.670	N.Q.
SUBJ005,1.5,12-24	4.321	0.376	1.330	1.657	0.314
SUBJ005,1.5,24-48	0.112	N.Q.	0.065	0.044	N.D.
SUBJ006,1.5,-12-0	N.D.	N.Q.	N.D.	N.D.	N.D.
SUBJ006,1.5,0-12	5.873	42.430	7.340	15.070	0.886
SUBJ006,1.5,12-24	7.939	1.495	1.697	2.062	0.290
SUBJ006,1.5,24-48	0.291	0.027	0.109	0.075	N.D.
SUBJ007,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ007,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ007,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ007,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.

- a The sample description includes the following components:
Example: SUBJ005,1.5,0-12 SUBJ005 is the study subject's identification number supplied by Inveresk Corp. 1.5 is the subject's dosing level in mg/kg (i.e. 1.5 mg/kg). [Placebo indicated as PL.] 0-12 is the sample interval in hours: -12-0 hrs; 0-12 hrs; 12-24 hrs; 24-48 hrs.
- b DCA = Malathion Dicarboxylic Acid; MCA = Malathion Monocarboxylic Acid; DMP = Dimethylphosphate; DMTP = Dimethylthiophosphate; DMDTP = Dimethyldithiophosphate
ppm = parts per million
- d ND = Not Detected. Limit of detection of DCA and MCA is 0.002 ppm. Limit of detection of DMP, DMTP, and DMDTP is 0.0125 ppm.
- e NQ = Not Quantitated. Limit of quantitation of DCA and MCA is 0.020 ppm. Limit of quantitation for DMP, DMTP, and DMDTP is 0.025 ppm.

Summary of Malathion Dicarboxylic Acid, Malathion Monocarboxylic Acid, Dimethylphosphate, Dimethylthiophosphate, and Dimethyldithiophosphate Metabolites in Human Urine Samples (Continued)

Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
SUBJ008,1.5,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ008,1.5,0-12	3.356	30.703	6.108	11.434	0.730
SUBJ008,1.5,12-24	0.917	0.021	0.258	0.255	0.883
SUBJ008,1.5,24-48	0.061	N.D.	0.082	0.058	N.D.
SUBJ009,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ009,5.0,0-12	7.010	0.477	3.608	4.625	0.828
SUBJ009,5.0,12-24	4.678	7.235	24.047	33.812	14.755
SUBJ009,5.0,24-48	0.228	0.177	0.279	0.190	0.045
SUBJ010,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ010,5.0,0-12	4.856	21.959	0.996	0.910	0.324
SUBJ010,5.0,12-24	3.395	1.072	0.612	1.484	0.352
SUBJ010,5.0,24-48	0.196	0.069	0.318	0.264	0.118
SUBJ011,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ011,5.0,0-12	34.277	3.320	12.003	18.095	5.274
SUBJ011,5.0,12-24	7.525	1.738	2.137	2.656	0.899
SUBJ011,5.0,24-48	0.648	1.045	0.342	0.494	0.113
SUBJ012,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ012,5.0,0-12	43.575	152.852	14.726	46.794	9.449
SUBJ012,5.0,12-24	23.909	2.495	4.013	7.625	3.106
SUBJ012,5.0,24-48	0.792	0.294	0.346	0.369	0.080
SUBJ013,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ013,PL,0-12	0.440	0.553	0.051	0.142	0.055
SUBJ013,PL,12-24	N.D.	N.D.	N.D.	0.033	N.D.
SUBJ013,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ014,5.0,-12-0	N.D.	N.Q.	N.D.	N.D.	N.D.
SUBJ014,5.0,0-12	33.443	77.797	16.960	41.420	4.269
SUBJ014,5.0,12-24	8.854	4.439	2.509	2.120	1.295
SUBJ014,5.0,24-48	0.218	0.042	0.178	0.185	0.037
SUBJ015,PL,-12-0	N.D.	N.D.	0.035	0.041	N.D.
SUBJ015,PL,0-12	0.579	0.475	0.081	0.159	0.031
SUBJ015,PL,12-24	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ015,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ016,PL,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ016,PL,0-12	N.Q.	N.Q.	N.D.	N.D.	N.D.
SUBJ016,PL,12-24	N.D.	N.D.	N.D.	0.027	N.D.
SUBJ016,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ917,5.0,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ917,5.0,0-12	15.064	73.484	7.036	21.706	3.270
SUBJ917,5.0,12-24	0.164	0.021	0.208	0.160	0.026
SUBJ917,5.0,24-48	0.182	1.735	2.263	2.526	0.352
SUBJ018,5.0,-12-0	0.028	N.Q.	N.Q.	N.Q.	N.D.
SUBJ018,5.0,0-12	8.529	32.391	7.790	14.797	0.928
SUBJ018,5.0,12-24	12.065	2.362	2.433	3.427	0.506
SUBJ018,5.0,24-48	0.631	0.108	0.229	0.294	0.067

Summary of Malathion Dicarboxylic Acid, Malathion Monocarboxylic Acid, Dimethylphosphate, Dimethylthiophosphate, and Dimethyldithiophosphate Metabolites in Human Urine Samples (Continued)

Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
SUBJ019,10.0,-12-0	N.D.	N.D.	N.Q.	N.D.	0.196
SUBJ019,10.0,0-12	69.601	274.951	69.257	123.417	7.683
SUBJ019,10.0,12-24	11.611	1.367	3.224	4.747	0.587
SUBJ019,10.0,24-48	2.158	0.303	1.400	0.832	0.998
SUBJ020,10.0,-12-0	N.D.	N.D.	N.Q.	N.Q.	N.D.
SUBJ020,10.0,0-12	24.220	224.275	74.790	47.755	4.044
SUBJ020,10.0,12-24	17.343	7.452	5.600	9.338	0.588
SUBJ020,10.0,24-48	0.085	0.110	0.965	1.248	0.097
SUBJ021,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ021,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ021,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ021,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ022,10.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ022,10.0,0-12	19.015	309.165	44.503	49.060	2.749
SUBJ022,10.0,12-24	7.895	10.620	3.758	7.281	1.604
SUBJ022,10.0,24-48	1.565	0.637	0.487	0.409	0.054
SUBJ023,10.0,-12-0	0.108	0.076	0.032	0.029	N.D.
SUBJ023,10.0,0-12	24.915	303.562	31.203	14.000	3.605
SUBJ023,10.0,12-24	12.566	4.036	3.422	5.562	0.958
SUBJ023,10.0,24-48	0.345	0.081	0.448	0.183	0.156
SUBJ024,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ024,PL,0-12	0.090	0.120	N.D.	N.D.	N.D.
SUBJ024,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ024,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ025,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ025,PL,0-12	N.D.	N.D.	N.Q.	N.D.	N.D.
SUBJ025,PL,12-24	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ025,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ026,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ026,15.0,0-12	32.457	296.546	115.085	53.172	9.321
SUBJ026,15.0,12-24	5.253	7.605	9.377	12.000	1.093
SUBJ026,15.0,24-48	0.180	0.137	0.386	0.249	0.057
SUBJ027,10.0,-12-0	N.D.	N.D.	0.347	N.D.	0.295
SUBJ027,10.0,0-12	8.811	168.587	30.431	62.508	2.745
SUBJ027,10.0,12-24	28.250	16.117	4.431	14.022	2.045
SUBJ027,10.0,24-48	3.977	2.519	0.538	3.535	0.715
SUBJ028,10.0,-12-0	N.D.	N.D.	0.275	0.053	0.095
SUBJ028,10.0,0-12	8.766	58.679	37.532	45.460	3.182
SUBJ028,10.0,12-24	2.424	2.887	3.672	5.629	0.677
SUBJ028,10.0,24-48	0.403	0.479	0.416	0.835	0.159
SUBJ029,15.0,-12-0	N.D.	N.D.	0.325	N.D.	N.D.
SUBJ029,15.0,0-12	15.152	264.142	57.861	164.924	9.322
SUBJ029,15.0,12-24	7.558	3.357	3.802	8.489	0.796
SUBJ029,15.0,24-48	1.074	0.197	0.734	0.785	0.082

Summary of Malathion Dicarboxylic Acid, Malathion Monocarboxylic Acid, Dimethylphosphate, Dimethylthiophosphate, and Dimethyldithiophosphate Metabolites in Human Urine Samples (Continued)

Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
SUBJ030,10.0,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ030,10.0,0-12	12.476	100.518	39.184	47.183	2.184
SUBJ030,10.0,12-24	4.190	2.262	2.762	3.979	0.385
SUBJ030,10.0,24-48	0.212	0.139	0.142	0.170	0.032
SUBJ031,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ031,15.0,0-12	22.973	242.167	43.293	71.286	5.628
SUBJ031,15.0,12-24	19.168	8.887	3.159	4.804	1.505
SUBJ031,15.0,24-48	0.897	0.329	0.910	1.022	0.308
SUBJ032,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ032,PL,0-12	N.Q.	N.Q.	N.D.	N.D.	N.D.
SUBJ032,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ032,PL,24-48	N.Q.	N.D.	N.D.	N.D.	N.D.
SUBJ033,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ033,15.0,0-12	56.141	492.237	43.642	136.535	8.172
SUBJ033,15.0,12-24	39.573	13.671	5.330	10.013	1.866
SUBJ033,15.0,24-48	2.803	0.890	0.800	1.152	0.358
SUBJ034,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ034,15.0,0-12	244.283	56.226	51.377	43.819	1.632
SUBJ034,15.0,12-24	38.638	43.932	6.235	12.190	1.339
SUBJ034,15.0,24-48	0.746	0.559	0.526	0.561	0.069
SUBJ035,PL,-12-0	N.Q.	N.D.	N.D.	N.D.	N.D.
SUBJ035,PL,0-12	0.022	0.026	N.D.	N.D.	N.D.
SUBJ035,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ035,PL,24-48	N.Q.	N.D.	N.D.	N.D.	N.D.
SUBJ036,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ036,PL,0-12	N.Q.	N.Q.	N.D.	N.D.	N.D.
SUBJ036,PL,12-24	N.D.	N.Q.	N.D.	N.D.	N.D.
SUBJ036,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ037,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ037,15.0,0-12	62.040	896.963	197.150	424.825	11.828
SUBJ037,15.0,12-24	8.049	7.377	5.869	16.477	3.203
SUBJ037,15.0,24-48	0.405	0.192	0.609	0.645	0.241
SUBJ038,15.0,-12-0	N.D.	N.D.	0.034	N.Q.	N.D.
SUBJ038,15.0,0-12	102.042	860.760	279.044	85.063	1.844
SUBJ038,15.0,12-24	16.953	10.502	5.205	8.870	1.370
SUBJ038,15.0,24-48	0.683	0.581	0.362	0.403	0.076
SUBJ039,15.0,-12-0	N.D.	N.D.	0.036	0.028	N.Q.
SUBJ039,15.0,0-12	18.813	181.273	55.411	15.484	2.656
SUBJ039,15.0,12-24	1.321	0.629	2.430	2.952	0.312
SUBJ039,15.0,24-48	0.201	0.064	0.317	0.259	0.051
SUBJ040,15.0,-12-0	N.D.	N.D.	0.069	0.039	0.026
SUBJ040,15.0,0-12	16.909	155.970	18.162	67.650	22.921
SUBJ040,15.0,12-24	14.944	3.999	3.335	4.934	0.447
SUBJ040,15.0,24-48	0.772	0.328	0.504	0.519	0.135

Summary of Malathion Dicarboxylic Acid, Malathion Monocarboxylic Acid, Dimethylphosphate, Dimethylthiophosphate, and Dimethyldithiophosphate Metabolites in Human Urine Samples (Continued)

Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
SUBJ041,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ041,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ041,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ041,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ042,15.0,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
SUBJ042,15.0,0-12	25.959	178.856	4.718	13.619	3.948
SUBJ042,15.0,12-24	6.463	1.303	4.943	4.111	1.194
SUBJ042,15.0,24-48	0.212	0.237	0.431	0.476	0.152
SUBJ043,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ043,15.0,0-12	6.074	204.058	50.916	73.732	2.666
SUBJ043,15.0,12-24	1.872	2.603	1.780	2.982	0.575
SUBJ043,15.0,24-48	0.870	1.253	0.320	1.077	0.112
SUBJ044,15.0,-12-0	0.023	0.073	0.041	0.047	N.D.
SUBJ044,15.0,0-12	33.767	59.882	126.575	175.959	3.037
SUBJ044,15.0,12-24	9.326	4.483	5.889	10.948	2.276
SUBJ044,15.0,24-48	0.485	0.275	1.412	0.961	0.122
SUBJ045,PL,-12-0	0.029	0.089	N.Q.	0.026	N.Q.
SUBJ045,PL,0-12	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ045,PL,12-24	N.Q.	N.Q.	N.D.	0.028	N.Q.
SUBJ045,PL,24-48	0.243	0.218	N.D.	N.D.	N.D.
SUBJ046,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ046,15.0,0-12	61.687	308.218	10.248	7.553	1.257
SUBJ046,15.0,12-24	5.362	11.767	4.325	13.035	1.115
SUBJ046,15.0,24-48	3.168	1.237	0.973	1.975	0.494
SUBJ047,PL,-12-0	N.D.	N.Q.	0.027	0.037	N.D.
SUBJ047,PL,0-12	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ047,PL,12-24	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ047,PL,24-48	N.D.	N.D.	N.D.	N.Q.	N.D.
SUBJ948,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
SUBJ948,15.0,0-12	30.985	302.771	26.003	92.939	8.213
SUBJ948,15.0,12-24	6.727	12.786	3.568	7.637	0.240
SUBJ948,15.0,24-48	1.497	2.043	0.716	1.074	0.238

The methods utilized were validated by fortifying normal pooled urine samples at approximately 30, 300 or 600 ppb for carboxylic acid metabolites, and approximately 25 or 1000 ppb for dimethyl phosphate metabolites. The results of these fortifications are summarized below.

Summary of Method Validation Recovery Data for Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid in Human Urine

Fortification	Malathion Dicarboxylic Acid			Malathion Monocarboxylic Acid		
	Fort. Level (ppb)	RSD (%)	Recovery (%)	Fort. Level (ppb)	RSD (%)	Recovery (%)
Low	30	3	103	30	3	96
Mid	300	4	91	300	9	95
High	600	2	106	600	2	106

n=7

Summary of Method Validation Recovery Data for Dimethyl Phosphate, Dimethyl Thiophosphate, and Dimethyl Dithiophosphate in Human Urine

Fortification	Dimethyl Phosphate			Dimethyl Thiophosphate			Dimethyl Dithiophosphate		
	Fort. Level (ppb)	RSD (%)	Recovery (%)	Fort. Level (ppb)	RSD (%)	Recovery (%)	Fort. Level (ppb)	RSD (%)	Recovery (%)
Low	25	16	98	25	16	100	25	26	126
High	1000	11	99	1000	12	101	1000	18	132

n=9

Samples were stored frozen for up to 13 months from the date of collection. Storage stability data indicate each metabolite to be stable under frozen conditions for at least this length of time.

2.0 INTRODUCTION

Pacific Toxicology Laboratories (PTL) conducted the analyses of human urine samples to evaluate the magnitude of five malathion metabolites: malathion dicarboxylic acid, malathion monocarboxylic acid, dimethyl phosphate, dimethyl thiophosphate, and dimethyl dithiophosphate. Urine samples were collected from human subjects as part of a clinical study conducted by Cheminova A/S at Inveresk Research (Inveresk), Tranent, Scotland; study title: "A Randomized Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity" (ICR Study No: 013177).

In this study, groups of human volunteers were given single oral doses of malathion at 0.5, 1.5, 5.0, 10.0, 15.0, or 0.0 mg/kg (placebo) levels. Urine samples were collected and pooled for the following durations: -12-0 hours (12 hours prior to dosing to 0 time—time of dosing), and 0-12, 12-24, and 24-48 hours after dosing. Total urine volume during each time period was measured and aliquots of these samples were shipped frozen from

Inveresk to PTL in California, where they were stored between -6°C and -15°C until analysis.

The study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards regulation (40 CFR 160) and complies with protocols, methods, standard operating procedures, and sponsor-specified guidelines. The protocol, protocol amendments, and protocol deviations are provided in Appendix 1 of this report.

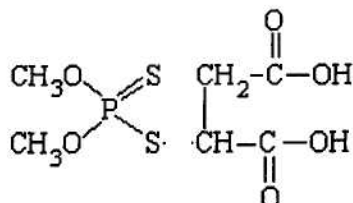
3.0 IDENTIFICATION OF REFERENCE STANDARDS

3.1 Reference Standards

Analytical standards for the five metabolites of malathion were provided by the sponsor. Certificates of analysis and other physical and chemical information of each standard are included in Appendix 5. Analytical standards of the two internal standards were obtained from ChemService of West Chester, PA. Certificates of analysis of each standard are included in Appendix 5. The following information is available regarding these standards.

3.1.1 Malathion Dicarboxylic Acid

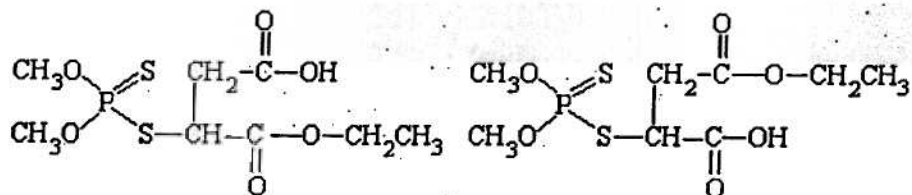
Name:	Malathion Dicarboxylic Acid
Chemical name:	Butanedioic acid, [(dimethoxyphosphinothioyl) thio]
CAS No:	1190-28-9
Chemical structure:	



Source:	Cheminova Agro A/S
Lot #:	167-BSe-71C
Storage Condition:	<0°C
Expiration Date:	June 9, 2003
Purity:	99.0%
Date of Receipt:	11/03/98
Molecular Weight:	274.26 g/mol
Physical Appearance:	white crystal

3.1.2 Malathion Monocarboxylic Acid

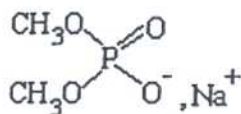
Name: Malathion Monocarboxylic Acid (& mixture)
Chemical name: Butanedioic acid, [(dimethoxyphosphinothioyl) thio]-, monoethyl ester
CAS No: 35884-76-5
Chemical structure:
Source: Cheminova Agro A/S



Lot #: 275-MJH-82-1
Storage Condition: <0°C
Expiration Date: June 22, 2003
Purity: 91.0%
Date of Receipt: 11/03/98
Molecular Weight: 302.31 g/mol
Physical Appearance: clear liquid

3.1.3 Dimethyl Phosphate

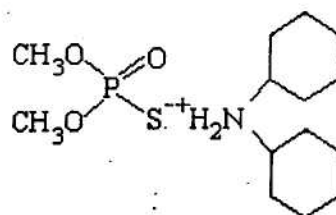
Name: Dimethyl Phosphate
Chemical name: Phosphoric acid, dimethyl ester, sodium salt
CAS No: 7351-83-9
Chemical structure:



Source: Cheminova Agro A/S
Lot #: 302-OSJ-50B
Storage Condition: ambient Temp., dark, in dessicator
Expiration Date: March 18, 2001
Purity: 98.4%
Date of Receipt: 11/30/98
Molecular Weight: 148.03 g/mol
Physical Appearance: white crystals

3.1.4 Dimethyl Thiophosphate

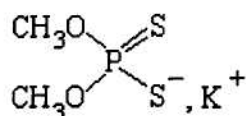
Name: Dimethyl Thiophosphate
Chemical name: O,O-dimethyl-thiophosphoric acid, dicyclohexylammonium salt
CAS No: 1112-38-5
Chemical structure:



Source: Cheminova Agro A/S
Lot #: 267-OSJ-54B
Storage Condition: <0°C
Expiration Date: May 21, 2001
Purity: 97.9%
Date of Receipt: 11/30/98
Molecular Weight: 323.2 g/mol
Physical Appearance: white crystals

3.1.5 Dimethyl Dithiophosphate

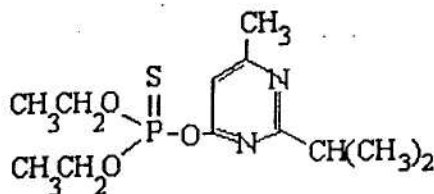
Name: Dimethyl Dithiophosphate
Chemical name: Phosphorodithioic acid, O,O-dimethyl ester, potassium salt
CAS No: 16001-68-6
Chemical structure:



Source: Cheminova Agro A/S
Lot #: 291-BSe-62A
Storage Condition: <0°C
Expiration Date: September 3, 2003
Purity: 99.1%
Date of Receipt: 11/30/98
Molecular Weight: 196.27 g/mol
Physical Appearance: white crystals

Diazinon

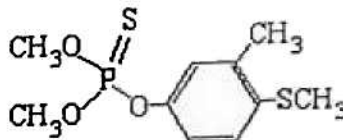
Name: Diazinon
Chemical name: O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate
CAS No: 333-41-5
Chemical structure:



Source: ChemService (West Chester, PA)
Lot #: 214-64A
Storage Condition: <0°C
Expiration Date: October, 2001
Purity: 99.3%
Date of Receipt: 05/05/98
Molecular Weight: 304.26
Physical Appearance: pale liquid

Fenthion

Name: Fenthion
Chemical name: O,O-Dimethyl O-[3-methyl-4-(methylthio)phenyl] phosphorothioate
CAS No: 55-38-9
Chemical structure:



Source: ChemService (West Chester, PA)
Lot #: 208-52B
Storage Condition: <0°C
Expiration Date: July, 2001
Purity: 98.0%
Date of Receipt: 01/16/99
Molecular Weight: 278.34
Physical Appearance: colorless liquid

4.0 EXPERIMENTAL MATERIALS AND METHODS

4.1 Sample Processing

Urine samples in 25 mL plastic urine screw cap cups were stored frozen and shipped, packed in dry ice, by international courier from Inveresk Research, Tranent, Scotland. Deliveries took on average two days to arrive. Samples were confirmed to have been maintained in a frozen state upon receipt and were processed (logged in) without thawing. Samples were accessioned upon receipt into PTL's ANTRIM Laboratory Information System according to PTL SOP: 1-FLOW-910515-2. This procedure assigns each sample a unique identifier number (accession number) starting with an "X" and followed by seven numerical digits. Additionally, the following information was recorded for each sample: sample name, date of receipt, date of collection, time of collection, sample type, container type, condition of sample upon receipt if abnormal, and specific test(s) to be performed. Complete list of accession numbers and the sample assigned to each is listed in Appendix 2.

4.2 Sample Label Code

An explanation of the label code for the samples used in the results tables (Tables III and IV) is as follows:

Example: SUBJ005,1.5,0-12
 (1) (2) (3)

- (1) Study subject's identification number supplied by Inveresk Corp.
- (2) Subject dosing level in mg/kg (i.e., 1.5 mg/kg). Placebo indicated as PL.
- (3) Sample interval in hours: -12-0 hrs, 0-12 hrs, 12-24 hrs, and 24-48 hrs.

4.3 Sample Storage Conditions

All samples were stored frozen until analysis. Samples were stored between -6 °C and -15 °C at all storage locations. During sample analysis, samples were brought to room temperature to be aliquotted, then stored in the laboratory refrigerator between 5°C and 6°C until the analysis was completed. Any remaining urine was then returned to the freezer.

4.4 Sample Mixing

Frozen urine samples were thawed in the refrigerator (overnight) then brought to room temperature. Once thawed, the samples were vigorously shaken by hand to achieve thorough mixing (for 5 seconds). Samples were aliquotted only after complete mixing (confirmed by visual inspection). After the urine aliquots were removed for analysis, the remaining samples were returned to the refrigerator. Once satisfactory analytical results were obtained, the remaining samples were returned to the freezer.

Residue Analytical Method

See Appendix 3 for a complete description of the residue analytical methods.

4.5.1 Malathion Carboxylic Acid Residue Analytical Method

4.5.1.1 Quality Control Samples

Quality control samples were prepared in batches of normal pooled urine collected from laboratory personnel. Fortification and aliquotting of quality control samples occurred at PTL on December 11, 1998. Quality control samples were considered stable for one year unless the stability of the analyte in the controls showed deterioration beyond SOP requirements. At that point, new quality control samples were prepared and validated. These requirements are set forth in SOP 2-QAP-891122-2, Section X, located in Appendix 4.

4.5.1.2 Extraction and Cleanup Procedure

On the day of extraction, the samples were removed from the refrigerator and allowed to come to room temperature. Quality control samples fortified at 0 (urine blank), approximately 30 ppb, and 300 ppb of DCA and MCA were removed from the storage freezer at the same time and allowed to reach room temperature. These fortified samples were extracted and analyzed in a manner identical to the study samples.

Once all the samples reached room temperature, a 5 mL aliquot of thoroughly mixed urine sample and quality control sample were dispensed into 16 x 125 mm screw cap tubes (for acid residue analysis). [At this time a second 1 mL aliquot was removed and kept aside for analysis for malathion alkyl phosphate residues (see section 4.5.2.1).] Into the 5 mL

aliquot of urine sample, two drops of concentrated HCl were added to bring the pH to 1-2. Fifty μL of the internal standard solution (Diazinon 10 $\mu\text{g/L}$) was added to each sample and control tubes.

Phenyl solid phase extraction cartridges were pre-conditioned by passing one column volume of acetone ($\sim 6\text{ mL}$) followed by two column volumes ($\sim 12\text{ mL}$) of distilled water through each column. The acidified urine samples were loaded onto the columns and drawn through at a rate of 3-4 mL/min. Each column was then washed with two column volumes ($\sim 12\text{ mL}$) of 1 mM HCl. The cartridges were centrifuged at 25,000 rpm for five minutes to remove any residual HCl. The malathion acid metabolites were eluted with 2 mL ethyl acetate into 12 x 75 mm test tubes. The organic layer of the eluant was passed through a pasteur pipet packed with 1 gram of anhydrous sodium sulfate and was collected in a 15 mL graduated centrifuge tube. The original 12 x 75 mm tube was then rinsed with additional 0.5 mL ethyl acetate, passed through the anhydrous sodium sulfate, and added to the extract.

For derivatizing, 250 μL of acidified ethyl acetate followed by 3 mL of ethereal diazomethane were added to the extracts, mixed, and allowed to stand for 15 minutes. After 15 minutes, excess diazomethane was removed via a slow steady stream of nitrogen gas and the sample was concentrated to approximately 0.5 mL under nitrogen, transferred to a glass autosampler vial, and analyzed by gas chromatography.

In addition to urine samples and quality control samples, a reagent blank and six calibration standards were prepared. Calibration standards were prepared by fortifying normal pooled urine with specified volumes of Intermediate Stock Standard Solution of DCA and MCA to achieve concentrations ranging from 10 ppb to 600 ppb. These standards were prepared and run daily to determine instrument linearity. The 200 ppb calibration standard was used for daily single point calibration and sample quantitation. Calibration standards were extracted, derivatized, and analyzed in the same manner as urine samples.

4.5.1.3 Gas Chromatographic Analysis

Typical instrument parameters are listed below.

Instrument:	Perkin Elmer 8500 Gas Chromatograph
Autosampler:	Perkin Elmer
Inlet:	Split/Splitless

Column: DB-210

Detector: FPD in Phosphorus mode

Temperatures: Injector (°C): 250
Column (°C): 185 Isothermal 15 minutes
Detector (°C): 300

Gas Flow Rates: N₂ carrier: 7.0 mL/min
Air: 90 mL/min
H₂: 65 mL/min

Attenuation: 6

Injection volume: 2µL

4.5.2 Malathion Alkyl Phosphate Residue Analytical Method

4.5.2.1 Quality Control Samples

Quality control samples were prepared by fortifying normal pooled urine collected from laboratory personnel. Fortification and aliquotting of quality control samples occurred at PTL on January 22, 1999. Quality control samples were considered stable for one year unless the stability of the analyte showed deterioration beyond SOP requirements. At that point, new quality control samples were prepared and validated. These requirements are set forth in SOP 2-QAP-891122-2, Section X, located in Appendix 4. On the day of extraction, quality control (QC) samples fortified at 0 (urine blank), approximately 25 ppb, and 1000 ppb of DMP, DMTP, and DMDTP, were removed from the storage freezer and allowed to reach room temperature.

4.5.2.2 Extraction and Cleanup Procedure

A 1.0 mL aliquots of each urine sample (section 4.5.1.2.), QC samples and urine blank samples were dispensed into 16 x 125 mm screw cap tubes. Each tube was prepared for freeze-drying by "shell freezing" the sample. A thin layer of urine was frozen to the wall of the tube by quickly stirring the tube in a beaker of acetone and dry ice. Once the samples were frozen, they were placed in the freeze-drying unit overnight.

The next day, the samples were removed from the freeze-drying unit. If a sample was not completely desiccated at the time of removal from the freeze dryer, a new 1.0 mL aliquot was refreeze-dried. For derivatization, 1 mL of acetone, 3 carborundum boiling chips, and 300µL of 10% 1-

Benzyl-3-p-Tolyltriazine (BTT) were added to each tube, vortexed for 30 seconds, capped tightly, and placed in a 70°C heating block for two hours. After derivatization, samples were allowed to stand overnight at room temperature.

On the following day, five drops of 6N HCl was added to each tube, mixed, followed by 10 mL of saturated NaCl. Again the samples were mixed. Next, 0.5 mL of 0.5 µg/mL internal standard (fenthion) in cyclohexane was added to the salt saturated extract, shaken vigorously for 1 minute, followed by centrifuging at 2000 rpm for 3 minutes. The upper organic layer was transferred to a 15 mL graduated centrifuge tube. This procedure was repeated one more time with 1.5 mL of cyclohexane. The extracts were combined and concentrated to 0.5 mL under a gentle stream of nitrogen. The sample was then transferred to a glass autosampler vial and analyzed by gas chromatography.

In addition to urine samples and quality control samples, a reagent blank and six calibration standards were prepared. Calibration standards were prepared by fortifying normal pooled urine with specified volumes of Intermediate Stock Standard Solution of DMP, DMTP, and DMDTP to produce concentrations ranging from 25 ppb to 1000 ppb. These standards were prepared and run daily to determine instrument linearity. The 200 ppb calibration standard was used for daily single point calibration and sample quantitation. Calibration standards were extracted, derivatized, and analyzed in the same manner as urine samples.

Figures 1 through 17 show example calibration curves and chromatograms for malathion dicarboxylic acid, malathion monocarboxylic acid, dimethyl phosphate, dimethyl thiophosphate (this compound gives two peaks due to isomerism between the phosphorothiolic and phosphorothionic forms), and dimethyl dithiophosphate in urine.

4.5.2.3 Gas Chromatographic Analysis

Typical instrument parameters are listed below.

Instrument:	Perkin Elmer 8500 Gas Chromatograph
Autosampler:	Perkin Elmer
Inlet:	Split/Splitless
Column:	DB-210

Detector:	FPD in Phosphorus mode
Temperatures:	Injector (°C): 250
	Column (°C): 160, hold for 6 min., 5°/min to 210°C
	hold for 5 min.

Gas Flow Rates:	N ₂ carrier:	7 mL/min
	Air:	90 mL/min
	H ₂ :	65 mL/min

Attenuation:	6
Injection volume:	2µL

4.6 Calculations

4.6.1 Concentration of Residues in Samples

Residue concentrations were quantitated using the relative response of the analyte of interest to the response of the internal standard. This relative response ratio was multiplied by the response factor of the analyte in the 200 µg/L standard and the dilution factor, if the sample was diluted, to determine the concentration of the analyte.

4.6.1.1 Calculation of Response Factor

Response Factor of analyte in 200 µg/L standard:

$$\frac{\text{response ISTD}}{\text{response A}} \times \text{conc. A} = \text{RF}$$

response ISTD = Area response of the internal standard

response A = Area response of analyte A

conc. A = Concentration (µg/L) of analyte A in the 200 µg/L standard

RF = Response Factor

An example calculation for sample with PTL ID X0017250 (Figure 11), sample code SUBJ023,10.0,0-12 follows:

Response Factor of DCA for the run in which sample X0017250 was analyzed (values taken from 204 µg/L calibration standard):

$$\frac{532328}{259713} \times 204 (\mu\text{g/L}) = 418$$

4.6.1.2 Calculation of Concentration of Analyte

Concentration of analyte A in a sample:

$$\frac{\text{response } A}{\text{response } ISTD} \times RF \times DF = \text{conc. } A$$

response A = Area response of analyte A

response ISTD = Area response of the internal standard

RF = Response Factor of the 200 standard

DF = Dilution Factor

conc. A = Concentration (µg/L) of analyte A in the sample

The concentration of DCA for sample with PTL ID X0017250, sample code SUBJ023, 10.0, 0-12 is (this sample was diluted 1:350):

$$\frac{73920}{434192} \times 418 \times 350 = 24,907 \text{ (}\mu\text{g/L)}$$

4.6.2 Percent Recovery From Fortified Samples

Percent recovery was calculated using the following equation:

$$\% \text{ recovery} = \frac{\text{ppm found}}{\text{ppm added}} \times 100$$

An example calculation for MCA recovery in low control run with Load 7089901:

$$87\% = \frac{26}{30} \times 100$$

4.7 Method Validation

4.7.1 Validation of Analytical Procedures for Malathion Carboxylic Acid Metabolites and Malathion Alkyl Phosphate Metabolites Methods

The following criteria were evaluated in order to provide a validation of the methods described in Appendix 3.

System Suitability

Assay Specificity in Human Urine

Assay Linearity in Human Urine

Assay Limit of Detection in Human Urine

Assay Limit of Quantitation in Human Urine

Intraday Assay Accuracy and Precision in Human Urine

4.7.2 System Suitability

System suitability calculations were performed manually utilizing Microsoft Excel version 5.0/97 database software and data obtained from the Shimadzu Integrator Model C-R5A.

4.7.2.1 Column Efficiency

The column efficiency for the malathion acid metabolite method was determined by injecting a derivatized and extracted blank urine sample fortified with DCA, MCA, and internal standard onto the GC system described in Appendix 3. The column efficiency for the malathion alkyl phosphate metabolite method was determined by injecting a derivatized and extracted blank urine sample fortified with DMP, DMTP, DMDTP, and internal standard onto the GC system described in Appendix 3.

The number of theoretical plates $N_{w_{1/2}}$, calculated at half peak height was determined for each analyte by the following equation:

$$N_{w_{1/2}} = 5.54 \{t/w_{1/2}\}^2$$

Where t is the retention time (sec) of the analyte and $w_{1/2}$ is the peak width at half the peak height (sec). The minimum acceptable column efficiency of 5000 theoretical plates was met for all peaks of interest. The column efficiency of DCA and MCA was 24189 $N_{w_{1/2}}$, and 24721 $N_{w_{1/2}}$.

respectively, and 11315 $Nw_{\frac{1}{2}}$ for DMP, 6109 and 6126 $Nw_{\frac{1}{2}}$ for the two DMTP peaks, and 10249 $Nw_{\frac{1}{2}}$ for DMDTP.

4.7.2.2 Tailing Factor

The tailing factor for the malathion acid metabolite method was determined by injecting a derivatized and extracted blank urine sample fortified with DCA, MCA, and internal standard onto the GC system described in Appendix 3. The tailing factor for the malathion alkyl phosphate metabolite method was determined by injecting a derivatized and extracted blank urine sample fortified with DMP, DMTP, DMDTP, and internal standard onto the GC system described in Appendix 3.

The tailing factor, T , for each analyte was determined by the following equation:

$$T = W_{0.05}/2f$$

Where $W_{0.05}$ is the width of the peak at 5% peak height (sec) and f is a measure of the width of the peak at 5% peak height from the center to the leading edge (sec). The tailing factors for DCA and MCA were 0.21 and 0.49, respectively, 0.10 for DMP, 0.20 and 0.25 for the two DMTP peaks, and 0.16 for DMDTP.

4.7.2.3 Resolution Factor

The resolution factors between DCA and MCA and between DCA and the internal standard for the malathion acid metabolite method were determined by injecting a derivatized and extracted blank urine sample fortified with DCA, MCA, and internal standard onto the GC system described in Appendix 3. The resolution factors between the first DMTP peak and DMP, between DMP and DMDTP, between DMDTP and the second DMTP peak, and between the second DMTP peak and the internal standard for the malathion alkyl phosphate metabolite method were determined by injecting a derivatized and extracted blank urine sample was fortified with DMP, DMTP, DMDTP, and internal standard onto the GC system described in Appendix 3.

The resolution, R , between each set of peaks was determined by the following equation:

$$R = 2(t_2 - t_1) / W_2 + W_1$$

Where t_1 and t_2 are the retention times (sec) of the two components and W_1 and W_2 are the corresponding widths of the bases of the peaks (sec), obtained by extrapolating the relatively straight sides of the peaks to the baseline. Resolution between all peak pairs measured was never less than 4 and was as large as 29. This surpasses the 1.5 resolution value required for baseline resolution.

4.7.2.4 System Precision

The system precision for the malathion acid metabolite method was determined during method validation by seven replicate injections of a derivatized and extracted blank urine sample fortified with DCA, MCA, and internal standard onto the GC system described in Appendix 3. The system precision for the malathion alkyl phosphate metabolite method was determined during method validation by nine replicate injections of a derivatized and extracted blank urine sample fortified with DMP, DMTP, DMDTP, and internal standard onto the GC system described in Appendix 3.

The coefficient of variation, CV (%), for each analyte was determined by the following equation:

$$CV (\%) = (SD/Mean) \times 100$$

Where SD is the standard deviation of the results from the mean, and the mean is the average of the results. The CV (%) for the malathion carboxylic acid metabolites ranged from 2% to 9% and from 11% to 26% for the malathion alkyl phosphate metabolites. Results are presented in Tables I and II.

4.7.3 Assay Specificity in Human Urine

4.7.3.1 Malathion Carboxylic Acid Metabolite Method

The Malathion Acid Metabolite assay specificity was examined in pooled human urine collected from members of the laboratory staff at PTL. Seven replicates of blank urine fortified with internal standard and seven replicates of double blank urine (urine with no DCA, MCA, or internal standard) were prepared and analyzed according to the method presented in Appendix 3. The chromatograms were examined for possible interferences. Results are presented in Table I.

4.7.3.2 Malathion Alkyl Phosphate Metabolite Method

The Malathion Alkyl Phosphate Metabolite assay specificity was examined in pooled human urine collected from members of the laboratory staff at PTL. Nine replicates of blank urine fortified with internal standard and seven replicates of double blank urine (urine with no DMP, DMTP, DMDTP, or internal standard) were prepared and analyzed according the method presented in Appendix 3. The chromatograms were examined for possible interferences. Results are presented in Table II.

4.7.4 Assay Linearity in Human Urine

4.7.4.1 Malathion Carboxylic Acid Metabolite Method

Appropriate quantities of intermediate stock solutions of DCA and MCA were added to a series of 5 mL aliquots of pooled human urine to yield concentrations in the range of approximately 10 to 600 µg/L urine for both analytes. Following the addition of internal standard, the samples were extracted, derivatized, and analyzed according to the method described in Appendix 3.

The peak area ratios for DCA:internal standard and MCA:internal standard were calculated for each sample. A calibration curve was prepared for each compound by plotting the peak area ratios of the calibration standards versus the concentration of each compound in the urine and the least squares linear regression parameters determined. Least squares linear regression parameters were determined with every batch of urine samples analyzed. Correlation coefficients (R^2) determined for the malathion carboxylic acid metabolite runs performed for this study ranged from 0.960 to 1.000.

4.7.4.2 Malathion Alkyl Phosphate Metabolite Method

Appropriate quantities of intermediate stock solutions of DMP, DMTP, and DMDTP were added to a series of 1 mL aliquots of pooled human urine to yield concentrations in the range of approximately 25 to 1000 µg/L urine for all alkyl phosphate analytes. Following the addition of internal standard, the samples were extracted, derivatized, and analyzed according to the method described in Appendix 3.

The peak area ratios for DMP:internal standard, the sum of the areas of the two DMTP peaks:(DMTP1+DMTP2):internal standard, and the peak areas for DMDTP:internal standard were calculated for each sample. A calibration curve was prepared for each compound by plotting the peak area ratios of the calibration standards versus the concentration of each compound in the urine and the least squares linear regression parameters determined. Least squares linear regression parameters were determined with every batch of urine samples analyzed. Correlation coefficients (R^2) determined for the malathion alkyl phosphate metabolite runs performed for this study ranged from 0.983 to 1.000.

4.7.5. Assay Limit of Detection in Human Urine

4.7.5.1 Malathion Carboxylic Acid Metabolite Method

The limit of detection was determined during method validation. Seven replicates of normal pooled blank human urine were prepared according to the method presented in Appendix 3. The mean of the baseline noise plus three standard deviations was established. This value was calculated to be 2 ppb for both DCA and MCA as compared to standards also prepared and analyzed according to the method described previously.

4.7.5.2 Malathion Alkyl Phosphate Metabolite Method

The limit of detection was determined during method validation. Seven replicates of normal pooled blank human urine were prepared according to the method presented in Appendix 3. The mean of the baseline noise plus five standard deviations was established. This value was calculated to be 12.5 ppb for DMP, DMTP, and DMDTP, as compared to standards also prepared and analyzed according to the method described previously.

4.7.6 Assay Limit of Quantitation in Human Urine

4.7.6.1 Malathion Carboxylic Acid Metabolite Method

The limit of quantitation was established from the same normal pooled blank urine samples and standards evaluated for the limit of detection. The limit of quantitation was calculated as at least the mean of the baseline noise plus 10 standard deviations and was established at 10 times the limit of detection (mean + 30 s.d.) or 20 ppb for both DCA and MCA.

4.7.6.2 Malathion Alkyl Phosphate Metabolite Method

The limit of quantitation was established from the same normal pooled blank urine samples and standards evaluated for the limit of detection. The limit of quantitation was calculated as at least the mean of the baseline noise plus 10 standard deviations and was established at two times the limit of detection (mean + 10 s.d.) or 25 ppb for all three analytes.

4.7.7 Intraday Assay Accuracy and Precision in Human Urine

4.7.7.1 Malathion Carboxylic Acid Metabolite Method

Determination of assay accuracy (% recovery) and precision (% relative standard deviation) was conducted according to the Quality Assurance SOP: 2-QAP-891122-2. Seven replicates of reagent blanks, urine blanks, and urine samples fortified at approximately 30 ppb and 300 ppb DCA and MCA each were extracted and analyzed according to the method presented in Appendix 3. The urine utilized for the fortified and blank urine samples was normal pooled urine collected from members of the laboratory staff. Results of the validation of the Malathion Carboxylic Acid Method are presented in Table I.

4.7.7.2 Malathion Alkyl Phosphate Metabolite Method

The method for the analysis of alkyl phosphate metabolites has been in continuous use at PTL for 12 years. The method is revalidated when significant changes to the method are made or when a new batch of standards or fortified control material is made. In order to validate the performance of the method with the new set of fortified control material, a "crossover" is performed, where the old and new fortified controls are run simultaneously. Once the laboratory director is satisfied that the recoveries of the new controls are within the specified parameters, the new controls are put into use. Continuous monitoring of the performance of the method is accomplished by analyzing these fortified samples with each batch of patient samples and recording the results in the Laboratory Information System. This system tracks the recovery of the fortified samples and evaluates those values against a set of Quality Assurance parameters called the Westgard Rules. These rules are set forth in the SOP for the laboratory Quality Assurance Program included in Appendix 4. The urine utilized for the fortified and blank urines was pooled urine collected from members of the laboratory staff and fortified at 25 ppb and 1000 ppb of DMP, DMTP, and DMDTP. Results of the "crossover" established at the beginning of this study are presented in Table II.

4.7.8 Concurrent Recoveries

4.7.8.1 Malathion Carboxylic Acid Metabolite Method

Method recovery of DCA and MCA was evaluated for each analytical set using two fortification (or "control") levels. A batch of normal pooled blank urine was fortified at the beginning of the study at approximately 30 ppb and 300 ppb, then aliquotted into 5 mL aliquots, and stored frozen. For each set of study samples, a reagent blank, urine blank, and 30 ppb and 300 ppb fortified sample were analyzed concurrently. Percent recovery data for DCA and MCA fortified urine samples are presented in Table III.

4.7.8.2 Malathion Alkyl Phosphate Metabolite Method

Method recovery of DMP, DMTP, and DMDTP was evaluated for each analytical set using two fortification (or "control") levels. A batch of normal pooled blank urine was fortified at the beginning of the study at approximately 25 ppb and 1000 ppb, and then aliquotted into 1 mL aliquots and stored frozen. For each set of study samples, a reagent blank, urine blank, and 25 ppb and 1000 ppb fortified sample was analyzed concurrently. Percent recovery data for DMP, DMTP, and DMDTP fortified samples are presented in Table IV.

Quantities of malathion carboxylic and metabolites and alkyl phosphate metabolites for each subject during each sample collection interval are presented in Table V.

4.8 Analytical Raw Data Included with Report

Examples of chromatograms are presented in Figures 1 through 17. In addition, pertinent raw data are also included in the report.

5.0 STORAGE STABILITY

Twenty 5 mL samples of normal pooled urine were fortified with 40 ppb and 200 ppb of DCA and MCA on January 7, 1999. One-hundred 1 mL samples of normal pooled urine were fortified with approximately 50 and 250 ppb of DMP, DMTP, and DMDTP on January 13, 1999. The fortified samples were stored at approximately -6°C to -15°C , same as the study samples. At approximate intervals of 10, 35, 45, 55, and 65 weeks, triplicate fortified samples (when

possible) were thawed and analyzed for the above analytes. In addition, quality control samples fortified at 30 and 300 ppb DCA and MCA, and quality control samples fortified at 25 and 1000 ppb DMP, DMTP, and DMDTP were thawed and analyzed according to the method SOPs.

6.0 CREATININE ANALYSES

The concentration of creatinine in each urine samples was determined by the method of Jaffé et al.¹ Creatinine is determined in 30 µL of urine by measuring the change in absorbance at 520 nm of an alkaline picrate solution following the addition of the sample. The test is performed on a Beckman CX7 analyzer using Beckman Coulter Synchron CX® Systems Creatinine Reagent Kit (P/N 443340, Beckman Coulter, Inc. Fullerton, CA). The detection limit for this analyte is 0.14 g/L. The CX7 instrument is calibrated every six months. The calibration curve generated for the time during which the samples in this study were analyzed is presented in Figure 18. The range over which the method was evaluated was 0.1 to 4.0 g/L. Linearity is determined by serially diluting a urine sample known to contain a Creatinine concentration greater than 4.0 g/L. PTL successfully participates in the external proficiency testing program administered by the College of American Pathologists for this analyte three times a year.

The concentrations of creatinine found in each urine sample are presented in Table VI.

7.0 RESULTS AND DISCUSSION

7.1 Analytical Phase

7.1.1 Method Validation

7.1.1.1 Malathion Acid Metabolites

The method validation data for the analysis of malathion dicarboxylic acid and malathion monocarboxylic acid in fortified and blank urine samples are presented in Table I. The recovery ranges, means, and standard deviations are summarized below.

Summary of Method Validation Recovery Data for Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid in Human Urine

Fortification Level (ppb)	<u>Malathion Dicarboxylic Acid^a</u>		<u>Malathion Monocarboxylic Acid^a</u>	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
30	103	3	96	3
300	91	4	95	9
600	106	2	106	2

^a n=7

7.1.1.2 Malathion Alkyl Phosphate Metabolites

The method validation data for the analysis of dimethyl phosphate, dimethyl thiophosphate, and dimethyl dithiophosphate in fortified and blank urine are presented in Table II. The recovery ranges, means, and standard deviations from the set of nine "crossover" samples function as our method validation and are summarized below.

Summary of Method Validation Recovery Data for Dimethyl Phosphate, Dimethyl Thiophosphate, and Dimethyl Dithiophosphate in Human Urine

Fortification Level (ppb)	<u>Dimethyl Phosphate^a</u>		<u>Dimethyl Thiophosphate^a</u>		<u>Dimethyl Dithiophosphate^a</u>	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
25	98	16	100	16	126	26
1000	99	11	101	12	132	18

^a n=9

7.1.2 Concurrent Recoveries

Concurrent recovery data for the analysis of malathion acid metabolites and malathion alkyl phosphate metabolites are presented in Tables III and IV. Summaries of the means, standard deviation of the values collected during the course of the study, and overall average recovery data are presented below. For the malathion acid method, 17 analytical sets were run, while 16 analytical sets were run for the malathion alkyl phosphate method.

7.1.2.1 Malathion Acid Metabolites

Summary of Concurrent Recovery Data of Malathion Acid Metabolites in Fortified Human Urine

Fortification Level (ppb)	<u>Malathion Dicarboxylic Acid^a</u>		<u>Malathion Monocarboxylic Acid^a</u>	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
30	108	16	102	13
300	104	18	99	11

^a n=17

7.1.2.2 Malathion Alkyl Phosphate Metabolites

Summary of Concurrent Recovery Data of Malathion Alkyl Phosphate Metabolites in Fortified Human Urine

Fortification Level (ppb)	<u>Dimethyl Phosphate^a</u>		<u>Dimethyl Thiophosphate^a</u>		<u>Dimethyl Dithiophosphate^a</u>	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
25	112	14	103	12	106	17
1000	110	19	99	17	120	23

^a n=16

7.2 Urine Analysis Results

7.2.1 Metabolite Levels in Treated Subjects

The qualitative determination of DCA, MCA, DMP, DMTP, and DMDTP in urine samples was based upon comparison of relative retention times (RRT) of the metabolites of interest to the internal standard. Residues in samples with the same RRT as the metabolites in the calibration standard were considered positive samples. Residues of DCA, MCA, DMP, DMTP, and DMDTP in urine samples calculated on a concentration basis (mg/L or ppm) are presented in Table V. The analyses indicated that the majority of malathion metabolites were excreted extremely rapidly. Approximately 90% of the urinary excretion occurred within the first 12 hours of dose administration and excretion was essentially complete within 24 to 48 hours. Total amounts of all metabolites were proportional to the dose levels.

In all but one subject (SUBJ009), the largest quantity of metabolite excretion occurred within 12 hours after dosing. In subject 9 (SUBJ009), who was dosed at 5 mg/kg, the majority of metabolite excretion occurred 12 to 24 hours after administration of the dose. By far the most abundantly excreted metabolite was MCA. This metabolite was excreted in greatest total concentration in the urines

of 30 of 34 subjects receiving oral doses of malathion. Of the other metabolites excreted, in order from greatest concentration to smallest, were DMTP, DCA, DMP, and DMDTP. Some variations in this pattern did occur.

7.2.2 Metabolite Levels in Control Subjects

Of the 14 control subjects given placebos, seven had urine samples containing trace levels of malathion metabolites. Four subjects had minor levels in their 0-12 hour urine samples; two of these subjects had all five metabolites present, and in the other two subjects only the acid metabolites were present. Of the three of seven remaining control subjects, two had various metabolites in their -12-0 hour urine samples and one had a single phosphate metabolite in his 12-24 hour urine sample.

Compared to the high amounts of metabolites found in the urine of dosed subjects, the quantities found in the control subjects were insignificant and could have been the result of possible contamination. The possible source of contamination in two control subjects that had all five metabolites in their urine samples could be cross contamination during handling of placebo/malathion capsules or technical activities during collection and pooling of urine specimens.

Among the other control subjects in which trace metabolites of various concentrations were detected, the possible contamination might have occurred during the laboratory analysis phase. It is unlikely that a contaminated sample with all 5 metabolites was produced by laboratory practices because the two sets of metabolites are determined by different analytical methods.

Figures 1 through 17 show example calibration curves and chromatograms for malathion dicarboxylic acid, malathion monocarboxylic acid, dimethyl phosphate, dimethyl thiophosphate, and dimethyl dithiophosphate detected in urine.

7.3 Storage Stability Results

Samples of frozen control urine were fortified at two levels of acids or alkyl phosphate and analyzed as previously described for storage stability. Stability samples for all metabolites were analyzed at 68, 92, 244, 301, 303, 372, and 462 days for the alkyl phosphate metabolites and at 98, 261, 308, 366, and 467 days for the carboxylic acids. With regard to the phosphate metabolites, only DMDTP had levels below 80% of nominal after 372 days. DMDTP fell to 67% of nominal after 372 days. For the carboxylic acids, neither compound was below 88% of nominal after 467 days. The results of all intervals are presented in Table VII. Based on these findings, no metabolite degradation was noted and the metabolites were deemed stable over the duration of the storage period during this study. Additionally, quality control samples, which were aliquoted as previously

described at the beginning of the study, were run with every sample set. The results of these samples are presented as Concurrent Recovery Data in Tables III and IV.

8.0 CONCLUSIONS

Residues of malathion metabolites from urine samples of human volunteers were analyzed using PTL validated analytical methods 3-MALA-90710-4 (for DCA and MCA) and 3-MOPPS-990115-1 (for DMP, DMTP, and DMDTP). The methods were validated through successful recovery of fortifications of urine at high and low levels. Average concurrent recoveries of fortified urine ranged from 91% to 132%. Samples were stored frozen for up to 13 months before analysis. Storage stability data indicated each metabolite is stable under frozen conditions for at least this length of time.

The maximum concentration of malathion acid metabolites, DCA and MCA, found in any single urine sample were 244 ppm and 897 ppm, respectively. The maximum concentration of DMP, DMTP, and DMDTP, malathion alkyl phosphate metabolites, in any single urine sample were 197 ppm, 425 ppm, and 23 ppm, respectively. On average, 66% of the total metabolites excreted were the acid metabolites. This amount ranged between 9% and 81% of the total metabolites excreted for the 34 subjects receiving malathion doses up to 15 mg/kg body weight.

The majority of these residues were excreted within the first 12 hours after dosing. Based on these results, in humans, metabolism and excretion of malathion appears to be extremely rapid with the majority of the metabolites formed and excreted within the first 12 hours of ingestion. Furthermore, metabolism and excretion appears to be essentially complete within 24 hours after dosing regardless of the gender of the subject or the level of dose (from 0.5 mg/kg body weight to 15 mg/kg body weight).

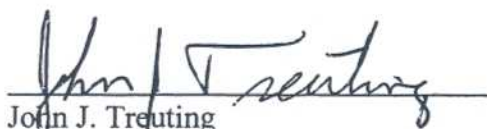
9.0 CERTIFICATION

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures described herein, and that this report provides a true and accurate record of the results obtained.



Linda S. Aston, Ph.D.
Author and Study Director
Pacific Toxicology Laboratories

10/11/00
Date



John J. Treuting
Chief Executive Officer
Pacific Toxicology Laboratories

10/11/00
Date

10.0 REFERENCES

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For further references, refer to the references contained within the methods included in Appendix 3.

Table I. Method Validation Recovery Data for Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid in Human Urine

PTL ID	Malathion Dicarboxylic Acid (µg/L)		Malathion Monocarboxylic Acid (µg/L)	
RB #1 ^a	Not Detected		Not Detected	
RB #2	ND		ND	
RB #3	ND		ND	
RB #4	ND		ND	
RB #5	ND		ND	
RB #6	ND		ND	
RB #7	ND		ND	
RBB #1 ^b	Not Detected		Not Detected	
RBB #2	ND		ND	
RBB #3	ND		ND	
RBB #4	ND		ND	
RBB #5	ND		ND	
RBB #6	ND		ND	
RBB #7	ND		ND	
UB #1 ^c	Not Detected		Not Detected	
UB #2	ND		ND	
UB #3	ND		ND	
UB #4	ND		ND	
UB #5	ND		ND	
UB #6	ND		ND	
UB #7	ND		ND	
UBB #1 ^d	Not Detected		Not Detected	
UBB #2	ND		ND	
UBB #3	ND		ND	
UBB #4	ND		ND	
UBB #5	ND		ND	
UBB #6	ND		ND	
UBB #7	ND		ND	
Low Fort. #1 ^e	33		28	
Low Fort. #2	33		28	
Low Fort. #3	31		32	
Low Fort. #4	32	Mean = 31	28	Mean = 30
Low Fort. #5	30	Std Dev = 1	28	Std Dev = 2
Low Fort. #6	32	%RSD = 3%	29	%RSD = 3%
Low Fort. #7	34	Ave % Rec = 103%	28	Ave % Rec = 96%

^a RB = Reagent spiked with internal standard.

^b RBB = Reagent not spiked with internal standard.

^c UB = Blank urine spiked with internal standard.

^d UBB = Blank urine not spiked with internal standard.

^e Amount fortified for Low Fort: DCA = 31 µg/L; MCA = 30 µg/L; Mid Fort.: DCA = 312 µg/L; MCA = 300 µg/L; High Fort: DCA = 624 µg/L; MCA = 600 µg/L.

Table I. Method Validation Recovery Data for Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid in Human Urine (Continued)

PTL ID	Malathion Dicarboxylic Acid (µg/L)		Malathion Monocarboxylic Acid (µg/L)	
Mid Fort. #1	262		242	
Mid Fort. #2	293		272	
Mid Fort. #3	284		266	
Mid Fort. #4	286	Mean = 285	299	Mean = 284
Mid Fort. #5	283	Std Dev = 11	296	Std Dev = 25
Mid Fort. #6	296	%RSD = 4%	314	%RSD = 9%
Mid Fort. #7	288	Ave %Rec = 91%	298	Ave %Rec = 95%
High Fort. #1	653		632	
High Fort. #2	662		638	
High Fort. #3	673		657	
High Fort. #4	661	Mean = 660	631	Mean = 638
High Fort. #5	678	Std Dev = 12	645	Std Dev = 10
High Fort. #6	651	%RSD = 2%	635	%RSD = 2%
High Fort. #7	643	Ave %Rec = 106%	627	Ave %Rec = 106 %

^a RB = Reagent spiked with internal standard.

^b RBB= Reagent not spiked with internal standard.

^c UB = Blank urine spiked with internal standard.

^d UBB = Blank urine not spiked with internal standard.

^e Amount fortified for Low Fort: DCA = 31 µg/L; MCA = 30 µg/L; Mid Fort.: DCA = 312 µg/L; MCA = 300 µg/L; High Fort: DCA = 624 µg/L; MCA = 600 µg/L.

Table II. Method Validation Recovery Data for Dimethyl Phosphate, Dimethyl Thiophosphate, and Dimethyl Dithiophosphate in Human Urine

	Dimethyl Phosphate (DMP)		Dimethyl Thiophosphate (DMTP)		Dimethyl Dithiophosphate (DMDTP)	
	(µg/L)		(µg/L)		(µg/L)	
RB ^a	ND ^c	N= 9	ND	N= 9	ND	N= 9
RBB ^b	ND	N= 7	ND	N= 7	ND	N= 7
UB ^c	ND	N= 9	ND	N= 9	ND	N= 9
UBB ^d	ND	N= 7	ND	N= 7	ND	N= 7
Low Fort #1 ^f	26		24		30	
Low Fort #2	30		23		44	
Low Fort #3	23		26		37	
Low Fort #4	20		30		42	
Low Fort #5	22	N= 9	30	N= 9	39	N= 9
Low Fort #6	30	Mean = 25	22	Mean = 25	49	Mean = 35
Low Fort #7	27	Std Dev = 4	23	Std Dev = 4	27	Std Dev = 9
Low Fort #8	18	%RSD = 16%	20	%RSD = 16%	22	%RSD = 26%
Low Fort #9	25	%Rec = 98%	28	%Rec = 100%	27	%Rec = 126%
High Fort #1	966		944		1156	
High Fort #2	1041		981		1108	
High Fort #3	883		1198		1848	
High Fort #4	905		1218		1723	
High Fort #5	826	N= 9	1009	N= 9	1670	N= 9
High Fort #6	1181	Mean = 975	1007	Mean = 1012	1434	Mean = 1452
High Fort #7	1050	Std Dev = 105	991	Std Dev = 123	1473	Std Dev = 254
High Fort #8	959	%RSD = 11%	914	%RSD = 12%	1297	%RSD = 18%
High Fort #9	961	%Rec = 99%	845	%Rec = 101%	1356	%Rec = 132%

^a RB = Reagent spiked with internal standard.

^b RBB= Reagent not spiked with internal standard.

^c UB = Blank urine spiked with internal standard.

^d UBB = Blank urine not spiked with internal standard.

^e ND = Not Detected.

^f Amount fortified for Low Fort: DMP = 25 µg/L; DMTP= 25 µg/L; DMDTP = 28 µg/L.
High Fort: DMP = 980 µg/L; DMTP= 1000 µg/L; DMDTP = 1100 µg/L.

Table III. Concurrent Recovery Data for Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid in Human Urine

Malathion Dicarboxylic Acid			Malathion Monocarboxylic Acid		
	Low Control. ^a		Low Control. ^a		
Run Date	(µg/L)		(µg/L)		
6/23/99	37		30		
7/1/99	30		31		
6/28/99	27		36		
7/1/99	32		27		
7/6/99	39		35		
7/9/99	34		26		
7/15/99	33		27		
7/19/99	37		27		
7/21/99	36		30		
7/30/99	26		28		
8/3/99	30		37		
7/17/99	48		34		
8/10/99	28	N= 17	35	N= 17	
8/11/99	31	Mean = 33	32	Mean = 31	
8/19/99	28	Std Dev = 5	30	Std Dev = 4	
12/7/99	36	%RSD = 16%	32	%RSD = 13%	
12/29/99	35	%Rec = 108%	24	%Rec = 102%	
	High Control. ^b		High Control. ^b		
Run Date	(µg/L)		(µg/L)		
6/23/99	328		295		
7/1/99	293		346		
6/28/99	261		352		
7/1/99	272		253		
7/6/99	310		286		
7/9/99	374		280		
7/15/99	347		263		
7/19/99	385		277		
7/21/99	373		300		
7/30/99	249		291		
8/3/99	387		352		
7/17/99	452		321		
8/10/99	286	N= 17	337	N= 17	
8/11/99	255	Mean = 324	289	Mean = 299	
8/19/99	277	Std Dev = 57	305	Std Dev = 32	
12/7/99	313	%RSD = 18%	283	%RSD = 11%	
12/29/99	352	%Rec = 104%	253	%Rec = 99%	

^aAmount fortified for Low Control: DCA = 31 µg/L; MCA = 30 µg/L.

^bAmount fortified for High Control: DCA = 312 µg/L; MCA = 300 µg/L

Table IV. Concurrent Recovery Data for Dimethyl Phosphate, Dimethyl Thiophosphate, and Dimethyl Dithiophosphate in Human Urine

	<u>Dimethyl Phosphate</u>		<u>Dimethyl Thiophosphate</u>		<u>Dimethyl Dithiophosphate</u>	
	Low Control ^a		Low Control		Low Control	
Run Date	(µg/L)		(µg/L)		(µg/L)	
6/25/99	23		22		32	
6/28/99	33		23		29	
7/2/99	28		21		31	
7/17/99	30		26		24	
7/14/99	28		24		20	
7/16/99	33		29		30	
7/22/99	24		22		26	
8/4/99	24		25		36	
7/29/99	25		23		37	
8/6/99	28		30		35	
8/15/99	31		30		28	
8/13/99	26	N= 16	29	N= 16	29	N= 16
8/25/99	33	Mean = 28	29	Mean = 26	32	Mean = 30
8/26/99	26	Std Dev = 4	25	Std Dev = 3	37	Std Dev = 5
12/9/99	32	%RSD = 14%	31	%RSD = 12%	27	%RSD = 17%
1/5/00	22	%Rec = 112%	22	% Rec=103%	23	% Rec = 106%
	High Control ^b		High Control		High Control	
Run Date	(µg/L)		(µg/L)		(µg/L)	
6/25/99	723		855		1473	
6/28/99	915		972		1617	
7/2/99	1101		1000		1441	
7/17/99	977		768		1505	
7/14/99	1245		899		748	
7/16/99	1127		978		1060	
7/22/99	917		808		1403	
8/4/99	820		891		1606	
7/29/99	906		881		1786	
8/6/99	937		925		1612	
8/15/99	1177		1122		1243	
8/13/99	1322	N= 16	1147	N= 16	1319	N= 16
8/25/99	1265	Mean = 1071	1079	Mean = 990	1244	Mean = 1322
8/26/99	983	Std Dev =	915	Std Dev =	1380	Std Dev = 298
12/9/99	1224	208	1461	170	807	%RSD = 23%
1/5/00	1500	%RSD = 19%	1134	%RSD = 17%	969	% Rec = 120%
		%Rec = 110%		% Rec = 99%		

^aAmount fortified for Low Control: DMP = 25 µg/L; DMTP= 25 µg/L; DMDTP= 28 µg/L.

^bAmount fortified for High Control: DMP = 980 µg/L; DMTP= 1000 µg/L; DMDTP = 1100µg/L.

Table V. Malathion Dicarboxylic Acid, Malathion Monocarboxylic Acid, Dimethylphosphate, Dimethylthiophosphate, and Dimethyldithiophosphate Metabolites in Human Urine Samples

PTL ID #	Sample Description ^a	DCA ^b Metabolite (ppm) ^c	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
X0016922	SUBJ001,0.5,-12-0	N.D. ^d	N.D.	N.Q. ^e	N.D.	N.D.
X0016931	SUBJ001,0.5,0-12	2.615	6.544	0.764	0.431	N.D.
X0016941	SUBJ001,0.5,12-24	1.636	0.099	0.203	0.209	0.052
X0016950	SUBJ001,0.5,24-48	N.Q.	N.D.	N.Q.	N.D.	N.D.
X0016969	SUBJ002,0.5,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
X0016978	SUBJ002,0.5,0-12	1.106	6.483	0.813	1.596	0.258
X0016987	SUBJ002,0.5,12-24	0.859	0.070	0.089	0.113	0.053
X0016996	SUBJ002,0.5,24-48	N.Q.	N.D.	N.Q.	N.D.	N.D.
X0017008	SUBJ003,PL,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
X0017017	SUBJ003,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
X0017026	SUBJ003,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0017071	SUBJ003,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0017035	SUBJ004,0.5,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
X0017044	SUBJ004,0.5,0-12	2.779	9.314	1.458	3.548	0.721
X0017053	SUBJ004,0.5,12-24	0.022	N.D.	0.190	0.196	0.039
X0017062	SUBJ004,0.5,24-48	0.892	0.089	0.177	0.202	0.040
X0017081	SUBJ005,1.5,-12-0	N.D.	N.D.	N.Q.	N.Q.	N.D.
X0017090	SUBJ005,1.5,0-12	2.314	28.753	7.086	15.670	N.Q.
X0017106	SUBJ005,1.5,12-24	4.321	0.376	1.330	1.657	0.314
X0017115	SUBJ005,1.5,24-48	0.112	N.Q.	0.065	0.044	N.D.
X0017124	SUBJ006,1.5,-12-0	N.D.	N.Q.	N.D.	N.D.	N.D.
X0017133	SUBJ006,1.5,0-12	5.873	42.430	7.340	15.070	0.886
X0017142	SUBJ006,1.5,12-24	7.939	1.495	1.697	2.062	0.290
X0017151	SUBJ006,1.5,24-48	0.291	0.027	0.109	0.075	N.D.
X0017161	SUBJ007,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017170	SUBJ007,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
X0017189	SUBJ007,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0017198	SUBJ007,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.

^a The sample description includes the following components:

Example: SUBJ005,1.5,0-12 SUBJ005 is the study subject's identification number supplied by Inveresk Corp. 1.5 is the subject's dosing level in mg/kg (i.e. 1.5 mg/kg). Placebo indicated as PL. 0-12 is the sample interval in hours: -12-0 hrs; 0-12 hrs; 12-24 hrs; 24-48 hrs.

^b DCA = Malathion Dicarboxylic Acid; MCA = Malathion Monocarboxylic Acid; DMP = Dimethylphosphate; DMTP = Dimethylthiophosphate; DMDTP = Dimethyldithiophosphate

^c ppm = parts per million

^d ND = Not Detected. Limit of detection of DCA and MCA is 0.002 ppm. Limit of detection of DMP, DMTP and DMDTP is 0.0125 ppm.

^e NQ = Not Quantitated. Limit of quantitation of DCA and MCA is 0.020 ppm. Limit of quantitation for DMP, DMTP and DMDTP is 0.025 ppm.

Table V. Malathion Metabolites in Human Urine Samples (Continued)

PTL ID #	Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
X0017204	SUBJ008,1.5,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017213	SUBJ008,1.5,0-12	3.356	30.703	6.108	11.434	0.730
X0017222	SUBJ008,1.5,12-24	0.917	0.021	0.258	0.255	0.883
X0017231	SUBJ008,1.5,24-48	0.061	N.D.	0.082	0.058	N.D.
X0016360	SUBJ009,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016379	SUBJ009,5.0,0-12	7.010	0.477	3.608	4.625	0.828
X0016388	SUBJ009,5.0,12-24	4.678	7.235	24.047	33.812	14.755
X0016397	SUBJ009,5.0,24-48	0.228	0.177	0.279	0.190	0.045
X0013403	SUBJ010,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016412	SUBJ010,5.0,0-12	4.856	21.959	0.996	0.910	0.324
X0016421	SUBJ010,5.0,12-24	3.395	1.072	0.612	1.484	0.352
X0016431	SUBJ010,5.0,24-48	0.196	0.069	0.318	0.264	0.118
X0016440	SUBJ011,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016459	SUBJ011,5.0,0-12	34.277	3.320	12.003	18.095	5.274
X0016468	SUBJ011,5.0,12-24	7.525	1.738	2.137	2.656	0.899
X0016477	SUBJ011,5.0,24-48	0.648	1.045	0.342	0.494	0.113
X0016486	SUBJ012,5.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016495	SUBJ012,5.0,0-12	43.575	152.852	14.726	46.794	9.449
X0016501	SUBJ012,5.0,12-24	23.909	2.495	4.013	7.625	3.106
X0016511	SUBJ012,5.0,24-48	0.792	0.294	0.346	0.369	0.080
X0016520	SUBJ013,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016539	SUBJ013,PL,0-12	0.440	0.553	0.051	0.142	0.055
X0016548	SUBJ013,PL,12-24	N.D.	N.D.	N.D.	0.033	N.D.
X0016557	SUBJ013,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0016566	SUBJ014,5.0,-12-0	N.D.	N.Q.	N.D.	N.D.	N.D.
X0016575	SUBJ014,5.0,0-12	33.443	77.797	16.960	41.420	4.269
X0016584	SUBJ014,5.0,12-24	8.854	4.439	2.509	2.120	1.295
X0016593	SUBJ014,5.0,24-48	0.218	0.042	0.178	0.185	0.037
X0016600	SUBJ015,PL,-12-0	N.D.	N.D.	0.035	0.041	N.D.
X0016619	SUBJ015,PL,0-12	0.579	0.475	0.081	0.159	0.031
X0016628	SUBJ015,PL,12-24	N.D.	N.D.	N.D.	N.Q.	N.D.
X0016637	SUBJ015,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0016646	SUBJ016,PL,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
X0016655	SUBJ016,PL,0-12	N.Q.	N.Q.	N.D.	N.D.	N.D.
X0016664	SUBJ016,PL,12-24	N.D.	N.D.	N.D.	0.027	N.D.
X0016673	SUBJ016,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0016682	SUBJ917,5.0,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
X0016691	SUBJ917,5.0,0-12	15.064	73.484	7.036	21.706	3.270
X0016708	SUBJ917,5.0,12-24	0.164	0.021	0.208	0.160	0.026
X0016717	SUBJ917,5.0,24-48	0.182	1.735	2.263	2.526	0.352
X0016726	SUBJ018,5.0,-12-0	0.028	N.Q.	N.Q.	N.Q.	N.D.
X0016735	SUBJ018,5.0,0-12	8.529	32.391	7.790	14.797	0.928
X0016744	SUBJ018,5.0,12-24	12.065	2.362	2.433	3.427	0.506
X0016753	SUBJ018,5.0,24-48	0.631	0.108	0.229	0.294	0.067

Table V. Malathion Metabolites in Human Urine Samples (Continued)

PTL ID #	Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
X0016762	SUBJ019,10.0,-12-0	N.D.	N.D.	N.Q.	N.D.	0.196
X0016771	SUBJ019,10.0,0-12	69.601	274.951	69.257	123.417	7.683
X0016781	SUBJ019,10.0,12-24	11.611	1.367	3.224	4.747	0.587
X0016790	SUBJ019,10.0,24-48	2.158	0.303	1.400	0.832	0.998
X0016806	SUBJ020,10.0,-12-0	N.D.	N.D.	N.Q.	N.Q.	N.D.
X0016815	SUBJ020,10.0,0-12	24.220	224.275	74.790	47.755	4.044
X0016824	SUBJ020,10.0,12-24	17.343	7.452	5.600	9.338	0.588
X0016833	SUBJ020,10.0,24-48	0.085	0.110	0.965	1.248	0.097
X0016842	SUBJ021,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016851	SUBJ021,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
X0016861	SUBJ021,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0016870	SUBJ021,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0016889	SUBJ022,10.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0016898	SUBJ022,10.0,0-12	19.015	309.165	44.503	49.060	2.749
X0016904	SUBJ022,10.0,12-24	7.895	10.620	3.758	7.281	1.604
X0016913	SUBJ022,10.0,24-48	1.565	0.637	0.487	0.409	0.054
X0017241	SUBJ023,10.0,-12-0	0.108	0.076	0.032	0.029	N.D.
X0017250	SUBJ023,10.0,0-12	24.915	303.562	31.203	14.000	3.605
X0017269	SUBJ023,10.0,12-24	12.566	4.036	3.422	5.562	0.958
X0017278	SUBJ023,10.0,24-48	0.345	0.081	0.448	0.183	0.156
X0017287	SUBJ024,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017296	SUBJ024,PL,0-12	0.090	0.120	N.D.	N.D.	N.D.
X0017302	SUBJ024,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0017311	SUBJ024,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0017321	SUBJ025,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017330	SUBJ025,PL,0-12	N.D.	N.D.	N.Q.	N.D.	N.D.
X0017349	SUBJ025,PL,12-24	N.D.	N.D.	N.D.	N.Q.	N.D.
X0017358	SUBJ025,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0017367	SUBJ026,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017376	SUBJ026,15.0,0-12	32.457	296.546	115.085	53.172	9.321
X0017385	SUBJ026,15.0,12-24	5.253	7.605	9.377	12.000	1.093
X0017394	SUBJ026,15.0,24-48	0.180	0.137	0.386	0.249	0.057
X0017401	SUBJ927,10.0,-12-0	N.D.	N.D.	0.347	N.D.	0.295
X0017410	SUBJ927,10.0,0-12	8.811	168.587	30.431	62.508	2.745
X0017429	SUBJ927,10.0,12-24	28.250	16.117	4.431	14.022	2.045
X0017438	SUBJ927,10.0,24-48	3.977	2.519	0.538	3.535	0.715
X0017447	SUBJ028,10.0,-12-0	N.D.	N.D.	0.275	0.053	0.095
X0017456	SUBJ028,10.0,0-12	8.766	58.679	37.532	45.460	3.182
X0017465	SUBJ028,10.0,12-24	2.424	2.887	3.672	5.629	0.677
X0017474	SUBJ028,10.0,24-48	0.403	0.479	0.416	0.835	0.159
X0017483	SUBJ029,15.0,-12-0	N.D.	N.D.	0.325	N.D.	N.D.
X0017492	SUBJ029,15.0,0-12	15.152	264.142	57.861	164.924	9.322
X0017509	SUBJ029,15.0,12-24	7.558	3.357	3.802	8.489	0.796
X0017518	SUBJ029,15.0,24-48	1.074	0.197	0.734	0.785	0.082

Table V. Malathion Metabolites in Human Urine Samples (Continued)

PTL ID #	Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
X0017527	SUBJ030,10.0,-12-0	N.D.	N.D.	N.D.	N.Q.	N.D.
X0017536	SUBJ030,10.0,0-12	12.476	100.518	39.184	47.183	2.184
X0017545	SUBJ030,10.0,12-24	4.190	2.262	2.762	3.979	0.385
X0017554	SUBJ030,10.0,24-48	0.212	0.139	0.142	0.170	0.032
X0017563	SUBJ031,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017572	SUBJ031,15.0,0-12	22.973	242.167	43.293	71.286	5.628
X0017581	SUBJ031,15.0,12-24	19.168	8.887	3.159	4.804	1.505
X0017591	SUBJ031,15.0,24-48	0.897	0.329	0.910	1.022	0.308
X0017607	SUBJ032,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017616	SUBJ032,PL,0-12	N.Q.	N.Q.	N.D.	N.D.	N.D.
X0017625	SUBJ032,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0017634	SUBJ032,PL,24-48	N.Q.	N.D.	N.D.	N.D.	N.D.
X0017643	SUBJ033,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017652	SUBJ033,15.0,0-12	56.141	492.237	43.642	136.535	8.172
X0017661	SUBJ033,15.0,12-24	39.573	13.671	5.330	10.013	1.866
X0017671	SUBJ033,15.0,24-48	2.803	0.890	0.800	1.152	0.358
X0017680	SUBJ034,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017699	SUBJ034,15.0,0-12	244.283	56.226	51.377	43.819	1.632
X0017705	SUBJ034,15.0,12-24	38.638	43.932	6.235	12.190	1.339
X0017714	SUBJ034,15.0,24-48	0.746	0.559	0.526	0.561	0.069
X0017732	SUBJ035,PL,-12-0	N.Q.	N.D.	N.D.	N.D.	N.D.
X0017741	SUBJ035,PL,0-12	0.022	0.026	N.D.	N.D.	N.D.
X0017751	SUBJ035,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0017760	SUBJ035,PL,24-48	N.Q.	N.D.	N.D.	N.D.	N.D.
X0017779	SUBJ036,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017788	SUBJ036,PL,0-12	N.Q.	N.Q.	N.D.	N.D.	N.D.
X0017797	SUBJ036,PL,12-24	N.D.	N.Q.	N.D.	N.D.	N.D.
X0017803	SUBJ036,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0017812	SUBJ037,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017821	SUBJ037,15.0,0-12	62.040	896.963	197.150	424.825	11.828
X0017831	SUBJ037,15.0,2-24	8.049	7.377	5.869	16.477	3.203
X0017840	SUBJ037,15.0,24-48	0.405	0.192	0.609	0.645	0.241
X0017859	SUBJ038,15.0,-12-0	N.D.	N.D.	0.034	N.Q.	N.D.
X0017868	SUBJ038,15.0,0-12	102.042	860.760	279.044	85.063	1.844
X0017877	SUBJ038,15.0,12-24	16.953	10.502	5.205	8.870	1.370
X0017886	SUBJ038,15.0,24-48	0.683	0.581	0.362	0.403	0.076
X0017895	SUBJ039,15.0,-12-0	N.D.	N.D.	0.036	0.028	N.Q.
X0017901	SUBJ039,15.0,0-12	18.813	181.273	55.411	15.484	2.656
X0017911	SUBJ039,15.0,12-24	1.321	0.629	2.430	2.952	0.312
X0017920	SUBJ039,15.0,24-48	0.201	0.064	0.317	0.259	0.051
X0017939	SUBJ040,15.0,-12-0	N.D.	N.D.	0.069	0.039	0.026
X0017948	SUBJ040,15.0,0-12	16.909	155.970	18.162	67.650	22.921
X0017957	SUBJ040,15.0,12-24	14.944	3.999	3.335	4.934	0.447
X0017966	SUBJ040,15.0,24-48	0.772	0.328	0.504	0.519	0.135

Table V. Malathion Metabolites in Human Urine Samples (Continued)

PTL ID #	Sample Description	DCA Metabolite (ppm)	MCA Metabolite (ppm)	DMP Metabolite (ppm)	DMTP Metabolite (ppm)	DMDTP Metabolite (ppm)
X0017975	SUBJ041,PL,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0017984	SUBJ041,PL,0-12	N.D.	N.D.	N.D.	N.D.	N.D.
X0017993	SUBJ041,PL,12-24	N.D.	N.D.	N.D.	N.D.	N.D.
X0018005	SUBJ041,PL,24-48	N.D.	N.D.	N.D.	N.D.	N.D.
X0018014	SUBJ042,15.0,-12-0	N.D.	N.D.	N.Q.	N.D.	N.D.
X0018023	SUBJ042,15.0,0-12	25.959	178.856	4.718	13.619	3.948
X0018032	SUBJ042,15.0,12-24	6.463	1.303	4.943	4.111	1.194
X0018041	SUBJ042,15.0,24-48	0.212	0.237	0.431	0.476	0.152
X0018051	SUBJ043,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0018060	SUBJ043,15.0,0-12	6.074	204.058	50.916	73.732	2.666
X0018079	SUBJ043,15.0,12-24	1.872	2.603	1.780	2.982	0.575
X0018088	SUBJ043,15.0,24-48	0.870	1.253	0.320	1.077	0.112
X0018121	SUBJ044,15.0,-12-0	0.023	0.073	0.041	0.047	N.D.
X0018131	SUBJ044,15.0,0-12	33.767	59.882	126.575	175.959	3.037
X0018140	SUBJ044,15.0,12-24	9.326	4.483	5.889	10.948	2.276
X0018159	SUBJ044,15.0,24-48	0.485	0.275	1.412	0.961	0.122
X0018168	SUBJ045,PL,-12-0	0.029	0.089	N.Q.	0.026	N.Q.
X0018177	SUBJ045,PL,0-12	N.D.	N.D.	N.D.	N.Q.	N.D.
X0018186	SUBJ045,PL,12-24	N.Q.	N.Q.	N.D.	0.028	N.Q.
X0018195	SUBJ045,PL,24-48	0.243	0.218	N.D.	N.D.	N.D.
X0018201	SUBJ046,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0018211	SUBJ046,15.0,0-12	61.687	308.218	10.248	7.553	1.257
X0018220	SUBJ046,15.0,12-24	5.362	11.767	4.325	13.035	1.115
X0018239	SUBJ046,15.0,24-48	3.168	1.237	0.973	1.975	0.494
X0018248	SUBJ047,PL,-12-0	N.D.	N.Q.	0.027	0.037	N.D.
X0018257	SUBJ047,PL,0-12	N.D.	N.D.	N.D.	N.Q.	N.D.
X0018266	SUBJ047,PL,12-24	N.D.	N.D.	N.D.	N.Q.	N.D.
X0018275	SUBJ047,PL,24-48	N.D.	N.D.	N.D.	N.Q.	N.D.
X0018284	SUBJ948,15.0,-12-0	N.D.	N.D.	N.D.	N.D.	N.D.
X0018293	SUBJ948,15.0,0-12	30.985	302.771	26.003	92.939	8.213
X0018300	SUBJ948,15.0,12-24	6.727	12.786	3.568	7.637	0.240
X0018319	SUBJ948,15.0,24-48	1.497	2.043	0.716	1.074	0.238

Table VI. Urine Creatinine Data

PTL ID #	Sample Description	Creatinine (g/L)	PTL ID #	Sample Description	Creatinine (g/L)
X0016922	SUBJ001,0.5,-12-0	0.83	X0016486	SUBJ012,5.0,-12-0	1.91
X0016931	SUBJ001,0.5,0-12	0.57	X0016495	SUBJ012,5.0,0-12	1.98
X0016941	SUBJ001,0.5,12-24	2.61	X0016501	SUBJ012,5.0,12-24	2.86
X0016950	SUBJ001,0.5,24-48	0.89	X0016511	SUBJ012,5.0,24-48	1.41
X0016969	SUBJ002,0.5,-12-0	1.59	X0016520	SUBJ013,PL,-12-0	1.82
X0016978	SUBJ002,0.5,0-12	0.66	X0016539	SUBJ013,PL,0-12	1.35
X0016987	SUBJ002,0.5,12-24	1.30	X0016548	SUBJ013,PL,12-24	2.74
X0016996	SUBJ002,0.5,24-48	0.59	X0016593	SUBJ013,PL,24-48	1.90
X0017008	SUBJ003,PL,-12-0	1.59	X0016566	SUBJ014,5.0,-12-0	1.23
X0017017	SUBJ003,PL,0-12	0.94	X0016575	SUBJ014,5.0,0-12	1.28
X0017026	SUBJ003,PL,12-24	1.92	X0016584	SUBJ014,5.0,12-24	1.31
X0017071	SUBJ003,PL,24-48	0.70	X0016593	SUBJ014,5.0,24-48	1.32
X0017035	SUBJ004,0.5,-12-0	1.38	X0016600	SUBJ015,PL,-12-0	1.82
X0017044	SUBJ004,0.5,0-12	1.16	X0016619	SUBJ015,PL,0-12	1.64
X0017053	SUBJ004,0.5,12-24	0.78	X0016628	SUBJ015,PL,12-24	2.59
X0017062	SUBJ004,0.5,24-48	1.25	X0016637	SUBJ015,PL,24-48	1.46
X0017081	SUBJ005,1.5,-12-0	1.39	X0016646	SUBJ016,PL,-12-0	1.65
X0017090	SUBJ005,1.5,0-12	1.26	X0016655	SUBJ016,PL,0-12	1.04
X0017106	SUBJ005,1.5,12-24	2.11	X0016664	SUBJ016,PL,12-24	2.13
X0017115	SUBJ005,1.5,24-48	1.03	X0016673	SUBJ016,PL,24-48	1.57
X0017124	SUBJ006,1.5,-12-0	1.74	X0016682	SUBJ017,5.0,-12-0	1.19
X0017133	SUBJ006,1.5,0-12	0.88	X0016691	SUBJ017,5.0,0-12	0.97
X0017142	SUBJ006,1.5,12-24	1.75	X0016708	SUBJ017,5.0,12-24	0.90
X0017151	SUBJ006,1.5,24-48	1.42	X0016717	SUBJ017,5.0,24-48	1.50
X0017161	SUBJ007,PL,-12-0	0.98	X0016726	SUBJ018,5.0,-12-0	1.24
X0017170	SUBJ007,PL,0-12	0.74	X0016735	SUBJ018,5.0,0-12	0.52
X0017189	SUBJ007,PL,12-24	1.12	X0016744	SUBJ018,5.0,12-24	1.31
X0017198	SUBJ007,PL,24-48	0.78	X0016753	SUBJ018,5.0,24-48	0.94
X0017204	SUBJ008,1.5,-12-0	0.76	X0016762	SUBJ019,10.0,-12-0	1.95
X0017213	SUBJ008,1.5,0-12	0.68	X0016771	SUBJ019,10.0,0-12	1.80
X0017222	SUBJ008,1.5,12-24	1.02	X0016781	SUBJ019,10.0,12-24	1.38
X0017231	SUBJ008,1.5,24-48	1.53	X0016790	SUBJ019,10.0,24-48	1.76
X0016360	SUBJ009,5.0,-12-0	1.76	X0016806	SUBJ020,10.0,-12-0	2.53
X0016379	SUBJ009,5.0,0-12	1.71	X0016815	SUBJ020,10.0,0-12	1.37
X0016388	SUBJ009,5.0,12-24	2.08	X0016824	SUBJ020,10.0,12-24	0.96
X0016397	SUBJ009,5.0,24-48	1.20	X0016833	SUBJ020,10.0,24-48	1.11
X0013403	SUBJ010,5.0,-12-0	0.78	X0016842	SUBJ021,PL,-12-0	1.08
X0016412	SUBJ010,5.0,0-12	0.37	X0016851	SUBJ021,PL,0-12	1.26
X0016421	SUBJ010,5.0,12-24	0.89	X0016861	SUBJ021,PL,12-24	0.53
X0016431	SUBJ010,5.0,24-48	1.44	X0016870	SUBJ021,PL,24-48	0.47
X0016440	SUBJ011,5.0,-12-0	0.97	X0016889	SUBJ022,10.0,-12-0	1.46
X0016459	SUBJ011,5.0,0-12	2.65	X0016898	SUBJ022,10.0,0-12	1.28
X0016468	SUBJ011,5.0,12-24	1.96	X0016904	SUBJ022,10.0,12-24	1.65
X0016477	SUBJ011,5.0,24-48	1.78	X0016913	SUBJ022,10.0,24-48	0.82

Table VI. Urine Creatinine Data (Continued)

PTL ID #	Sample Description	Creatinine (g/L)	PTL ID #	Sample Description	Creatinine (g/L)
X0017241	SUBJ023,10.0,-12-0	1.60	X0017680	SUBJ034,15.0,-12-0	0.97
X0017250	SUBJ023,10.0,0-12	0.66	X0017699	SUBJ034,15.0,0-12	0.70
X0017269	SUBJ023,10.0,12-24	1.30	X0017705	SUBJ034,15.0,12-24	2.23
X0017278	SUBJ023,10.0,24-48	0.78	X0017714	SUBJ034,15.0,24-48	0.95
X0017287	SUBJ024,PL,-12-0	0.56	X0017732	SUBJ035,PL,-12-0	1.46
X0017296	SUBJ024,PL,0-12	0.47	X0017741	SUBJ035,PL,0-12	0.72
X0017302	SUBJ024,PL,12-24	0.81	X0017751	SUBJ035,PL,12-24	1.51
X0017311	SUBJ024,PL,24-48	0.54	X0017760	SUBJ035,PL,24-48	0.85
X0017321	SUBJ025,PL,-12-0	1.64	X0017779	SUBJ036,PL,-12-0	1.24
X0017330	SUBJ025,PL,0-12	1.00	X0017788	SUBJ036,PL,0-12	1.47
X0017349	SUBJ025,PL,12-24	1.23	X0017797	SUBJ036,PL,12-24	1.46
X0017358	SUBJ025,PL,24-48	0.64	X0017803	SUBJ036,PL,24-48	0.72
X0017367	SUBJ026,15.0,-12-0	0.97	X0017812	SUBJ037,15.0,-12-0	0.85
X0017376	SUBJ026,15.0,0-12	0.54	X0017821	SUBJ037,15.0,0-12	0.90
X0017385	SUBJ026,15.0,12-24	1.40	X0017831	SUBJ037,15.0,12-24	1.31
X0017394	SUBJ026,15.0,24-48	0.85	X0017840	SUBJ037,15.0,24-48	0.76
X0017401	SUBJ027,10.0,-12-0	1.89	X0017859	SUBJ038,15.0,-12-0	2.11
X0017410	SUBJ027,10.0,0-12	0.75	X0017868	SUBJ038,15.0,0-12	1.99
X0017429	SUBJ027,10.0,12-24	2.22	X0017877	SUBJ038,15.0,12-24	1.84
X0017438	SUBJ027,10.0,24-48	2.11	X0017886	SUBJ038,15.0,24-48	1.15
X0017447	SUBJ028,10.0,-12-0	0.92	X0017895	SUBJ039,15.0,-12-0	2.14
X0017456	SUBJ028,10.0,0-12	0.79	X0017901	SUBJ039,15.0,0-12	0.56
X0017465	SUBJ028,10.0,12-24	0.85	X0017911	SUBJ039,15.0,12-24	0.45
X0017474	SUBJ028,10.0,24-48	0.87	X0017920	SUBJ039,15.0,24-48	0.45
X0017483	SUBJ029,15.0,-12-0	1.72	X0017939	SUBJ040,15.0,-12-0	1.97
X0017492	SUBJ029,15.0,0-12	0.84	X0017948	SUBJ040,15.0,0-12	0.55
X0017509	SUBJ029,15.0,12-24	1.22	X0017957	SUBJ040,15.0,12-24	1.39
X0017518	SUBJ029,15.0,24-48	1.12	X0017966	SUBJ040,15.0,24-48	0.86
X0017527	SUBJ030,10.0,-12-0	1.15	X0017975	SUBJ041,PL,-12-0	1.02
X0017536	SUBJ030,10.0,0-12	0.73	X0017984	SUBJ041,PL,0-12	0.80
X0017545	SUBJ030,10.0,12-24	0.94	X0017993	SUBJ041,PL,12-24	1.08
X0017554	SUBJ030,10.0,24-48	0.55	X0018005	SUBJ041,PL,24-48	0.88
X0017563	SUBJ031,15.0,-12-0	1.44	X0018014	SUBJ042,15.0,-12-0	1.38
X0017572	SUBJ031,15.0,0-12	0.90	X0018023	SUBJ042,15.0,0-12	0.57
X0017581	SUBJ031,15.0,12-24	1.25	X0018032	SUBJ042,15.0,12-24	1.16
X0017591	SUBJ031,15.0,24-48	1.02	X0018041	SUBJ042,15.0,24-48	0.65
X0017607	SUBJ032,PL,-12-0	1.42	X0018051	SUBJ043,15.0,-12-0	1.17
X0017616	SUBJ032,PL,0-12	0.96	X0018060	SUBJ043,15.0,0-12	0.92
X0017625	SUBJ032,PL,12-24	0.83	X0018079	SUBJ043,15.0,12-24	0.65
X0017634	SUBJ032,PL,24-48	0.67	X0018088	SUBJ043,15.0,24-48	0.58
X0017643	SUBJ033,15.0,-12-0	1.28	X0018121	SUBJ044,15.0,-12-0	0.68
X0017652	SUBJ033,15.0,0-12	1.16	X0018131	SUBJ044,15.0,0-12	1.48
X0017661	SUBJ033,15.0,12-24	1.57	X0018140	SUBJ044,15.0,12-24	1.40
X0017671	SUBJ033,15.0,24-48	1.47	X0018159	SUBJ044,15.0,24-48	1.36

Table VI. Urine Creatinine Data (Continued)

PTL ID #	Sample Description	Creatinine (g/L)	PTL ID #	Sample Description	Creatinine (g/L)
X0018168	SUBJ045,PL,-12-0	2.76	X0018248	SUBJ047,PL,-12-0	1.16
X0018177	SUBJ045,PL,0-12	3.61	X0018257	SUBJ047,PL,0-12	0.77
X0018186	SUBJ045,PL,12-24	1.02	X0018266	SUBJ047,PL,12-24	1.27
X0018195	SUBJ045,PL,24-48	1.13	X0018275	SUBJ047,PL,24-48	0.72
X0018501	SUBJ046,15.0,-12-0	0.74	X0018284	SUBJ948,15.0,-12-0	0.57
X0018211	SUBJ046,15.0,0-12	0.65	X0018293	SUBJ948,15.0,0-12	0.82
X0018220	SUBJ046,15.0,12-24	0.56	X0018300	SUBJ948,15.0,12-24	0.64
X0018239	SUBJ046,15.0,24-48	0.36	X0018319	SUBJ948,15.0,24-48	0.80

Table VII. Storage Stability Results for Malathion Acid and Phosphate Metabolites

Storage Interval (Weeks)	Average % Change from Nominal ^a									
	Metabolite ^b									
	DCA		MCA		DMP		DMTP		DMDTP	
	40 ^c	200	40	200	49	245	50	250	55	275
13	* ^d	*	*	*	+42	+62	+14	+4	-1	+24
32	*	*	*	*	+59 ^h	+39 ^h	+24 ^h	+18 ^h	-5 ^h	+14 ^h
37	+65 ^e	+68 ^e	-9 ^e	-14 ^e	*	*	*	*	*	*
43	*	*	*	*	-1 ^f	-3 ^f	+7 ^f	+19 ^f	-42 ^f	-31 ^f
44	+20	+23	+5	-7	*	*	*	*	*	*
52	-17	-9	-36	-36	*	*	*	*	*	*
53	*	*	*	*	-1	0	-11	-5	-33	-6
66	*	*	*	*	+58	+22	-11	-1	-15	+22
67	+19 ^f	+29 ^g	-13 ^f	-11 ^g	*	*	*	*	*	*

^a Values are an average of 3 replicates unless otherwise noted.

^b Metabolite Abbreviations:

DCA- Malathion Dicarboxylic Acid

MCA-Malathion Monocarboxylic Acid

DMP-Dimethylphosphate

DMTP-Dimethylthiophosphate

DMDTP-Dimethyldithiophosphate

^c Fortification level (in ppb)

^d *-No analysis taken at that interval.

^e -Average of 2 replicates

^f -Average of 6 replicates

^g -Average of 5 replicates

^h -Single Value

Figure 1 **Chromatogram of Malathion Carboxylic Acid Calibration Standard**

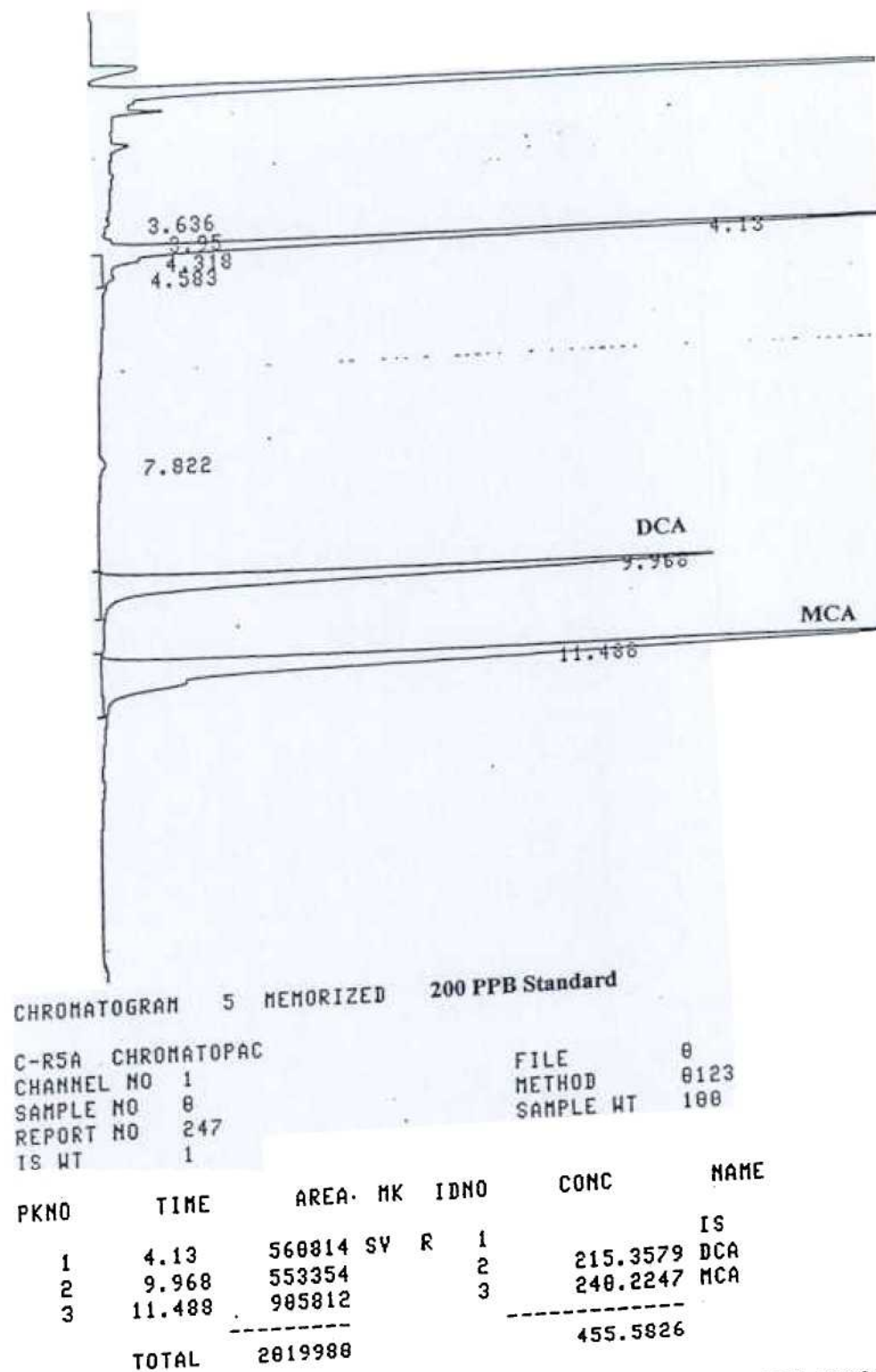
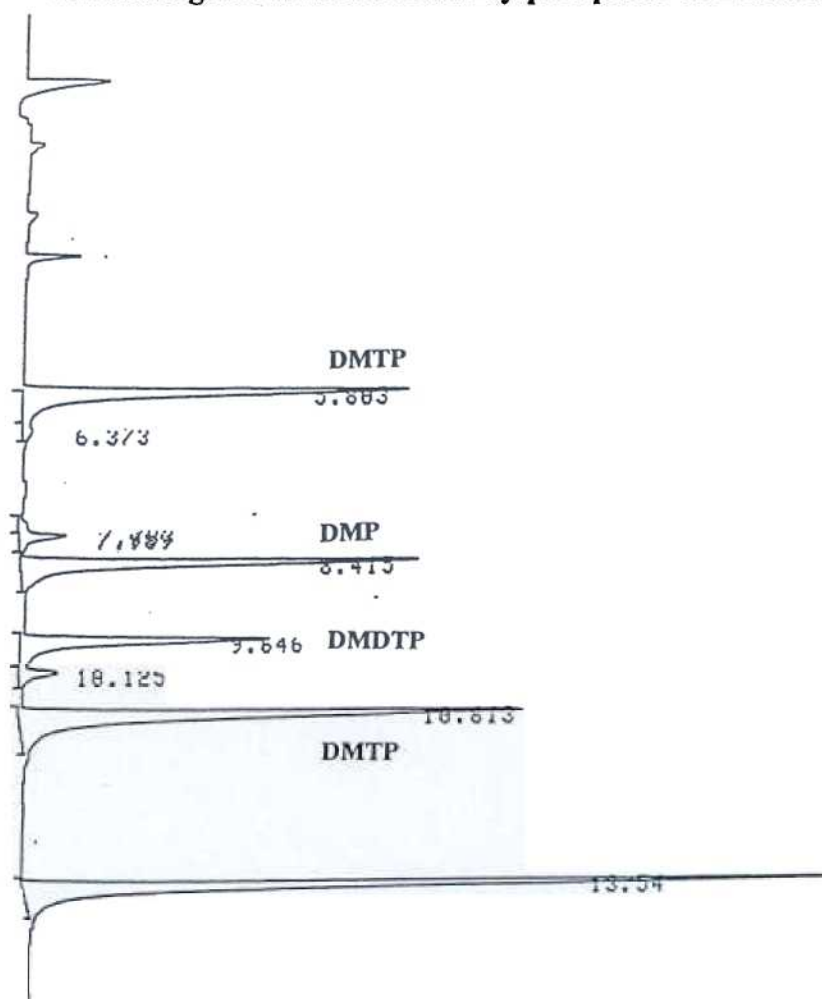


Figure 2 Chromatogram of Malathion Alkylphosphate Calibration Standard



CHROMATOGRAM 1 UNENRICHED 200 PPB Standard

C-KSA CHROMATOPAC
CHANNEL NO 1
SAMPLE NO 8
REPORT NO 1082
IS WT 1

FILE 0
METHOD 0123
SAMPLE WT 100

PKNU	TIME	AREA	PK	IDNO	CONC	NAME
1	5.883	225353		2	215.3359	DMTP
2	6.373	6198	Y			
3	7.783	7674				
4	7.989	21972	Y			
5	8.413	211898		3	165.2066	DMP
6	9.646	141975		4	209.498	DMDTP
7	10.125	20528	Y	4	38.291	DMDTP
8	10.813	348933		5	227.1253	DMTP
9	13.54	441465	R	1		IS
TOTAL		1417986			847.4569	

Figure 3 **Standard Linearity Curve: Malathion Dicarboxylic Acid**

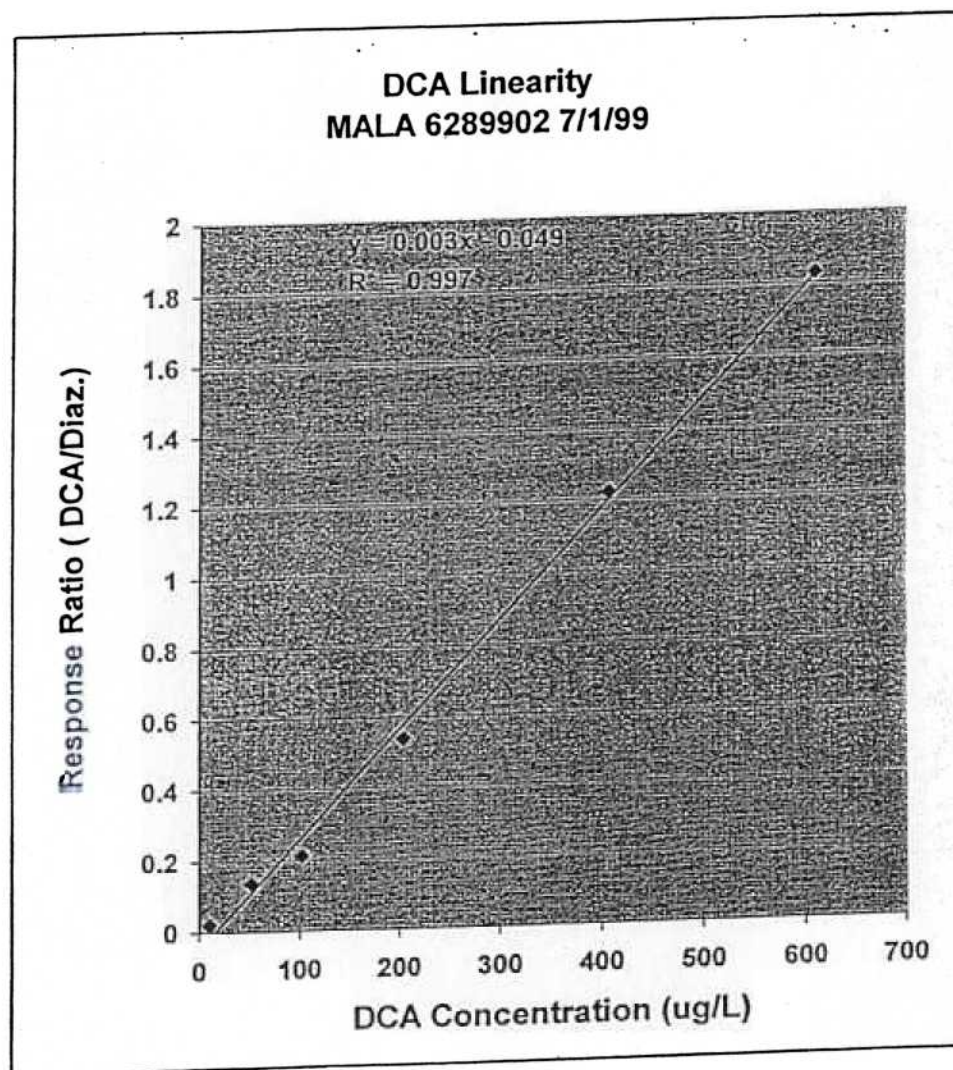


Figure 4 **Standard Linearity Curve: Malathion Monocarboxylic Acid**

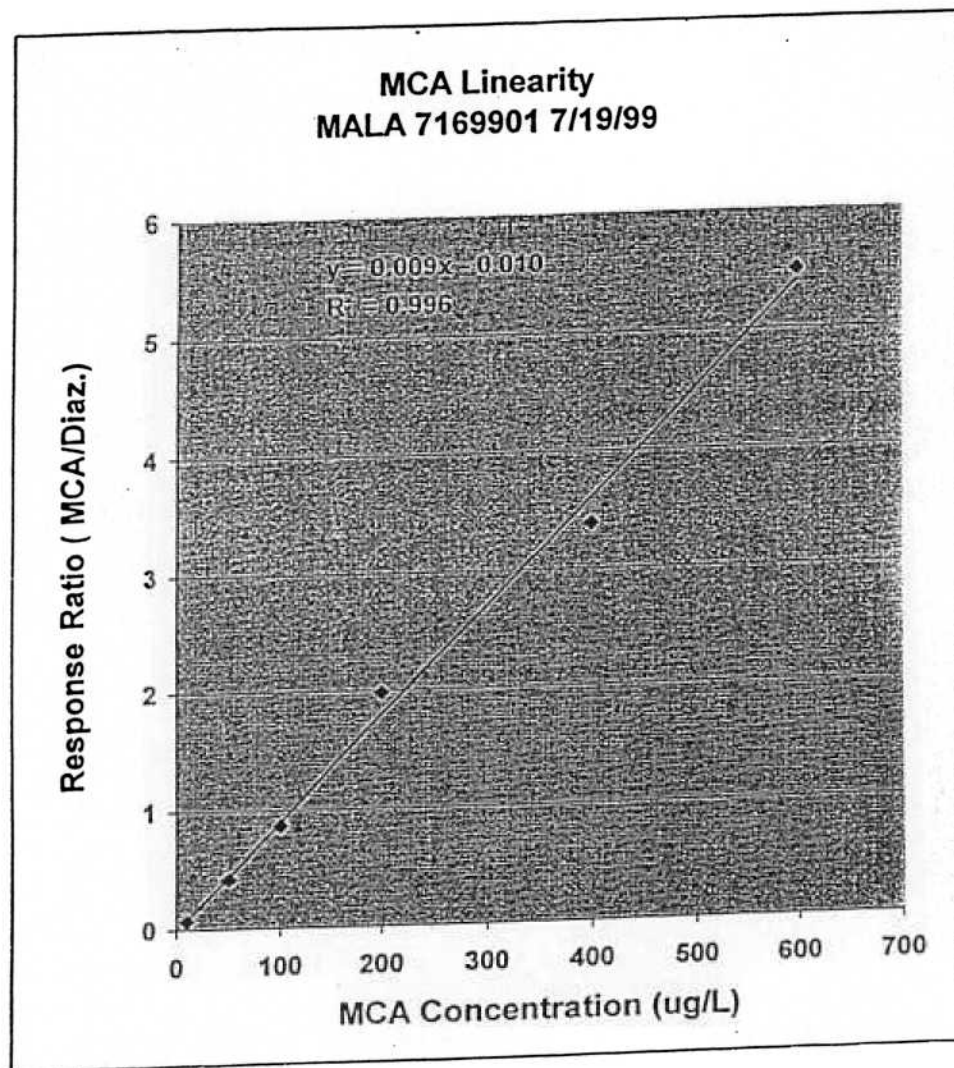


Figure 5 Standard Linearity Curve: Dimethyl Phosphate

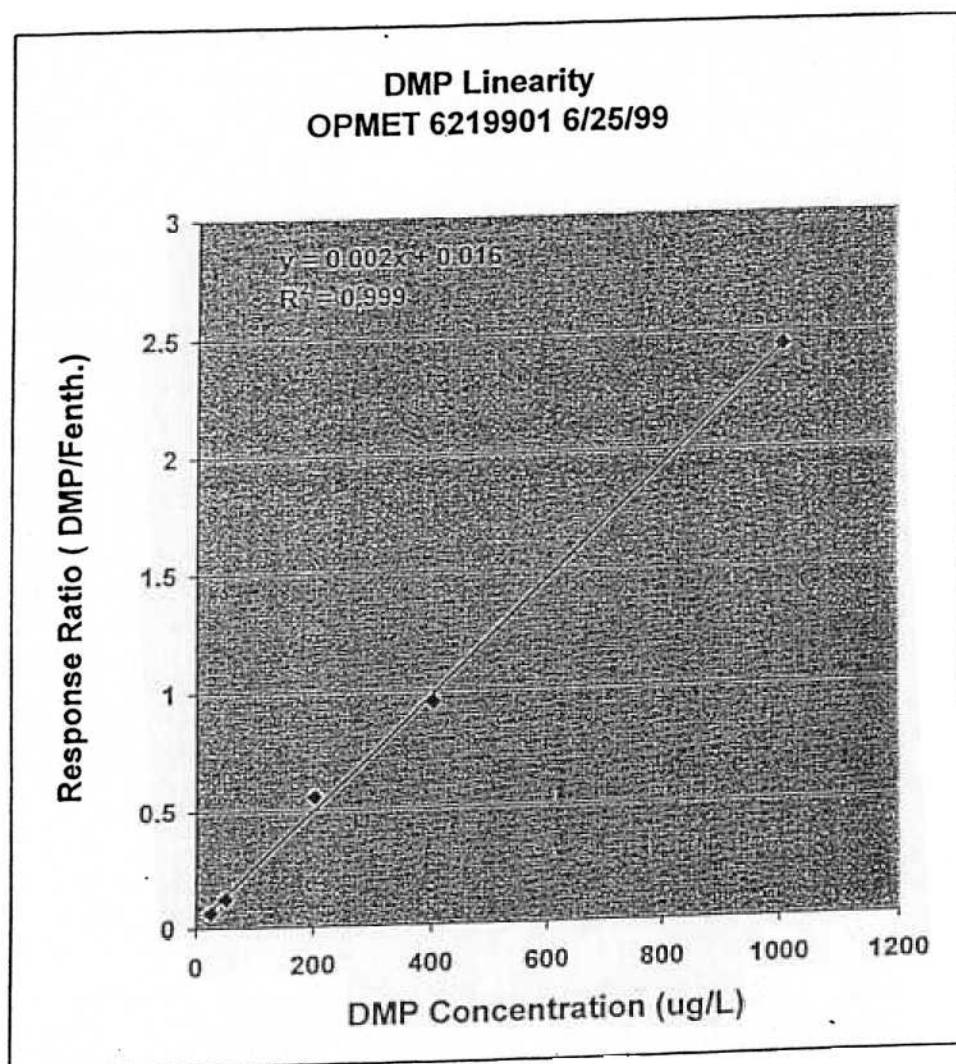


Figure 6 **Standard Linearity Curve: Dimethyl Thiophosphate**

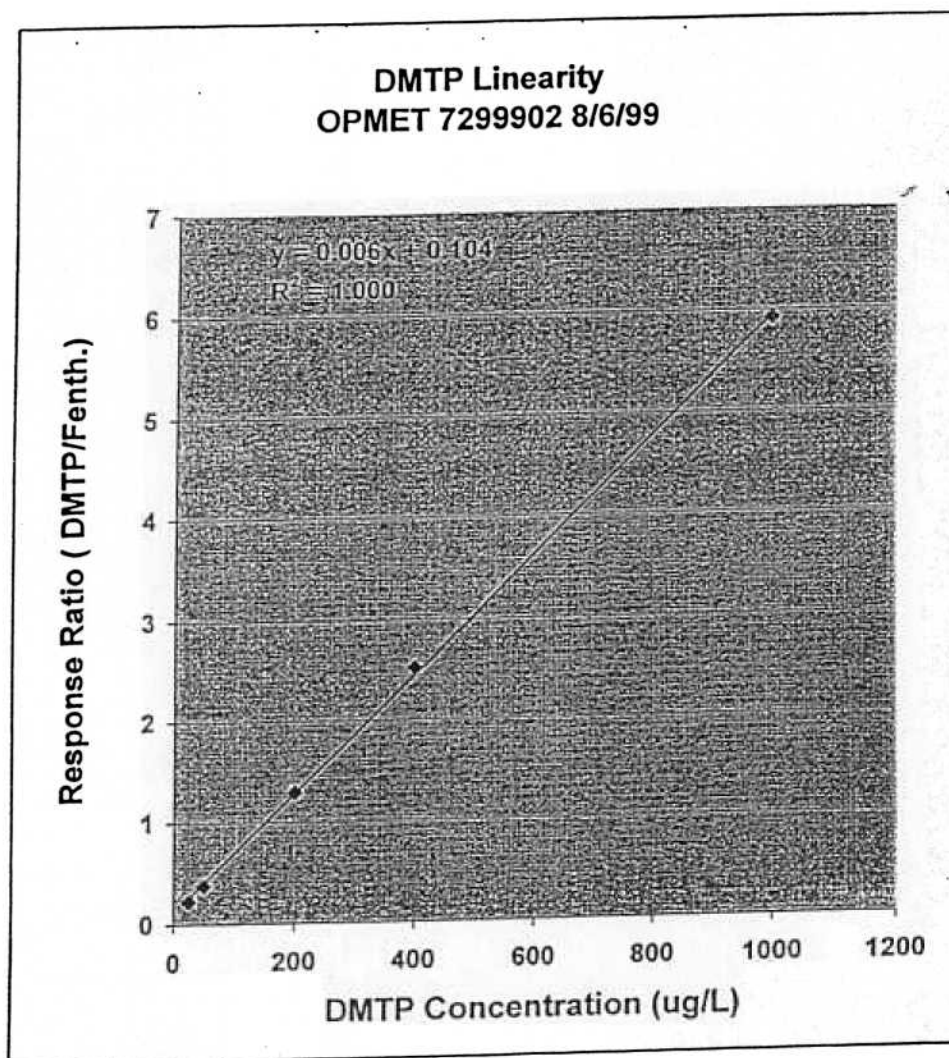


Figure 7 Standard Linearity Curve: Dimethyl Dithiophosphate

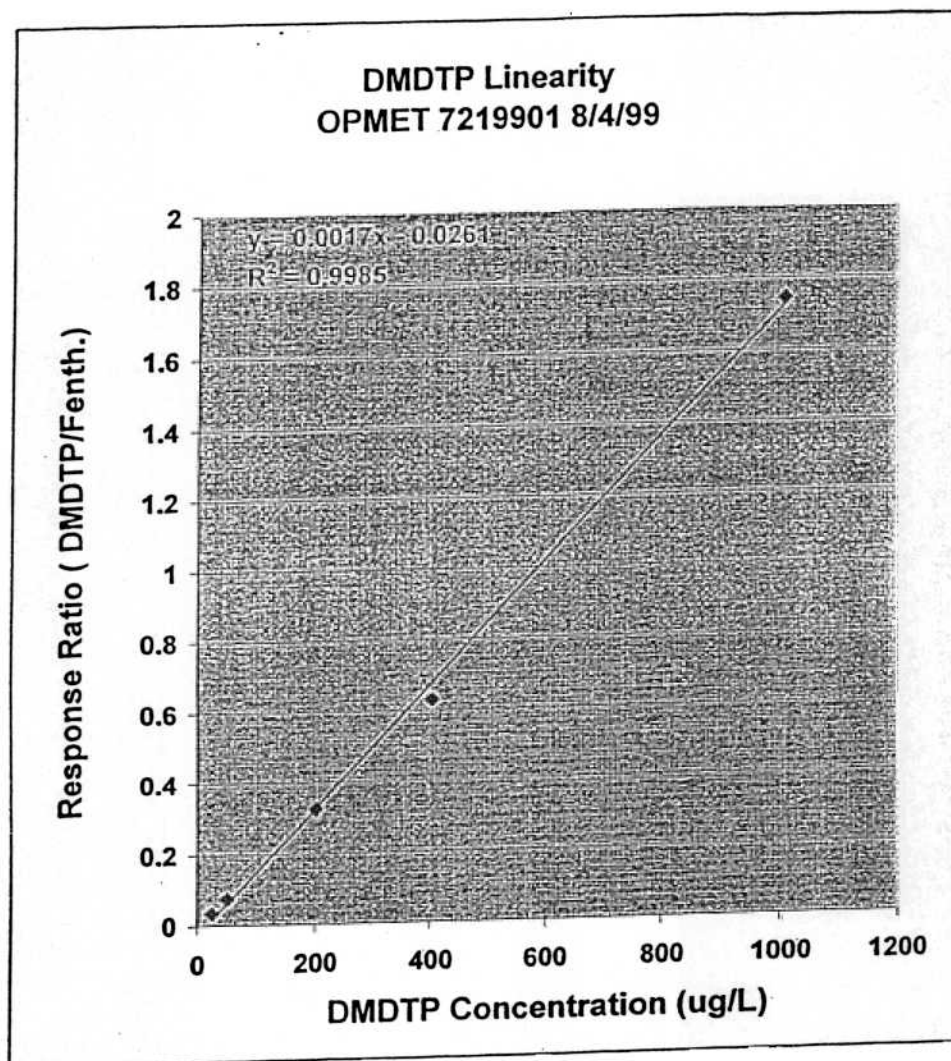
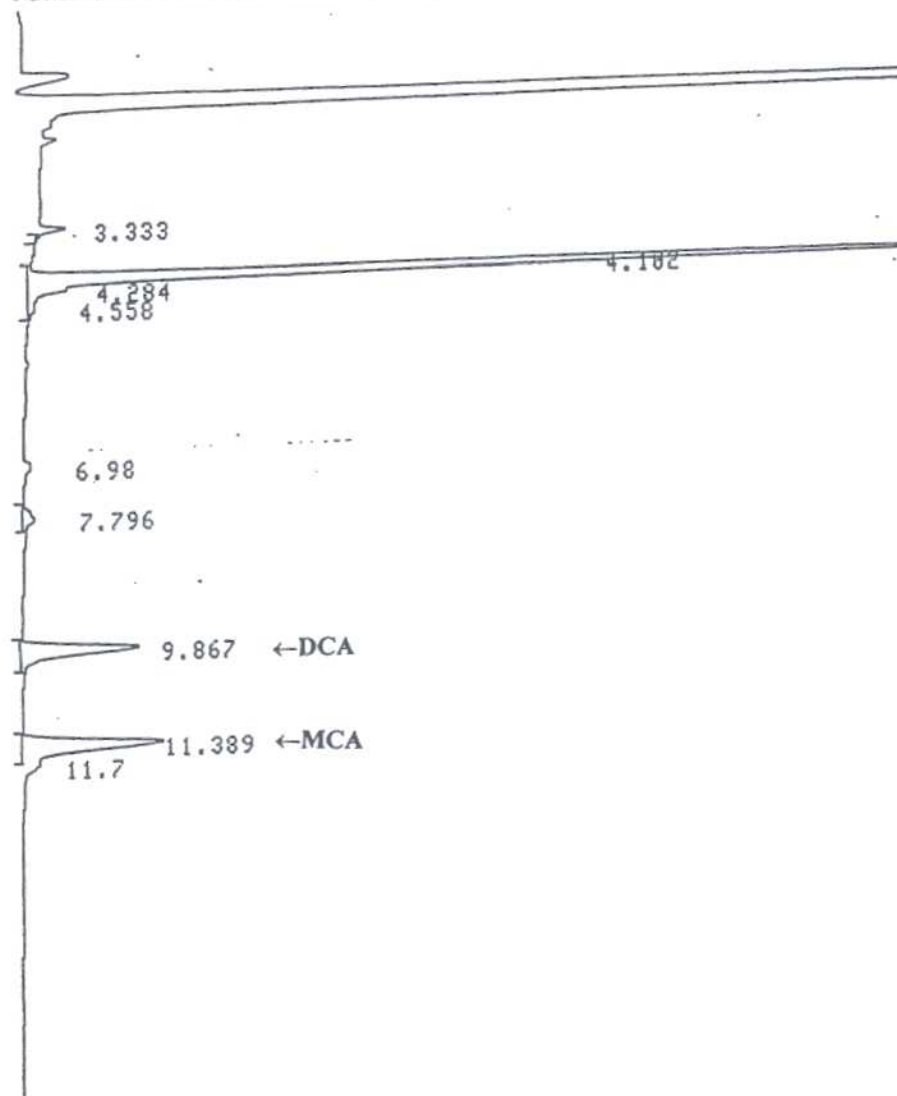


Figure 8

Malathion Acid Fortified (25 ppb) Human Urine Chromatogram



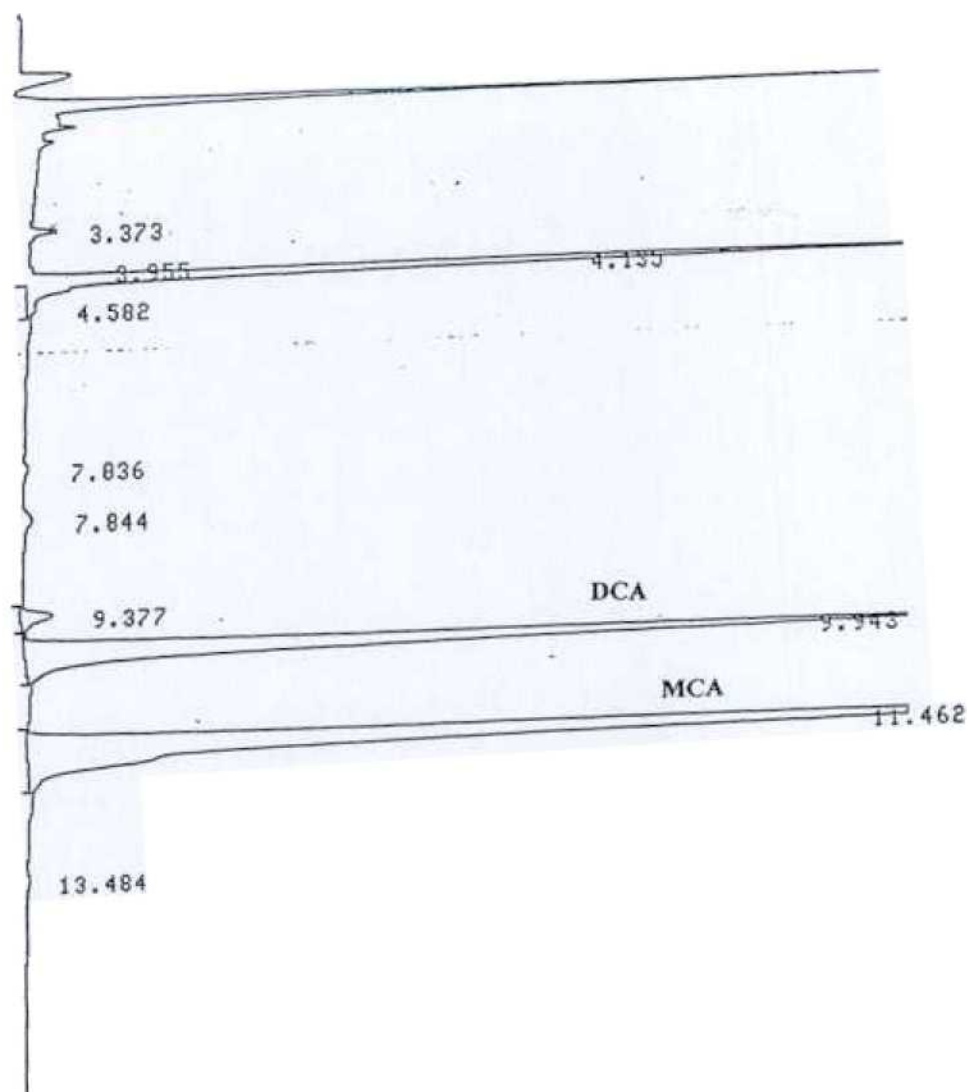
CHROMATOGRAM 10 MEMORIZED

C-R5A CHROMATOPAC
 CHANNEL NO 1
 SAMPLE NO 8
 REPORT NO 251
 IS WT 1

FILE 8
 METHOD 8123
 SAMPLE WT 100

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.333	10830				IS
2	4.102	508317	S	R 1		
3	7.796	11599				
4	9.867	98202		2	38.7389	DCA
5	11.389	121556			35.5665	MCA
TOTAL		742503			74.2974	

Figure 9 Malathion Acid Fortified (250 ppb) Human Urine Chromatogram



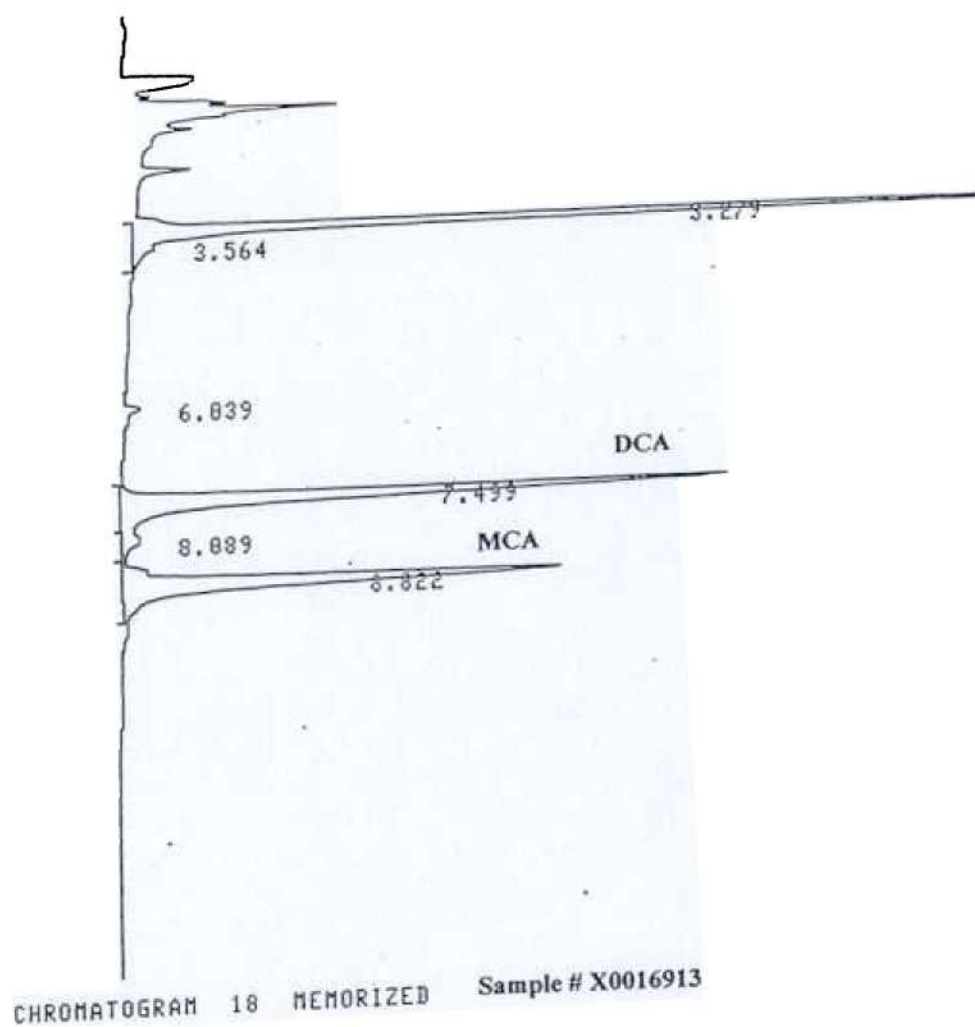
CHROMATOGRAM 34 MEMORIZED

C-R5A CHROMATOPAC
 CHANNEL NO 1
 SAMPLE NO 8
 REPORT NO 273
 IS WT 1

FILE 8
 METHOD 8123
 SAMPLE WT 180

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
	4.135	472682	SV	R 1		IS
2	9.377	24983			346.278	DCA
3	9.943	749796		2	354.1686	MCA
4	11.462	1125375		3		
TOTAL		2372677			788.4385	

Figure 10 Malathion Acid Human Urine Sample - DCA and MCA



C-RSA CHROMATOPAC
 CHANNEL NO 1
 SAMPLE NO 8
 REPORT NO 351
 IS WT 1

FILE 8
 METHOD 8123
 SAMPLE WT 188

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.279	547536	S	R 1	353.6926	DCA
2	7.499	489961	.	2	17.8453	DCA
3	8.089	19757	V	2	149.5164	MCA
4	8.822	343352		3		
TOTAL					528.2542	

Figure 11 Malathion Acid Human Urine Sample - DCA

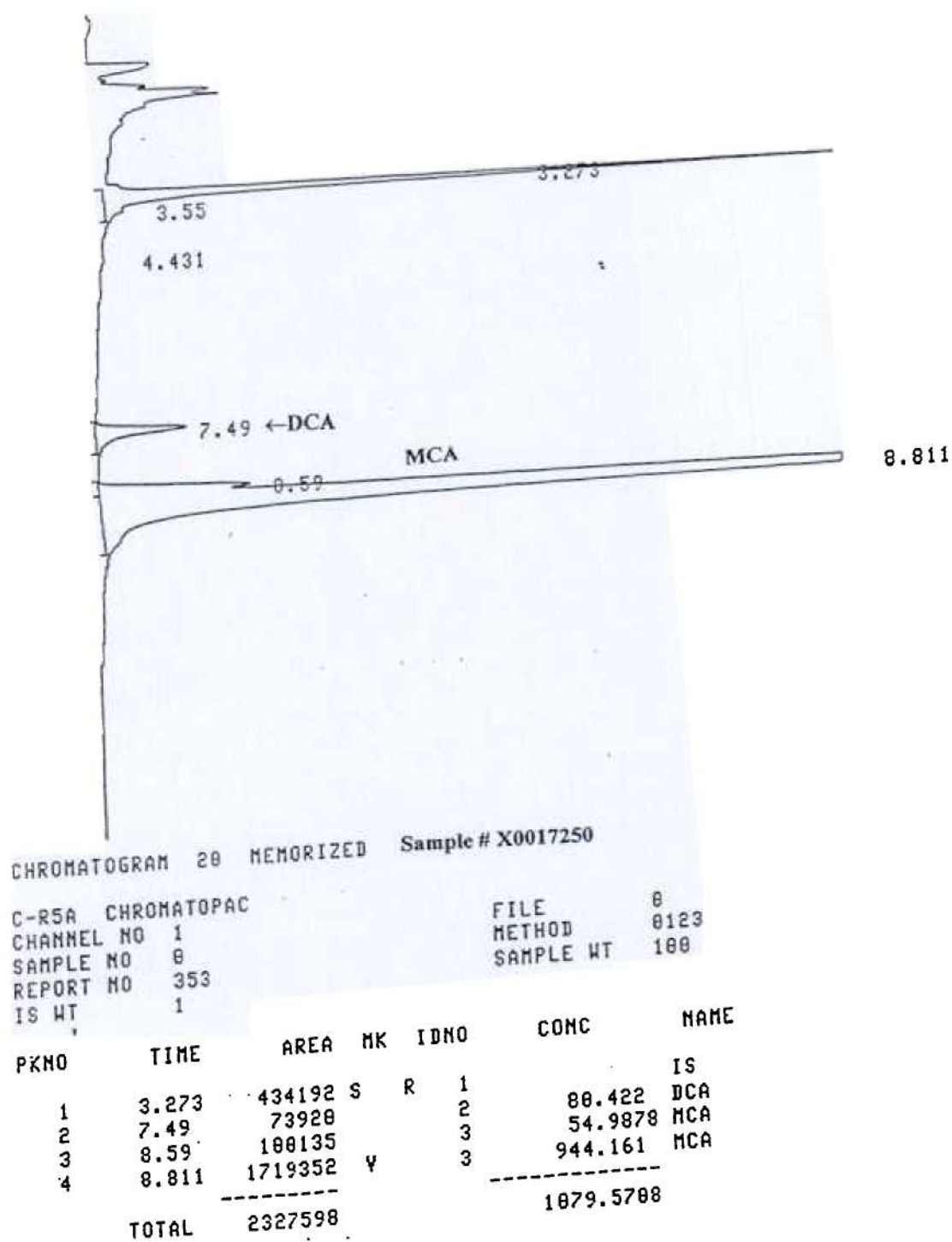
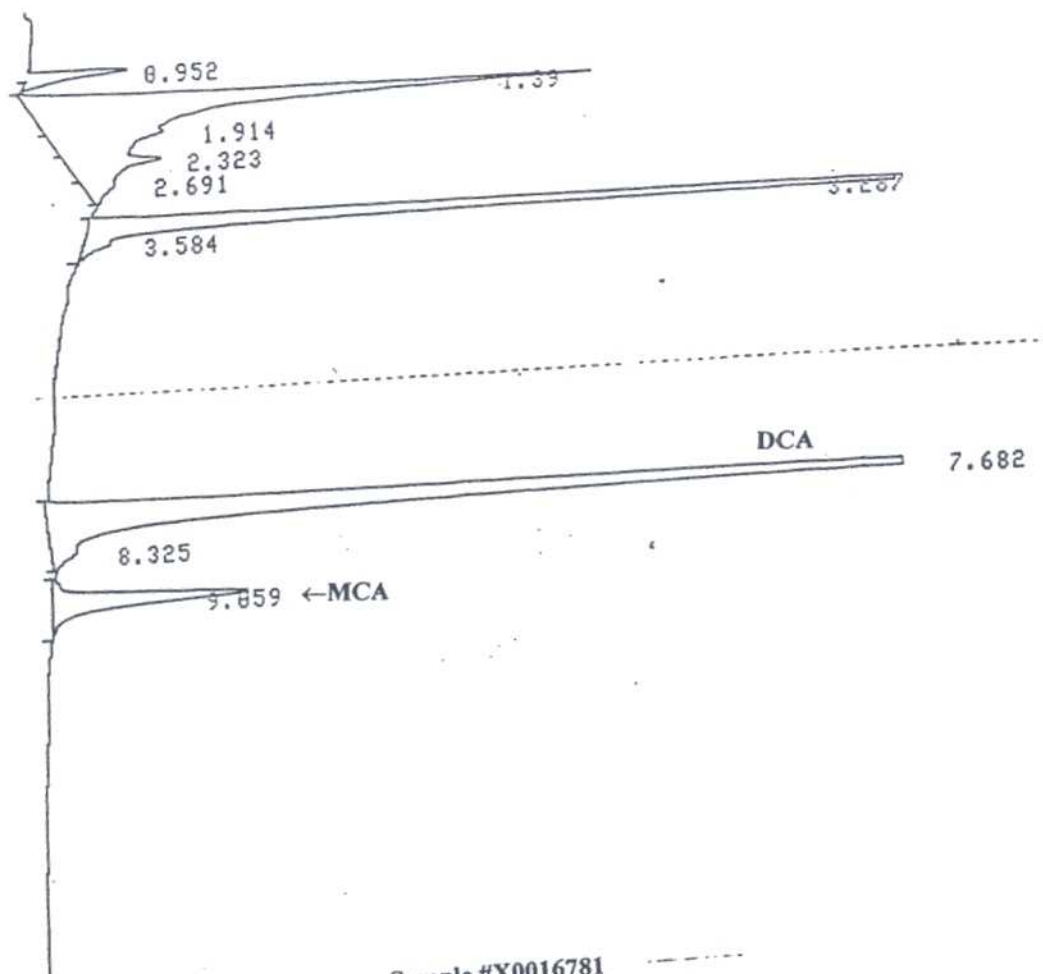


Figure 12 Malathion Acid Human Urine Sample-MCA



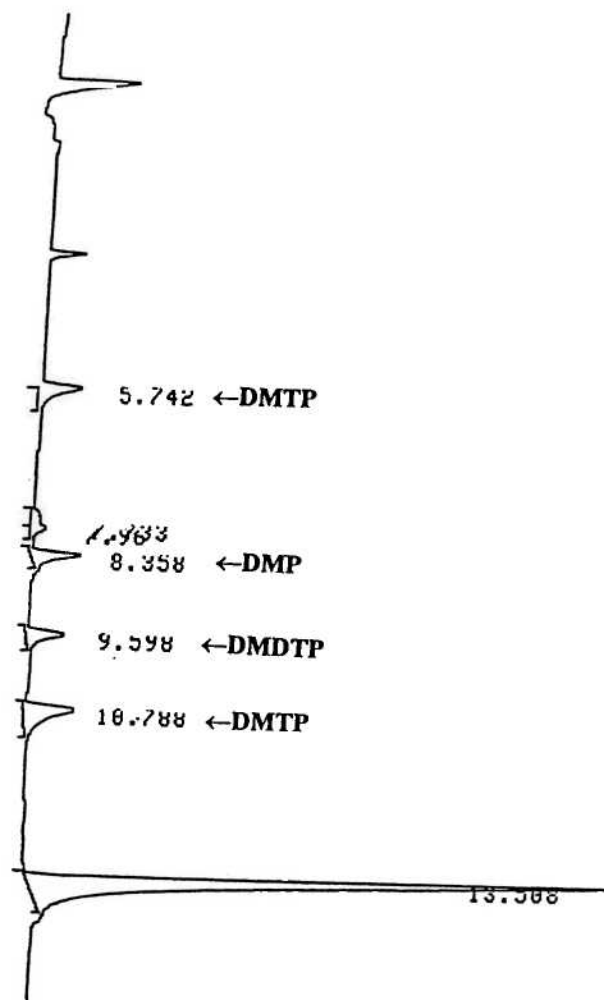
CHROMATOGRAM 27 MEMORIZED Sample #X0016781

C-R5A CHROMATOPAC
CHANNEL NO 1
SAMPLE NO 8
REPORT NO 298
IS WT 1

FILE 8
METHOD 8123
SAMPLE WT 100

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	0.952	56698				
2	1.39	587168				
3	1.914	129678	V			
4	2.323	187997	V			
5	2.691	25421	V			
6	3.287	615582	S	R 1	786.5557	IS
7	7.682	1229368	S	2	65.3338	DCA
8	9.859	187874		3		MCA
TOTAL		2938978			851.8894	

Figure 13 Malathion Alkyl Phosphate Fortified (30 ppb) Human Urine Chromatogram



CHROMATOGRAM 10 MEMORIZED

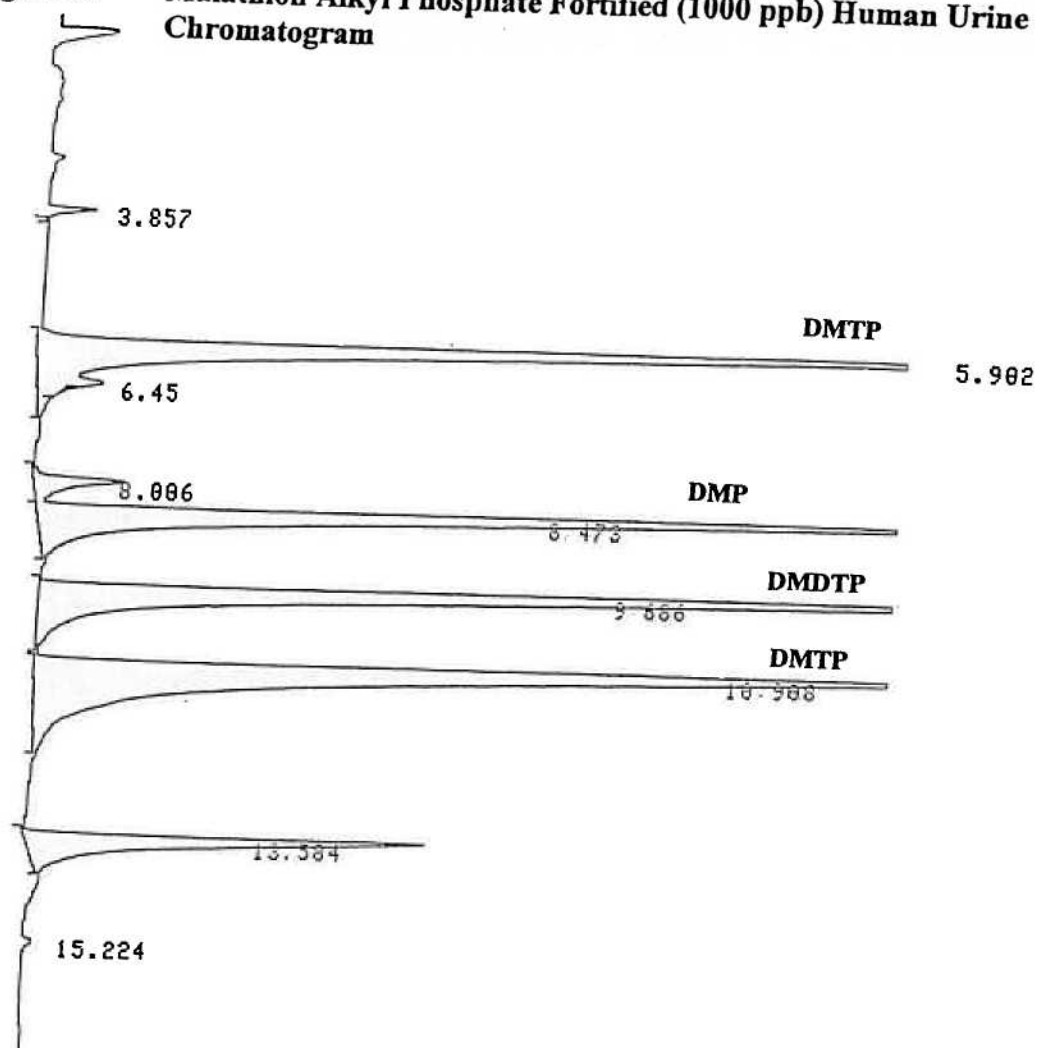
C-KSA CHROMATOPAC

CHANNEL NO 1
SAMPLE NO 8
REPORT NO 1098
IS WT 1

FILE 8
METHOD 8123
SAMPLE WT 188

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	5.742	29393		2	29.0782	DMTP
2	7.733	9573				
3	7.96	6863	Y			
4	8.358	26842		3	25.874	DMP
5	9.598	21879		4	30.3162	DMDTP
6	10.788	47886		5	30.7375	DMTP
7	13.588	396848	R	1		IS
TOTAL		536796			115.2858	

Figure 14 Malathion Alkyl Phosphate Fortified (1000 ppb) Human Urine Chromatogram



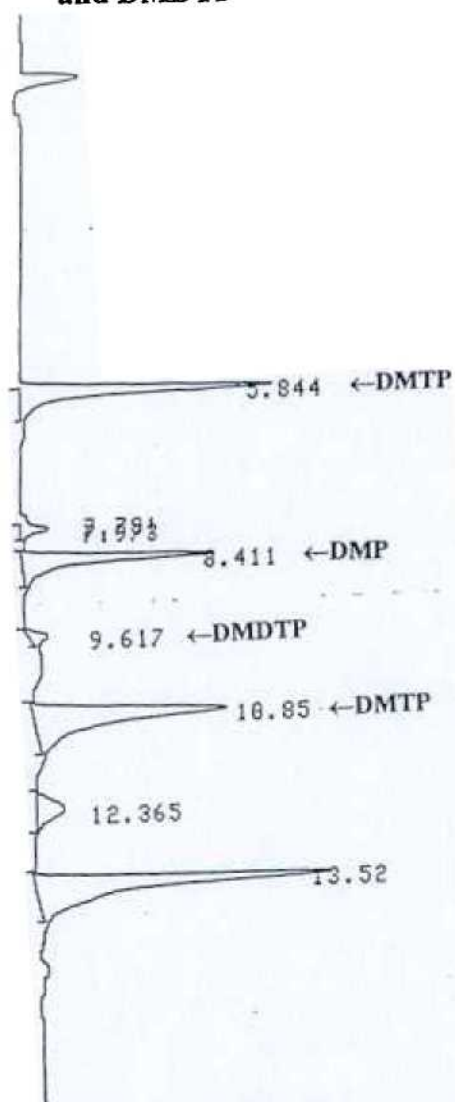
CHROMATOGRAM 1 MEMORIZED

C-RSA CHROMATOPAC
CHANNEL NO - 1
SAMPLE NO 8
REPORT NO 74
IS WT 1

FILE 8
METHOD 0123
SAMPLE WT 100

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.857	16442				
2	5.902	990464	S	2	989.5899	DMTP
3	6.45	20016	T			
4	8.006	64847				
5	8.473	796649	V	3	914.5411	DMP
6	9.686	946607		4	1616.7313	DMDTP
7	10.908	1220316		5	958.4697	DMTP
8	13.584	335483	R			IS
TOTAL		4390824			4479.332	

Figure 15 Malathion Alkyl Phosphate Human Urine Sample – DMP, DMTP, and DMDTP



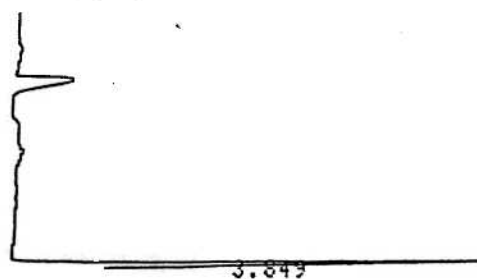
CHROMATOGRAM 1 MEMORIZED Sample #X0017142 (Diluted 1:9)

C-R5A CHROMATOPAC
CHANNEL NO 1
SAMPLE NO 8
REPORT NO 56
IS WT 1

FILE 8
METHOD 8123
SAMPLE WT 100

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	5.844	164503		2	199.3997	DMTP
2	7.973	14555	Y	3	169.7389	DMP
3	8.411	121874		4	22.8659	DMDTP
4	9.617	11035		5	211.5367	DMTP
5	10.85	221996				
6	12.365	39282				
7	13.52	276526	R	1		IS
TOTAL		849773			603.5411	

Figure 16 Human Urine Sample Negative for Malathion Alkyl Phosphate Metabolites



←DMTP (ND)

← 8.008

←DMP (ND)

←DMDTP (ND)

←DMTP (ND)

CHROMATOGRAM 1 MEMORIZED Sample #X0017204

C-R5A CHROMATOPAC

CHANNEL NO 1

SAMPLE NO 0

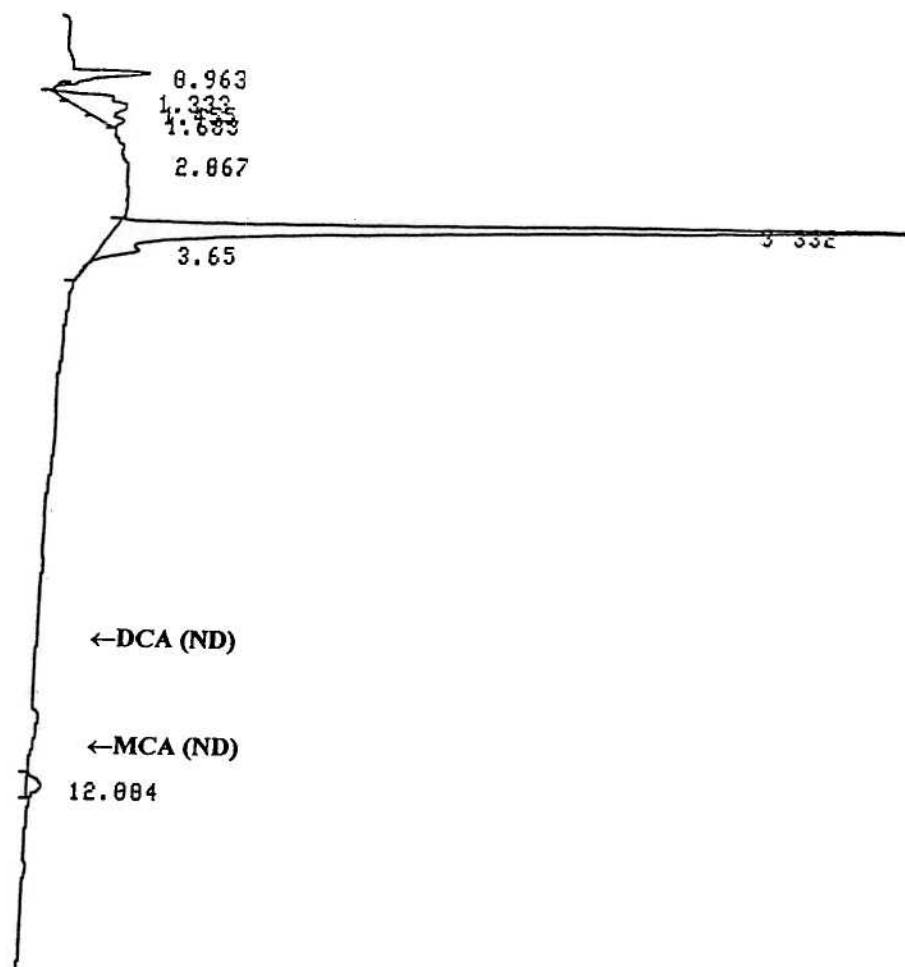
REPORT NO 62

IS WT 1

FILE 0
METHOD 0123
SAMPLE WT 100

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.849	136997				
2	8.008	29340				
	13.57	349429	R	1		IS
TOTAL		515766			0	

Figure 17 Human Urine Sample Negative for Malathion Acid Metabolites



CHROMATOGRAM 15 MEMORIZED Sample #X0016673

C-R5A CHROMATOPAC

CHANNEL NO 1
SAMPLE NO 0
REPORT NO 278
IS WT 1

FILE 0
METHOD 0123
SAMPLE WT 100

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.963	46816				
2	1.333	21883				
3	1.455	39320	Y			
4	1.683	12190	Y			
5	3.332	508770	S	R	1	IS
6	12.004	12642				

TOTAL 641621

0

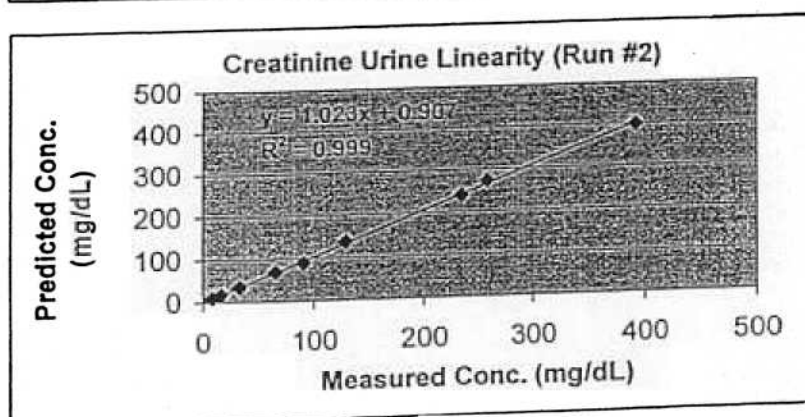
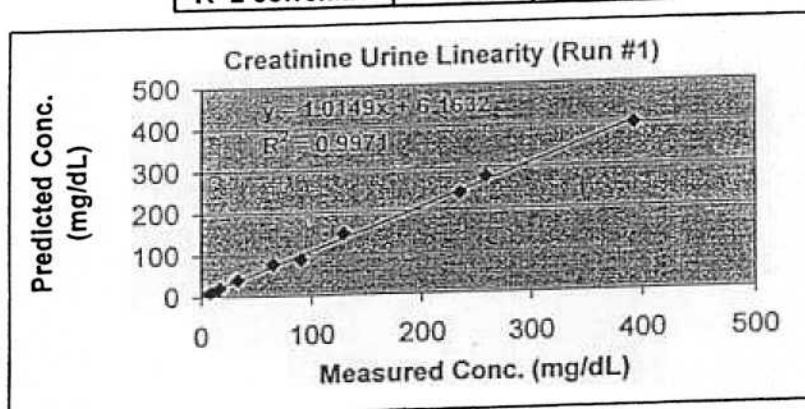
Figure 18 Creatinine Linearity in Human Urine

Linear Range: 10 to 400 mg/dL or 0.1 to 4.0 g/L

Original Concentration (after urine sample was diluted 1:2) was 516 mg/dL.
This urine was then diluted with DI water at the following ratios,
and measured against normal calibration and control materials.

DATA:

Dilution:	Original Conc.	Returned Conc.:	
	516		
	Linearity Conc.:	1st Run	2nd Run
3.8 : 5	392	399	399
1 : 2	258	278	271
high control	234	239	238
1 : 4	129	148	137
low control	90	87	88
1 : 8	65	77	68
1 : 16	32	40	33
1 : 32	16	20	17
1 : 64	8	11	9
slope		1.015	1.023
intercept		6.163	0.907
R^2 correlation		0.997	0.999



**APPENDIX 1-
PROTOCOL, PROTOCOL AMENDMENTS AND
DEVIATIONS**

STUDY PROTOCOL

STUDY NUMBER: PTL119801

STUDY TITLE:

Determination of Residues of Malathion Dicarboxylic Acid (DCA),
Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP),
Dimethyl Thiophosphate (DMTP) and Dimethyl Dithiophosphate
(DMDTP) in Human Urine.

STUDY DIRECTOR/TESTING FACILITY:

Linda Aston, Ph.D.
Director of Occupational Services
Pacific Toxicology Laboratories
6160 Varie! Ave
Woodland Hills, CA 91367
Telephone: 310-479-4911

SPONSOR:

Cheminova Agro A/S
P.O. Box 9
Lumvig Denmark

SPONSOR'S REPRESENTATIVE:

Matthew Brooks, Ph.D.
Senior Chemist
Jellineck, Schwartz and Connolly, Inc.
1525 Wilson Boulevard
Suite 600
Arlington, VA 22209
Telephone: 703-312-8557

PROPOSED STUDY SCHEDULE:

Analytical Start Date:	November 1998
Experiment Termination Date:	March 1999
Study Completion Date:	May 1999

OBJECTIVE:

To determine the magnitude of residue of malathion metabolites Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP) and Dimethyl Dithiophosphate (DMDTP) in Human Urine. The data will be used to further determine human exposure effects.

TEST SUBSTANCE:

The test substance was administered under separate protocol.

TEST SYSTEM:

Human urine collected from volunteers. The collection and justification for the test system is discussed under separate protocol.

ANALYTICAL PHASE

ANALYSIS FACILITY:

Pacific Toxicology Laboratories
6160 Varuel Ave
Woodland Hills, CA 91367

PRINCIPAL ANALYST:

To be named by protocol amendment.

SAMPLE PREPARATION PROCEDURES:

All samples will be prepared in accordance with applicable SOPs to provide a homogeneous sample for analysis. Any SOP or other procedure used for sample preparation as well as sample history and chain of custody information received with samples will be documented and placed in the study file.

REFERENCE SUBSTANCES:

Malathion Dicarboxylic Acid
Malathion Monocarboxylic Acid
Dimethyl Phosphate
Dimethyl Thiophosphate
Dimethyl Dithiophosphate

- All reference standards will be fully characterized and supplied by the Sponsor.
- All reference materials will be stored in the dark at -10° C or below.
- The Sponsor will supply material safety data sheets for all reference substances.

PROPOSED ANALYSIS PLAN:

Analysis of human urine for the listed malathion metabolites will be as prescribed in the analytical methods. The method limit of quantitation for malathion dicarboxylic acid and malathion monocarboxylic acid in human urine is targeted at 0.020 ppm and the method limit of quantitation for Dimethyl Phosphate, Dimethyl Thiophosphate, and Dimethyl Dithiophosphate in human urine is targeted at 0.025 ppm for each.

ANALYTICAL METHOD:

The analytical method to be used for determination of malathion dicarboxylic acid and malathion monocarboxylic acid in human urine is Pacific Toxicology Labs method 3-MALA-90710-3. The analytical method to be used for determination of Dimethyl Phosphate, Dimethyl Thiophosphate, Dimethyl Dithiophosphate in human urine is Pacific Toxicology Labs method 3-OPPS-870216-4. The analytical methods will be validated prior to initiation of sample analysis for the study. Fortifications of control urine will be employed with every analytical set. A 5-point calibration curve for quantitation of samples is to be run daily. A least squares coefficient of 0.99 or greater will be required to demonstrate quantitation of analytes within the concentrations bracketed by the calibration curve. The lowest standard on the calibration curve must be one-half the LOQ for each compound. A complete copy of the method will be placed in the study file. A complete list of all instrument operating conditions will be included in the final report.

Method Validation

The method will be fully validated with respect to system suitability, assay specificity, assay linearity, assay limit of detection, assay limit of quantification, intra-day assay accuracy and precision, and assay recovery as detailed below.

System Suitability and Linearity

The suitability and performance of the chromatographic and detection system will be evaluated. The column efficiency and tailing factor for all analytes will be defined. The detector linearity and system precision will be established.

Assay Specificity

The specificity of the assay will be examined by extraction and analysis of control human urine. The assay must be specific for all analytes and have no significant interfering substances eluting at the same retention time of the analytes.

Retention Time Window

The data produced during the validation will be assessed for variability in the retention times of all analytes. The intra-assay of inter-assay variabilities will be assessed and used to define a retention time window for each analyte for use during the analysis of routine samples.

Assay Recovery

In order to assure the validity of the method, untreated control samples of urine will be fortified with malathion dicarboxylic acid, malathion monocarboxylic acid, dimethyl phosphate, dimethyl thiophosphate, and dimethyl dithiophosphate at two different concentration levels. Two control samples of urine will be analyzed along with the spike samples:

0.0 (duplicate), 0.02 (triplicate) for Malathion dicarboxylic Acid and Malathion Monocarboxylic Acid OR 0.025 (triplicate) for Dimethyl Phosphate, Dimethyl thiophosphate, and Dimethyl Dithiophosphate, and 0.50 (triplicate, all analytes).

The average recovery of each fortification level must be within the range of 70-120% with a relative standard deviation of less than 15% (20% at the LOQ) for the set to be considered as acceptable. A complete list of all instrument operating conditions will be included in the final report.

System Linearity

The linearity of the gas chromatographic system will be demonstrated by utilization of a calibration curve of at least 5 concentrations over the analytical range. A least squares coefficient of 0.99 or greater will be required to demonstrate quantitation of analytes within the concentrations bracketed by the calibration curve. The lowest standard on the calibration curve must be one-half the LOQ for each compound.

Limit of Detection

A limit of detection must be established for each analyte. The analytical concentration can be determined from a standard injection but the value should take into account sample noise. The estimate should give a peak at least 3 times the magnitude of control sample noise. Fortification of a control extract with standard immediately prior to injection may be used to determine this value. The limit of detection must be no greater than one-half the limit of quantitation.

Limit of Quantitation

The limit of quantitation (LOQ) must be demonstrated by acceptable recoveries for each analyte at 0.02 ppm for Malathion dicarboxylic Acid and Malathion Monocarboxylic Acid and at 0.025 ppm for Dimethyl Phosphate, Dimethyl Thiophosphate, and Dimethyl Dithiophosphate. The criterion for recovery is described under method validation. The peak for each analyte must be free from chromatographic interference and a minimum of 6 times signal to noise.

STORAGE STABILITY

Control urine will be fortified with all analytes and frozen. Over subsequent periods of time stored and freshly fortified, samples will be analyzed in triplicate to determine the stability of each analyte in frozen urine. Fortification levels will be twice the limit of quantitation and 10 times the limit of quantitation. A record of sample numbers and analysis time intervals will be added by protocol amendment.

GENERAL RECORDS AND REPORTING**INSTRUMENTATION AND EQUIPMENT:**

Instruments and equipment that generate raw data or significantly impact the validity and results of a study should be maintained in proper working order. A logbook will be used to record maintenance, inspection, cleaning, and calibration of such equipment.

RECORDS TO BE MAINTAINED:

The Study Director must maintain all records necessary to support the study and to allow for reconstruction of the study in the study file. These records will be archived according to applicable SOPs and include, but are not limited to:

- The study protocol and all amendments.
- Data reporting forms that document test substance shipping, inventory, and use logs, sample storage temperature logs, sample receiving and storage records.
- A record of all SOP deviations.
- Laboratory equipment maintenance and calibration records.
- A list of all personnel involved with the study conduct.
- An exact copy of all applicable correspondence.
- An exact copy of the analytical method.
- Reference substance purity and preparation records.
- All laboratory raw data, including coversheets, chromatograms, and spreadsheets.
- Record of problems or protocol deviations and their justification and/or resolution.
- An exact copy of the final report.

STATISTICAL TREATMENT OF THE DATA:

Any statistical treatment of the data other than generation of means, standard deviations, linear correlations and/or correlation coefficients will be documented in the study file. An example of residue analytical calculations will be provided in the final study report.

GLP COMPLIANCE:

It is the policy of Pacific Toxicology Labs to conduct all studies in compliance with the Good Laboratory Practices and according to relevant SOPs. A GLPS compliance statement will be included in the final study report.

QUALITY ASSURANCE:

A Quality Assurance Unit of the laboratory will conduct inspections of the analytical phase of the study. Additional QAU may inspect the study and review the raw data and final report to verify the integrity of reported results and to assure compliance with existing regulations.

PROTOCOL ALTERATIONS/AMENDMENTS:

Any revisions to or deviations from the analytical phases of this protocol will be documented. The form must be completed, signed and sent promptly to the Study Director, Study Sponsor, and Quality Assurance Unit for authorization. A copy of any protocol alteration/amendment must be distributed by the Study Director, or delegate, to all study personnel.


REPORTING:

On completion of the study a draft report will be issued to the Sponsor's representative. The report will incorporate:

- Detailed descriptions of the methods used.
- Review/discussion of results obtained.
- Table/appendices of numerical data.
- Conclusions reached.

The data will be reported in ng/ml to 3 significant figures.

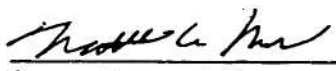
SIGNATURES:


Study Director
Linda S. Aston, Ph.D.

11-16-98
(Date)


Senior Quality Assurance Specialist
Terry Miller, MT

11/16/98
(Date)


Sponsor Representative
Matthew Brooks, Ph.D.

11/18/98
(Date)

STUDY PROTOCOL ALTERATION

Study Number: PTL119801

Alteration No. 1

Study Title: Determination of Residues of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP) and Dimethyl Dithiophosphate (DMDTP) in Human Urine.

☒ Amendment
☐ Deviation
(Choose one)

Revision:

Linda Aston is serving as Principal Analyst for this Project.

Reason for Revision:

Protocol states that a principal analyst will be named by protocol amendment

Impact on Study:

None.

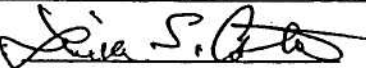
Initiator's
Signature:



Date: 9/30/99

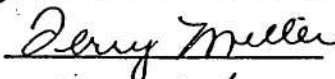
APPROVALS

Study Director:



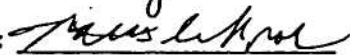
Date: 9/20/99

Quality Assurance:



Date: 10/1/99

Sponsor:



Date: 10/22/99

STUDY PROTOCOL ALTERATION

Study Number: PTL119801

Alteration No. 2

Study Title: Determination of Residues of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP) and Dimethyl Dithionphosphate (DMDTP) in Human Urine.

Amendment
Deviation
(Choose one)

Revision:

The following samples are being utilized for storage stability determination. The samples were fortified at the initiation of the study and are being analyzed as over the approximate time periods illustrated below.

Malathion Carboxylic Acid Metabolites		Malathion Alkyl Phosphate Metabolites	
Sample ID Number(s)	Storage Time	Sample ID Number (s)	Storage Time
SSB-1,2,3	5 Weeks	SSB-1,2,3	5 Weeks
SSB-4,5,6	10 Weeks	SSB-4,5,6	10 Weeks
SSB-7,8,9	15 Weeks	SSB-7,8,9	15 Weeks
SSB-10,11,12	35 Weeks	SSB-10,11,12	35 Weeks
SSB-13,14,15	45 Weeks	SSB-13,14,15	45 Weeks
SSB-16,17,18	55 Weeks	SSB-16,17,18	55 Weeks
SSB-19,20,21	65 Weeks	SS50-19,20,21	65 Weeks
SS40-1,2,3	5 Weeks	SS50-1,2,3	5 Weeks
SS40-4,5,6	10 Weeks	SS50-4,5,6	10 Weeks
SS40-7,8,9	15 Weeks	SS50-7,8,9	15 Weeks
SS40-10,11,12	35 Weeks	SS50-10,11,12	35 Weeks
SS40-13,14,15	45 Weeks	SS50-13,14,15	45 Weeks
SS40-16,17,18	55 Weeks	SS50-16,17,18	55 Weeks
SS40-19,20,21	65 Weeks	SS50-19,20,21	65 Weeks
SS200-1,2,3	5 Weeks	SS250-1,2,3	5 Weeks
SS200-4,5,6	10 Weeks	SS250-4,5,6	10 Weeks
SS200-7,8,9	15 Weeks	SS250-7,8,9	15 Weeks
SS200-10,11,12	35 Weeks	SS250-10,11,12	35 Weeks
SS200-13,14,15	45 Weeks	SS250-13,14,15	45 Weeks
SS200-16,17,18	55 Weeks	SS250-16,17,18	55 Weeks
SS200-19,20,21	65 Weeks	SS250-19,20,21	65 Weeks

Reason for Revision:

Protocol states that sample numbers for the storage stability urine samples and their storage times will be added to the protocol by amendment.

Impact on Study:

None.

Initiator's
Signature:

Julien S. Astor

Date: 8/3/00

APPROVALS

Study Director:

Julien S. Astor

Date: 8/3/00

Quality Assurance:

Jimmy L. Miller

Date: 8/4/00

Sponsor:

Pat C. H.

Date: 8/7/00

STUDY PROTOCOL ALTERATION

Study Number: PTL119801

Revision No. 3

Study Title: Determination of Residues of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP) and Dimethyl Dithiophosphate (DMDTP) in Human Urine

☐ Amendment

☒ Deviation

(Choose one)

Revision:

Change validation fortification levels as follows: For the acid mixture revise from triplicate analysis to 7 analytical runs and revise the acid concentrations as follows;

For monocarboxylic acids, revise from 0.02 ppm and 0.05 ppm to 0.03 ppm, 0.3 ppm and 0.6 ppm.

For dicarboxylic acids, revise from 0.02 ppm and 0.05 ppm to 0.03 ppm, 0.312 ppm and 0.624 ppm

For the phosphate mixture revise from triplicate analysis to 7 analytical runs and revise the phosphate concentrations as follows;

For dimethyl phosphate revise from 0.025 ppm and 0.05 ppm to 0.025 ppm and 0.98 ppm.

For dimethyl thiophosphate revise from 0.025 ppm and 0.05 ppm to 0.025 ppm and 1.0 ppm.

For dimethyl dithiophosphate revise from 0.025 ppm and 0.05 ppm to 0.028 ppm and 1.1 ppm.

Accept dimethyldithiophosphate recovery average of 129% and 20% RSD for the 1.1 ppm range and accept %RSD of 27% for .028 ppm fortification.

Reason for Revision:

Number of runs was increased to further increase confidence in the analytical method.

Laboratory utilized fortification levels they typically run routinely with this method. A 0.6

ppm level fortification was added for the acid metabolites to ensure accurate quantitation of high level urine sample acid metabolites.

Simultaneous analysis of the three phosphate metabolites requires freeze-drying and derivatization with benzyl-p-tolyltriazine that makes for a difficult, complex analysis. The average of the dimethyldithiophosphate recovery for the high level fortification was only 9%

above acceptable levels with a 20% rather than 15% RSD. The low fortification had a RSD only 7% above the acceptable 20% level. All other analytes (phosphates and acids) were within the acceptable range.

Impact on Study:

None.

All dosed patients had acid metabolites in their urine that were well above the lowest fortification level. Additionally the analytical data showed linearity for the acids via matrix spike/extracted standards through 0.01 ppm. The dimethyldithiophosphate metabolite is an extremely minor constituent and the accepted values were only slightly out of the acceptance criteria, consequently there is no impact on the overall study results.

Initiator's
Signature:

Timothy S. Oester

Date: 8/3/00

APPROVALS

Study Director:

Timothy S. Oester

Date: 8/3/00

Quality Assurance:

Jerry L. Miller

Date: 8/4/00

Sponsor:

David L. Hall

Date: 8/7/00

STUDY PROTOCOL ALTERATION

Study Number: PTL119801

Revision No. 4

Study Title: Determination of Residues of Malathion Dicarboxylic Acid (DCA), Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP), Dimethyl Thiophosphate (DMTP) and Dimethyl Dithiophosphate (DMDTP) in Human Urine

☐ **Amendment**

☒ **Deviation**

(Choose one)

Storage stability samples were analyzed at different intervals than stated in Protocol Alteration 2. Malathion acid mixture samples to be analyzed after 15, 35, 45, 55, and 65 weeks of storage were analyzed after 14, 37, 44, 52 and 67 weeks of storage. Malathion phosphate mixture samples to be analyzed after 5, 10, 15, 35, 45, 55 and 65 weeks of storage were analyzed 6, 10, 13, 32, 43, 53 and 66 weeks after storage.

For each analytical period, at least one replicate of each storage stability sample type was analyzed rather than three of each type.

For the acid mixture data from storage stability standards analyzed after 5 and 10 weeks of storage was not used.

For the phosphate mixture samples, no SSB samples were analyzed during week 10 and week 32 analyses.

Reason for Revision:

Timing of analysis of storage stability samples was dependent upon the availability of the gas chromatograph, and the laboratory technician. This analyses were performed as close to the schedule as possible.

Triplicate analyses of the storage stability samples was performed whenever possible. Circumstances (such as time, instrument availability, and materials available on hand) at times did not permit the analysis of all nine storage stability samples at once. It was deemed more important to analyze as many samples as possible rather than delay the test.

The week 5 and week 10 analyses of the malathion acid storage stability samples were performed with fenthion as the internal standard. It was determined that fenthion was not adequately resolved from the malathion dicarboxylic acid peak, and the internal standard was

switched to diazinon. All data collected before the internal standard was switched was determined to be not usable.

SSB samples were inadvertently omitted from malathion phosphate storage stability analysis during these two weeks.

Impact on Study:

None.

A sufficient number of data points were collected for all six storage stability samples for a period of time that more than adequately covered the storage interval of the samples. Additional information regarding the stability of the malathion metabolites in frozen urine is unlikely to be gained by further analysis of urine samples not fortified with those analytes.

**Initiator's
Signature:**

Lincoln S. Costen

Date: 8/3/00

APPROVALS

Study Director:

Lincoln S. Costen

Date:

8/3/00

Quality Assurance:

Denny L. Muller

Date:

8/4/00

Sponsor:

Robert L. Price

Date:

8/7/00

STUDY PROTOCOL ALTERATION

Study Number: PTL119801

Revision No. 5

Study Title: Determination of Residues of Malathion Dicarboxylic Acid (DCA),
Malathion Monocarboxylic Acid (MCA), Dimethyl Phosphate (DMP),
Dimethyl Thiophosphate (DMTP) and Dimethyl Dithiophosphate (DMDTP)
in Human Urine

☐ Amendment

☒ Deviation

(Choose one)

Data is reported in ppm ($\mu\text{g/mL}$) rather than in ng/mL. Data is reported to thousandths of a μg in numbers that have 4, 5, or 6 significant figures.

Reason for Revision:

Concentrations of analytes in the vast majority of the positive samples were greater than 1000 ng/mL. It was therefore more reasonable to change to a larger unit in order to maintain clear communication of the results. The accuracy of the instruments and calculations was originally determined to be 1 ng/mL or $\pm 0.001 \mu\text{g/mL}$. This accuracy was maintained throughout the data reporting.

Impact on Study:

None.

Numbers with fewer digits before the decimal point are inherently easier to read, compare and comprehend. The data in this format should therefore be easier to interpret.

Initiator's
Signature:

Linda S. Ostro

Date: 8/3/00

APPROVALS

Study Director:

Linda S. Ostro

Date:

8/3/00

Quality Assurance:

Debra L. Miller

Date:

8/4/00

Sponsor:

Robert C. King

Date:

8/7/00

APPENDIX 2- SAMPLE HISTORY

Samples were accessioned in the order in which they were received. Samples were analyzed in the order they appear here.

PTL Accession #	Sample ID	Sample Interval (hrs)
X0016922	SUBJ001	-12-0
X0016931	SUBJ001	0-12
X0016941	SUBJ001	12-24
X0016950	SUBJ001	24-48
X0016969	SUBJ002	-12-0
X0016978	SUBJ002	0-12
X0016987	SUBJ002	12-24
X0016996	SUBJ002	24-48
X0017008	SUBJ003	-12-0
X0017017	SUBJ003	0-12
X0017026	SUBJ003	12-24
X0017071	SUBJ003	24-48
X0017035	SUBJ004	-12-0
X0017044	SUBJ004	0-12
X0017053	SUBJ004	12-24
X0017062	SUBJ004	24-48
X0017081	SUBJ005	-12-0
X0017090	SUBJ005	0-12
X0017106	SUBJ005	12-24
X0017115	SUBJ005	24-48
X0017124	SUBJ006	-12-0
X0017133	SUBJ006	0-12
X0017142	SUBJ006	12-24
X0017151	SUBJ006	24-48
X0017161	SUBJ007	-12-0
X0017170	SUBJ007	0-12
X0017189	SUBJ007	12-24
X0017198	SUBJ007	24-48

PTL Accession #	Sample ID	Sample Interval (hrs)
X0017204	SUBJ008	-12-0
X0017213	SUBJ008	0-12
X0017222	SUBJ008	12-24
X0017231	SUBJ008	24-48
X0016360	SUBJ009	-12-0
X0016379	SUBJ009	0-12
X0016388	SUBJ009	12-24
X0016397	SUBJ009	24-48
X0016403	SUBJ010	-12-0
X0016412	SUBJ010	0-12
X0016421	SUBJ010	12-24
X0016431	SUBJ010	24-48
X0016440	SUBJ011	-12-0
X0016459	SUBJ011	0-12
X0016468	SUBJ011	12-24
X0016477	SUBJ011	24-48
X0016486	SUBJ012	-12-0
X0016495	SUBJ012	0-12
X0016501	SUBJ012	12-24
X0016511	SUBJ012	24-48
X0016520	SUBJ013	-12-0
X0016539	SUBJ013	0-12
X0016548	SUBJ013	12-24
X0016557	SUBJ013	24-48
X0016566	SUBJ014	-12-0
X0016575	SUBJ014	0-12
X0016584	SUBJ014	12-24
X0016593	SUBJ014	24-48
X0016600	SUBJ015	-12-0
X0016619	SUBJ015	0-12
X0016628	SUBJ015	12-24
X0016637	SUBJ015	24-48

PTL		Sample Interval
Accession #	Sample ID	(hrs)
X0016646	SUBJ016	-12-0
X0016655	SUBJ016	0-12
X0016664	SUBJ016	12-24
X0016673	SUBJ016	24-48
X0016682	SUBJ917	-12-0
X0016691	SUBJ917	0-12
X0016708	SUBJ917	12-24
X0016717	SUBJ917	24-48
X0016726	SUBJ018	-12-0
X0016735	SUBJ018	0-12
X0016744	SUBJ018	12-24
X0016753	SUBJ018	24-48
X0016762	SUBJ019	-12-0
X0016771	SUBJ019	0-12
X0016781	SUBJ019	12-24
X0016790	SUBJ019	24-48
X0016806	SUBJ020	-12-0
X0016815	SUBJ020	0-12
X0016824	SUBJ020	12-24
X0016833	SUBJ020	24-48
X0016842	SUBJ021	-12-0
X0016851	SUBJ021	0-12
X0016861	SUBJ021	12-24
X0016870	SUBJ021	24-48
X0016889	SUBJ022	-12-0
X0016898	SUBJ022	0-12
X0016904	SUBJ022	12-24
X0016913	SUBJ022	24-48
X0017241	SUBJ023	-12-0
X0017250	SUBJ023	0-12
X0017269	SUBJ023	12-24
X0017278	SUBJ023	24-48

PTL		Sample Interval
Accession #	Sample ID	(hrs)
X0017287	SUBJ024	-12-0
X0017296	SUBJ024	0-12
X0017302	SUBJ024	12-24
X0017311	SUBJ024	24-48
X0017321	SUBJ025	-12-0
X0017330	SUBJ025	0-12
X0017349	SUBJ025	12-24
X0017358	SUBJ025	24-48
X0017367	SUBJ026	-12-0
X0017376	SUBJ026	0-12
X0017385	SUBJ026	12-24
X0017394	SUBJ026	24-48
X0017401	SUBJ927	-12-0
X0017410	SUBJ927	0-12
X0017429	SUBJ927	12-24
X0017438	SUBJ927	24-48
X0017447	SUBJ028	-12-0
X0017456	SUBJ028	0-12
X0017465	SUBJ028	12-24
X0017474	SUBJ028	24-48
X0017483	SUBJ029	-12-0
X0017492	SUBJ029	0-12
X0017509	SUBJ029	12-24
X0017518	SUBJ029	24-48
X0017527	SUBJ030	-12-0
X0017536	SUBJ030	0-12
X0017545	SUBJ030	12-24
X0017554	SUBJ030	24-48
X0017563	SUBJ031	-12-0
X0017572	SUBJ031	0-12
X0017581	SUBJ031	12-24
X0017591	SUBJ031	24-48

PTL Accession #	Sample ID	Sample Interval (hrs)
X0017607	SUBJ032	-12-0
X0017616	SUBJ032	0-12
X0017625	SUBJ032	12-24
X0017634	SUBJ032	24-48
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X0017643	SUBJ033	-12-0
X0017652	SUBJ033	0-12
X0017661	SUBJ033	12-24
X0017671	SUBJ033	24-48
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X0017680	SUBJ034	-12-0
X0017699	SUBJ034	0-12
X0017705	SUBJ034	12-24
X0017714	SUBJ034	24-48
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X0017732	SUBJ035	-12-0
X0017741	SUBJ035	0-12
X0017751	SUBJ035	12-24
X0017760	SUBJ035	24-48
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X0017779	SUBJ036	-12-0
X0017788	SUBJ036	0-12
X0017797	SUBJ036	12-24
X0017803	SUBJ036	24-48
<hr/>		
X0017812	SUBJ037	-12-0
X0017821	SUBJ037	0-12
X0017831	SUBJ037	12-24
X0017840	SUBJ037	24-48
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X0017859	SUBJ038	-12-0
X0017868	SUBJ038	0-12
X0017877	SUBJ038	12-24
X0017886	SUBJ038	24-48
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X0017895	SUBJ039	-12-0
X0017901	SUBJ039	0-12
X0017911	SUBJ039	12-24
X0017920	SUBJ039	24-48

PTL Accession #	Sample ID	Sample Interval (hrs)
X0017939	SUBJ040	-12-0
X0017948	SUBJ040	0-12
X0017957	SUBJ040	12-24
X0017966	SUBJ040	24-48
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X0017975	SUBJ041	-12-0
X0017984	SUBJ041	0-12
X0017993	SUBJ041	12-24
X0018005	SUBJ041	24-48
<hr/>		
X0018014	SUBJ042	-12-0
X0018023	SUBJ042	0-12
X0018032	SUBJ042	12-24
X0018041	SUBJ042	24-48
<hr/>		
X0018051	SUBJ043	-12-0
X0018060	SUBJ043	0-12
X0018079	SUBJ043	12-24
X0018088	SUBJ043	24-48
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X0018121	SUBJ044	-12-0
X0018131	SUBJ044	0-12
X0018140	SUBJ044	12-24
X0018159	SUBJ044	24-48
<hr/>		
X0018168	SUBJ045	-12-0
X0018177	SUBJ045	0-12
X0018186	SUBJ045	12-24
X0018195	SUBJ045	24-48
<hr/>		
X0018201	SUBJ046	-12-0
X0018211	SUBJ046	0-12
X0018220	SUBJ046	12-24
X0018239	SUBJ046	24-48
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X0018248	SUBJ047	-12-0
X0018257	SUBJ047	0-12
X0018266	SUBJ047	12-24
X0018275	SUBJ047	24-48

PTL Accession #	Sample ID	Sample Interval (hrs)
X0018284	SUBJ948	-12-0
X0018293	SUBJ948	0-12
X0018300	SUBJ948	12-24
X0018319	SUBJ948	24-48

APPENDIX 3-

ANALYTICAL METHODS

SOP: 3-MALA-90710-4

Analytical Method for the Determination of Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid Metabolites in Human Urine by GC/FPD

SOP: 3-MOPPS-990115-1

Analytical Method for the Determination of Dimethyl Phosphate, Dimethyl Thiophosphate and Dimethyl Dithiophosphate Metabolites in Human Urine by GC/FPD

SOP: 3-MALA-90710-4

Analytical Method for the Determination of Malathion Dicarboxylic Acid and Malathion Monocarboxylic Acid Metabolites in Human Urine by GC/FPD

MALATHION CARBOXYLIC ACIDS, URINE GC/FPD

TEST CODE:	MCA, DCA
ANALYTES:	Malathion monocarboxylic acid (MCA) Malathion dicarboxylic acid (DCA)
METHOD:	Gas chromatography with flame photometric detection.
TYPE OF SPECIMEN:	Urine
AMOUNT OF SPECIMEN REQUIRED:	Optimum: 10 ml; Minimum: 5 ml
SPECIMEN COLLECTION AND HANDLING:	Place random or well mixed 24 hr collected urine sample in a 30 ml polypropylene vial with a screw cap. Freeze immediately. Deliver samples or ship by overnight delivery with dry ice or frozen refrigerant pack.
STORAGE:	Freezer (<-10°C)
STABILITY:	Freezer: > One year.
AVERAGE REPORTING TIME:	5 days
GENERAL POPULATION RANGE:	Not detected (MCA and DCA).
THERAPEUTIC RANGE:	Not applicable
NOTIFICATION VALUES:	None established
BIOLOGICAL EXPOSURE INDICES:	None established.

DETECTION LIMITS: 2.0 µg/L

QUANTITATION LIMITS: 20.0 µg/L

PRINCIPLE:

A 5 ml aliquot of the urine sample is acidified to pH 1-2 and passed through a polar solid phase extraction column (Phenyl, J. T. Baker). After washing the column with HCl acidified water and dried by centrifugation, the analytes are eluted from the column with 2 ml of ethyl acetate. The eluate is dried with sodium sulfate and derivatized with diazomethane. The volume is reduced to 1 ml and the methylated mono- and dicarboxylic acid metabolites are analyzed by gas chromatography with flame photometric detection (FPD).

CLINICAL APPLICABILITY:

The malathion carboxylic acid metabolites are the major human malathion metabolites excreted in urine and are the most specific and sensitive indicators of malathion exposure.

REFERENCES

- Bradway, D. E. and Shafik, T. M., J. Agr. Food Chem. 25, 1977, 1342-1344.
2. Cook, G. H., Moore, J. C., J. Agr. Food Chem. 24, 1976, 631-634.

Authors:

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(Former) Director of Research & Development

Linda S. Aston, Ph.D.
Director, Occupational Laboratory

SAFETY

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level using good laboratory practices such as working under a fume hood, wearing protective gloves and never pipetting by mouth.

All biological samples should be considered potentially infectious. Protective gloves should always be worn when handling specimens, even when the container is not open.

REAGENTS

1. Ethyl Acetate, J.T. Baker, Ultra Resi-Analyzed or equivalent.
2. Acidified ethyl acetate. Add 2 ml of conc. HCl to 100 ml of ethyl acetate.
3. Acetone, J.T. Baker, Resi-Analyzed or equivalent.
4. Reagent Grade Water. Water which has passed through the ion exchanger (California Purification and Recycling, Inc.) and Water I (Barnstead) water purification system (18 megohm, carbon trap).
5. Concentrated HCl, 36.5-38.0%, Baker Analyzed reagent, J.T. Baker, Inc. or equivalent.
6. Sodium sulfate, anhydrous (granular), Analytical reagent, Mallinkrodt or equivalent.
7. Diazomethane (methylating) reagent.

NOTES:

- a. Both the reagent and the diazoalkane gases are extremely toxic, carcinogenic and potentially explosive.
- b. Diazoalkane generation should be carried out in a high draft hood.
- c. Do not allow the nitroguanidine or the diazoalkane to come into contact with the skin. Disposable gloves and safety goggles should always be worn when handling.
- d. Diazoalkane solutions must not be pipetted by mouth.
- e. Do not use ground glass stoppered bottles or bottles with visible interior etching.
- f. Face shield or stationary shield must be used when preparing or handling the diazoalkane reagent.

PREPARATION:

- a. In a 125 ml Erlenmeyer flask, dissolve 5.5 g of KOH. A.R. grade in 5.5 ml of distilled water. When solution is complete, allow to cool to room temperature.
- b. Add 60 ml ethyl ether (J.T. Baker, Ultra Resi-Analyzed) and

- cool flask in a -10°C freezer for 15 min.
- c. In a high draft hood, add 3.84 g of N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical, Inc. Milwaukee, WI) in small portions at a time, mixing contents of flask after each addition.
 - d. Decant the ether layer into a bottle with a Teflon-lined screw cap. This may be stored for periods up to a week at -10°C

CALIBRATION STANDARDS

NOTE:

Label all standards and controls with the content's identity, concentration, preparation and expiration dates and preparator's initials. Pure reference standards: Obtained from Cheminova Agro A/S, P.O. Box 9 DK-7620 Lemvig Denmark.

Stock standards (1 mg/ml):

Weigh 25 mg of each MCA* and DCA standard into separate 25 ml volumetric flasks. Dilute to volume with acetone. Record the weights ($\pm 0.1\text{mg}$). Mix by gently inverting the flask and transfer to amber bottles with Teflon lined screw caps. Label as stock standards. Stable for one year at $2-8^{\circ}\text{C}$. (MCA* is available commercially as 10 $\mu\text{g/ml}$ solution, equivalent to intermediate level standard below, from Axact Standards and Chem Service.)

2. Intermediate standards (10 $\mu\text{g/ml}$):
With volumetric pipets, add 0.5 ml of each stock standard to a 50 ml volumetric flask. Dilute to the mark with acetone. Transfer to an amber glass bottle with a Teflon lined screw cap. Label as intermediate standard. Stable for six months at $2-8^{\circ}\text{C}$.
3. Calibration standards (Extracted standards)
Add the following volumes of the intermediate standard to 5 ml of blank urine to obtain the corresponding calibration standard concentrations:

<u>Calib. Std. Conc.</u>	<u>Interm. Std. Vol.</u>
10 $\mu\text{g/l}$	5 μl
50 $\mu\text{g/l}$	25 μl
100 $\mu\text{g/l}$	50 μl
200 $\mu\text{g/l}$	100 μl
400 $\mu\text{g/l}$	200 μl
600 $\mu\text{g/l}$	300 μl

The five extracted standards are used to check linearity. The 200 $\mu\text{g/l}$ standard is used for daily calibration. Calibration standards are extracted, derivatized and analyzed in the same manner as urine samples. If the number of samples to be analyzed requires the use of two or more vacuum manifolds, prepare a separate 200 $\mu\text{g/l}$ standard for each manifold to account for different extraction efficiencies.

INTERNAL STANDARD (Diazinon)

1. Stock standard (1 mg/ml):

Weigh 25 mg of Diazinon (ChemService) in a 25 ml volumetric flask. Dilute to volume with acetone. Record the weight (+/- 0.1mg). Mix by gently inverting the flask and transfer to an amber bottle with a Teflon lined screw cap. Label as stock standard. Stable for one year at 2-8°C.

2. Intermediate standards (10 µg/ml):
With volumetric pipets, add 0.5 ml of the stock standard to a 50 ml volumetric flask. Dilute to the mark with acetone. Transfer to an amber glass bottle with a Teflon lined screw cap. Label as intermediate standard. Stable for six months at 2-8°C.
3. Fifty µl of intermediate internal standard solution (10 µg/ml) is spiked into each extracted sample, calibration standard and control so as to correct to variation in extraction efficiency, extract volumes and instrument performance. The nominal concentration of the internal standard in a 5 ml urine sample is 100 µg/l.

CONTROLS

1. Stock control standards (1 mg/ml).
(Stock solutions for controls are made separately from those for calibration standards.)
Weigh 25 mg of each MCA* and DCA standard into separate 25 ml volumetric flasks. Dilute to volume with acetone. Record the weights (+/- 0.1mg). Mix by gently inverting the flask and transfer to amber bottles with Teflon lined screw caps. Label as stock standards for controls. Stable for one year at 2-8°C.
2. Intermediate control standards (10 mg/l).
With volumetric pipets, add 0.5 ml of each stock standard to a 50 ml volumetric flask. Dilute to the mark with acetone. Transfer to an amber glass bottle with a Teflon lined screw cap. Label as intermediate standard for controls. Stable for six months at 2-8°C.
3. Collect and pool at least 300 ml of blank urine into a liter beaker. Use within 48 hours for control preparation.
4. High Control (300 µg/l):
To a 100 ml volumetric flask add 50 ml blank urine. Add 3.0 ml of the intermediate standard to the flask with a volumetric pipet. Add sufficient blank urine to the mark and mix for several minutes by gentle inversion.
5. Low Control (30 µg/l):
To a 100 ml volumetric flask add 50 ml blank urine. Add 10.0 ml of the high control to the flask with a volumetric pipet. Add sufficient blank urine to the mark and mix for several minutes by gentle inversion.
6. Freeze the controls in 5.0 ml aliquots in labeled 16 x 125 mm screw cap tubes with Teflon lined caps. Stable for 1 year at less than -10°C.

EQUIPMENT AND MATERIALS

Gas Chromatograph, Perkin Elmer model 8500 equipped with a model AS 8300 auto sampler and flame photometric detector.

Volumetric pipets, Class A, 0.5, 1.0, 2.0 and 10.0 ml. Baxter Scientific Products.

3. Hamilton syringes. 100 μ l and 500 μ l.
4. Volumetric flasks, Class A, 25, 100 and 250 ml
5. Nitrogen slow-down evaporator concentration with 12 stations N-Evap Model III (Organomation Associates, Inc.) or equivalent.
6. Analytical Balance, capable of weighing to 0.1 mg, Sartorius 1712 or equivalent.
7. 16 x 125 mm culture tubes with teflon lined screw caps, Kimble or equivalent.
8. Disposable 13 x 100 mm culture tubes, glass (non screw cap).
9. Disposable 10 ml serological pipets.
10. Vortex mixer, Baxter Scientific Products #98220 or equivalent.
11. Pasteur pipets, disposable, 5 3/4" long, VWR Scientific, or equivalent.
12. Solid phase extraction system, AASP Vac-elut (12 positions), Analytichem International or SPE-21 (21 positions), J.T. Baker, Inc.
13. Solid phase extraction cartridge (6 ml-Phenyl). J.T. Baker, Inc.
14. Graduated centrifuge tubes, 15 ml, Kimble or equivalent.
15. Centrifuge, with head to accommodate 16 x 125 mm culture tubes, capable of speeds of 2,000 rpm. Damon/IEC Division Model HN/SII or equivalent.
16. Auto sampler vials, 1.0 ml, crimp cap, Teflon lined. Alltech Associates.

NOTE: All pipets and volumetric flasks used in this method must be thoroughly washed and then rinsed with acetone followed by hexane and dried in a 100°C oven. All non-volumetric glassware is thoroughly washed, rinsed with deionized water, and baked for two hours at 350°C.

INSTRUMENTAL PARAMETERS

1. Perkin-Elmer Series 8500 Gas Chromatograph with a Flame Photometric Detector.
FPD Detector:
 - a. Air flow: (approx.) 90 ml/min at 28.5 psi.

- b. Hydrogen flow: 65 ml/min at 22 psi.
 - c. Detector temperature: 300°C.
- 2. Column: 30M DB-210, 0.53 i.d., 1.0 µm film thickness (J&W Scientific).
 - a. Carrier gas, Nitrogen, 7.0 ml/min (instrument readout: 70).
 - b. Oven temperature: 185°C, isothermal.
- 3. Injection port temperature: 250°C.
- 4. Autosampler:
 - a. Pressures: Aux 1: 45-55 psi, Aux 2: 35-45 psi.
 - b. Injection volume: 2 µl
- 5. Data system: method 3: MALA
 - a. Peak height calculation
 - b. Internal standard method
 - c. Peak width: 5

SAMPLE PREPARATION

1. Thaw controls, standards and blank. Pipet 5.0 ml of patient sample into an appropriately labeled 16 x 125 mm screw cap tube. Include an empty tube to be used for a reagent blank.
2. Add 2 drops of concentrated HCl to each tube to bring the pH to 1 to 2.
3. Add 50 µl of the intermediate internal standard solution (Diazinon 10 µg/ml) to each tube.
4. Condition the solid phase extraction cartridge with one column volume of acetone followed by two column volumes of distilled water.
5. Load the urine sample onto the column. Draw the sample through the column at a flow rate of 3-4 ml/min.
6. Wash the column with two column volumes of 1 mM HCl and centrifuge at 25,000 rpm for 5 minutes to remove the residual water.
7. Elute the analytes from the cartridge with 2 ml of ethyl acetate into a 12 x 75 mm non-screw cap test tube. Some water will elute with the ethyl acetate.
8. Draw off the upper layer of ethyl acetate with a pasteur pipet and transfer solvent to a pasteur pipet packed with 1 g of sodium sulfate. Allow the eluate to pass into a 15 ml graduated centrifuge tube.
9. Add approximately 0.5 ml of ethyl acetate to the original tube, vortex briefly and transfer the remaining solvent to the sodium sulfate column and combine the eluates. Add 250 µl of the acidified ethyl acetate to the extracts.

10. Add 3 ml of ethereal diazomethane to each urine extract and allow the mixture to stand for 15 min.
11. Remove excess diazomethane by bubbling a slow stream of nitrogen into the tube below the surface of the ethyl acetate for 15 min. (Remove and rinse the needles with acetone before beginning.)
12. Pull the blow down apparatus needles out of the solution and continue to concentrate the extract down to 0.5 ml as measured in the graduated tube. (Remove and rinse the needles with acetone after the concentration step.)
13. Transfer the extracts to autosampler vials. The extracts are ready for gas chromatographic analysis.

GC PROCEDURE

1. Light the FPD flame and turn on the photomultiplier tube. Perform all necessary GC start-up checks and procedures.
2. Sample analysis:
 - a. Load method: MALA
 - b. Load the samples in the autosampler rack in the following sequence:
 1. Calibration standard: 200 µg/l
 2. Urine blank
 3. Low control
 4. Samples 1-20
 5. High control
 6. Reagent blank
 - c. In the automation section of the instrument prepare the "auto queue: with the method number, automation method number, initial vial and number of vials.
 - d. Build the sample table in the above order.
 - e. Start the auto queue.

CALCULATIONS

Daily calibration is performed using Calibration Standard 200. An example of the typical chromatogram of the 200 µg/l calibration standard is presented in Figure 1. Quantitation is performed by the internal standard method. The relative response factor (RF) for MCA and DCA is determined using the following formula:

$$RF(std) = H(is)/H(std) \times C(std) / C(is)$$

Where H(is) = Peak height of internal standard

H(std) = Peak height of analyte
C(std) = Known concentration of analyte
C(is) = Concentration of internal standard

2. The following formula is applied to each analyte to determine the concentration in the sample:

$$C(\text{sample}) = RF(\text{std}) \times \frac{H(s)}{H(is)} \times \frac{C(is)}{V(\text{Sample})}$$

Where C(sample) = concentration of analyte in sample.
V(sample) = Volume of sample (normally = 1)
H(s) = height of analyte peak in sample

3. Creatinine correction:

The sample concentration in mg/L is corrected for creatinine concentration by the following equation:

$$C (\mu\text{g/l})/\text{Creat. (g/l)} = C \text{ corr. } (\mu\text{g/g-creat})$$

4. Blank measurements:

With each set of samples or a maximum of 20 samples a reagent blank is analyzed (tube without urine that is carried through entire procedure). If the analysis produces a peak interfering with an analyte peak, its apparent concentration is subtracted from that of the urine blank controls and samples.

A urine blank is also analyzed with each set of samples. This is a pooled urine which was used to prepare the urine control. The apparent concentrations of any interfering peaks are subtracted from the concentrations of the controls only. An example of the typical chromatogram blank urine is presented in Figure 2.

5. Example calculation: MCA

a. Run the 200 $\mu\text{g/l}$ calibration standard:

<u>Retention time</u>	<u>Peak Name</u>	<u>Peak Height</u>	<u>Concentration</u>
7.17 min.	MCA	356.14	200 $\mu\text{g/l}$
5.99 min.	Diazinon	572.23	100 $\mu\text{g/l}$

b. Calculate the response factor for MCA.

$$= \frac{572.23}{356.14} \times \frac{200 \mu\text{g/l}}{100 \mu\text{g/l}} = 3.2134 \text{ (unitless)}$$

c. Run the patient sample:

<u>Retention time</u>	<u>Peak Name</u>	<u>Peak Height</u>	<u>Concentration</u>
7.14 min.	MCA	596.41	unknown
5.97 min.	Diazinon	494.09	100 $\mu\text{g/l}$

d. Calculate the concentration:

$$\frac{596.41}{494.09} \times \frac{100 \mu\text{g/l}}{3.2134} = 387.89 \mu\text{g/l} \text{ (round off to } 388 \mu\text{g/l)}$$

e. Correct for creatinine concentration (creat. conc. = 1.6 g/l)

$$388 \mu\text{g/l} \div 1.6 \text{ g/l} = 242 \mu\text{g/g-creat.}$$

REPORTING RESULTS

The concentration of each analyte is reported in $\mu\text{g/L}$ and $\mu\text{g/g-creatinine}$ to the nearest tenth $\mu\text{g/l}$, but with a maximum of three significant figures. See sample report (Figure 3).

QUALITY ASSURANCE

Detector Linearity

Detector linearity is checked (1) with each new set of standards or controls (2) when the controls have failed Westgard rules (3) when the daily instrument performance check is outside 15% limits (see below) (4) when a new column is installed and (5) at least every 6 months.

A linearity check is made with the five linearity standards ranging from 10 to 600 µg/l. Each standard level extracted from urine is analyzed and the response factor is calculated in the same manner as the calibration standard. Record the retention time, peak height, and response factor in the "Linearity Check" log. If the percent relative standard deviation (C.V.) of the response factors is less than 20% over the working range, linearity through the origin may be assumed.

If the linearity criteria are not met, plot the peak height versus the standard concentrations to diagnose the problem. If it appears that a single point is out of line, rerun the standard. If this does not correct the problem, one or two standards may be removed from the curve (three standards minimum for curve). If linearity is still not achieved, cut off a portion of the capillary column, clean the detector liner, replace the photomultiplier tube or remake the standards.

2. Instrument Performance Check

The 200 µg/l calibration standard is injected prior to each run to ensure proper GC conditions, detector response and retention times. Record the retention time, peak height and response factor of MCA in the instrument performance log. If the daily response factor differs from the response factor established in the most recent linearity check by more than 15%, repeat the instrument performance check. If the response factor is still outside limits, a new linearity check is performed. The retention time and peak height are recorded to observe trends to aid in diagnosing instrumental problems. The peak height should not drop by more than 50% from the last calibration standard run. If such a loss in sensitivity is apparent, examine the injection port liner and detector liner and clean or replace if necessary. Also check and adjust the detector gases for optimal sensitivity.

3. Blank measurements

With each set of samples a reagent blank using reagent water instead of urine is analyzed. If the analysis produces a peak interfering with an analyte peak, its apparent concentration must be subtracted from that of the urine blank, control and patient samples. A blank urine is also analyzed with each set of samples. This is a pooled urine which was used to prepare the urine control. The apparent concentrations of any interfering peaks are subtracted from the concentration of the controls only.

4. Quality Control

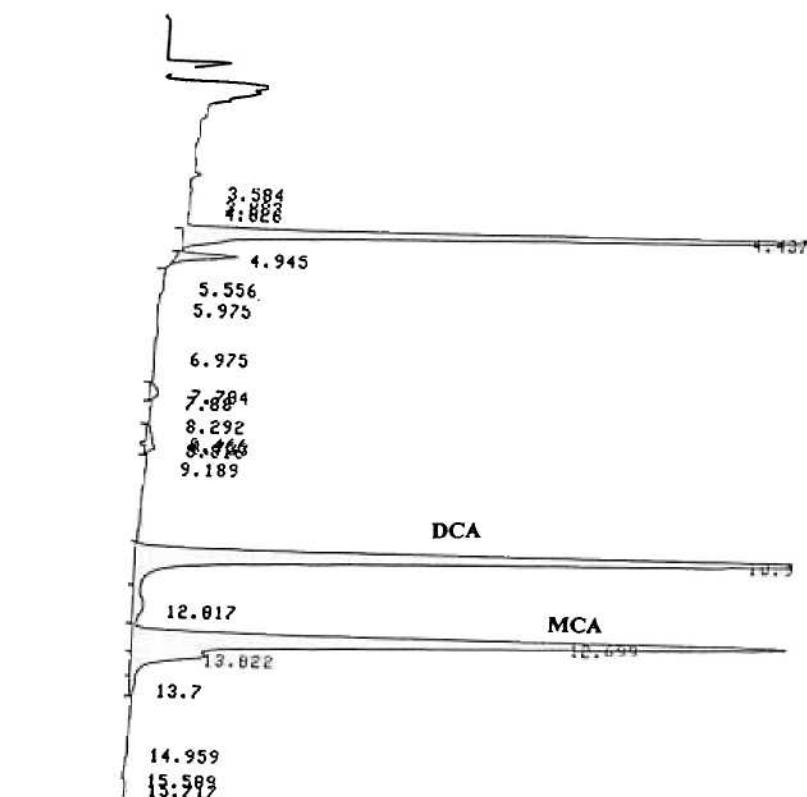
High and low urine controls are analyzed with each set of samples and the results are recorded on the sample work sheet and on QC Charts prepared using 2 standard deviation limits. Label each QC chart with the identity and/or source of control materials and with any "Out of Limits" log sheets, describing the cause of the problem and actions taken to correct it. Bring any such condition to the attention of the supervisor or laboratory director.

Method notes:

There appear to be two factors affecting the derivatization of the carboxylic acids.

- 1) Weak solutions of diazomethane reagent will result in lower yield of both derivatives equally. The yield is lower with urines with higher creatinine levels. It is therefore important to prepare daily diazomethane solutions of consistent strength. The periodic inclusion of malathion in a standard (which does not get derivatized) at the same level as the metabolites in the standard provides a marker to evaluate the derivatization efficiency (approximately equal heights means high efficiency).
- 2) Derivatization of unextracted standards (spiked ethyl acetate) results in poor yields of the DCA derivative compared to urine spiked at the same level and carried through the extraction procedure. Acidification prior to derivatization did not improve the yield. There appears to be something in the urine extract, which promotes the reaction. We therefore calibrated the GC using extracted urine standards.

Figure Typical chromatogram of 200 µg/l calibration standard.



CHROMATOGRAM 5 MEMORIZED

C-RSA CHROMATOPAC

CHANNEL NO 1

SAMPLE NO 0

REPORT NO 584

IS WT 1

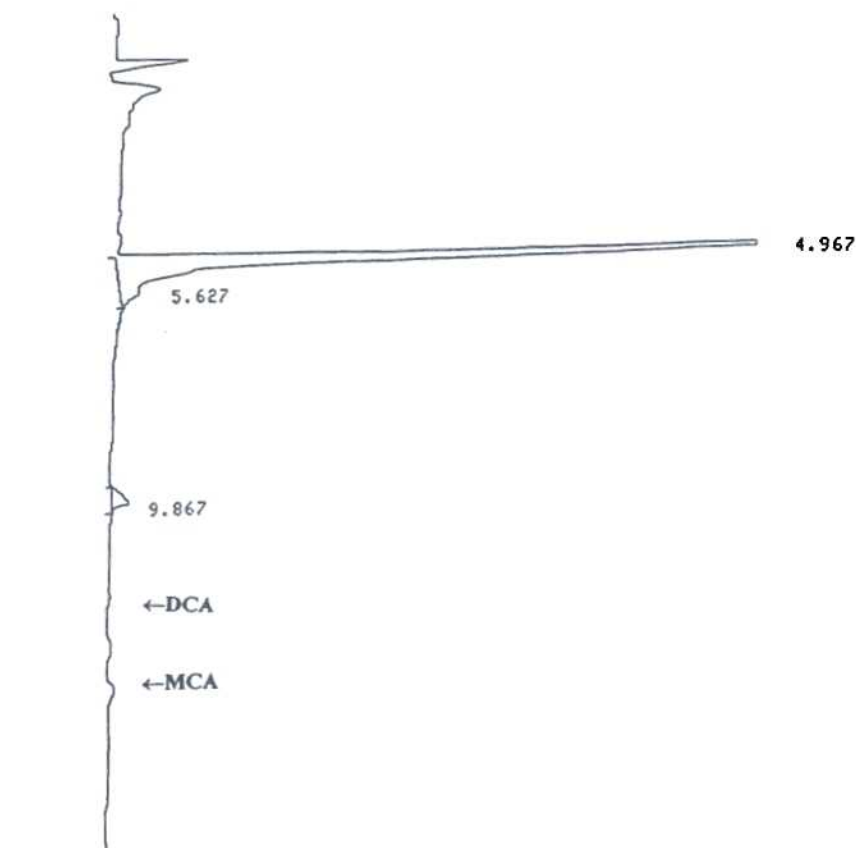
FILE 0

METHOD 0123

SAMPLE WT 100

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	4.437	385374				
2	4.945	39614				
3	7.704	10736				
4	8.466	10751	V			
5	8.816	5977	V			
6	10.99	861975		2	184.2077	DCA
7	12.017	28642	V			
8	12.699	813852	V	3	172.1654	MCA
9	13.022	85759	V	3	18.1418	MCA
10	13.7	5301	V			
TOTAL		2447981			374.5148	

Figure 2. Typical chromatogram of urine blank.



CHROMATOGRAM 11 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1
SAMPLE NO 8
REPORT NO 581
IS WT 1

FILE 8
METHOD 8123
SAMPLE WT 100

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	4.967	582448	S	R	1	IS
2	9.867	12591				
TOTAL		515031			8	

SOP: 3-MOPPS-990115-1

Analytical Method for the Determination of Dimethyl Phosphate, Dimethyl Thiophosphate and Dimethyl Dithiophosphate Metabolites in Human Urine by GC/FPD

**METHYL ORGANOPHOSPHATE PESTICIDE
METABOLITES (MOPPS), URINE, GC-FPD**

TEST CODE:	OPMETDMP
ANALYTES:	a) dimethylphosphate (DMP) b) dimethylthiophosphate (DMTP) c) dimethyldithiophosphate (DMDTP)
TRADE NAMES, SYNONYMS:	(b) dimethylphosphorothionate (c) dimethylphosphorodithioate
METHOD:	Gas chromatography with flame photometric detection.
TYPE OF SPECIMEN:	Urine
AMOUNT OF SPECIMEN REQUIRED:	Optimum: 10 ml; minimum: 3 ml
SPECIMEN COLLECTION AND HANDLING:	Random urine sample transferred to a plastic bottle or glass bottle with Teflon lined cap.
STORAGE:	Locked specimen refrigerator, 2-8°C.
STABILITY:	Ambient temp: 2 weeks Refrigerator (2-8°C): 3 months Freezer (less than -15°C): 12 months
AVERAGE REPORTING TIME:	7 days
GENERAL POPULATION RANGE:	Not detected (all analytes).
THERAPEUTIC RANGE:	Not applicable

NOTIFICATION VALUES: Dependent on organophosphate pesticide.

DETECTION LIMITS: 12.5 µg/L

QUANTITATION LIMITS 25 µg/L

PRINCIPLE: Freeze-dried urine sample is derivatized with a benzyltolyltriazine reagent to produce benzyl derivatives of alkylphosphate metabolites. A saturated salt solution is added to the tubes and the benzyl derivatives are extracted with cyclohexane and analyzed by gas chromatography with flame photometric detection.

CLINICAL APPLICABILITY: The measurement of alkylphosphate metabolites provides the most sensitive detection of organophosphate pesticide exposure available. One test detects exposure to over 100 commercially available organophosphate pesticides. However, even though the test specifically detects organophosphate pesticide exposure, only determination of the general class of organophosphate pesticide is possible and identification of the exact pesticide involved is difficult. The most common organophosphate pesticides are listed in Table I with their corresponding alkylphosphate metabolites. This narrowed group is often enough to confirm a pesticide when specific supporting information concerning the exposure is available. In cases where a phenolic metabolite is also produced (p-nitrophenol from parathion and methyl parathion) a more definitive identification of the parent can be made.

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6. Ito, G, Kilgore, W.W. and Seabury, J.J. "Effect of Freezer Storage on Alkyl Phosphate Metabolites in Urine" *Bull. Environm. Contam. Toxicol.* 22, 530-535, 1979.

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Laboratory

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Director, Occupational

Table I: Alkylphosphate Metabolites of Common Organophosphates

Pesticides

<u>Diethyl phosphates</u>		<u>Dimethyl phosphates</u>	
<u>Pesticide</u>	<u>Metabolite(s)*</u>	<u>Pesticide</u>	<u>Metabolite(s)*</u>
Chlorpyrifos (Dursban; Lorsban)	DEP, DETP, DEP	Diclotophos (Bidrin)	DMP
Diazinon (Spectracide)		Naled (Dibrom)	
<u>Fensulfothion</u> (Dansit)		<u>Mevinphos</u> (Phosdrin)	
<u>Parathion</u> (Ethyl parathion)		<u>Monocrotophos</u> (Azodrin)	
		<u>Phosphamidon</u> (Dimecron)	
<u>Disulfoton</u> (Di-Syston)	DEP, DETP, DEDTP	<u>Demeton-methyl</u> (Metasystox)	DMP, DMTP
Ethion		Methyl parathion (Dalf, Penncup-m)	
<u>Fonofos</u> (Dyfonate)		Azinphos-methyl (Guthion)	DMP, DMTP, DMDTP
<u>Phorate</u> (Thimet)		Dimethoate (Cygon, De-fend)	
Phosalone (Zolone)		<u>Methidathion</u> (Supacide)	
		Malathion (Cythion)	

Underlined pesticides are considered highly toxic [LD₅₀ values (rat) are less than 50 mg/Kg.]

* One or more of these metabolites would be expected.

+ These pesticides are considered the most common based on the California Department of Food and Agriculture "Pesticide Use Report Annual 1985".

SAFETY

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level using good laboratory practices such as working under a fume hood, wearing protective gloves and never pipetting by mouth.

All biological samples should be considered potentially infectious. Protective gloves should always be worn when handling specimens, even when the container is not open.

REAGENTS

1. Acetone, distilled in glass, pesticide grade (American Burdick and Jackson), flammable storage cabinet.

Cyclohexane, distilled in glass, pesticide grade (Burdick and Jackson), flammable storage cabinet.

3. 1-Benzyl-3-p-Tolyltriazene (BTT) (American Tokyo Kosei, Inc., Portland, OR (503)283-1681 product #B0949). The BTT reagent is recrystallized prior to use. Dissolve approximately 10 g. of the crude BTT reagent in approximately 1 liter of solvent solution (petroleum ether: isooctane, 10:1). Filter the solution with a paper filter and funnel by gravity. Evaporate half the volume under nitrogen at 30°C. Place in freezer (<10°C) and allow to crystallize undisturbed for 3 hours. Filter by vacuum and wash with cold (from freezer) isooctane. Dry crystals under vacuum for 30-45 minutes.

A 10% solution in acetone is prepared just prior to analysis. Make only slightly more than required for that days run, ie., if 20 tubes are being analyzed (including controls) make at least 2 ml of reagent $20 \times 100 \mu\text{L} = 2 \text{ ml}$. Dissolve 0.3g in 3 ml of acetone. (NOTE: BTT is suspect carcinogen).

4. Reagent Grade Water. Water which has passed through the ion exchanger (California Purification and Recycling, Inc.) and Water I (Barnstead) water purification system (18 megohm).
5. Saturated sodium chloride solution. Add 50g of NaCl to 100 ml of reagent grade water.
6. Carborundum Chips (VWR Scientific).
7. Dry ice (Cal. Ice Co., 3928 Ince Blvd., Culver City, CA 213-559-7333).
8. Sodium bitartrate, Pfaltz and Bauer, Waterbury, CN.

CALIBRATION STANDARDS

NOTE: Label all standards and controls with the content's identity, concentration, preparation and expiration dates and repairer's initials.

1. Pure reference standards: Obtained from Cheminova Agro A/S, P.O. Box 9 DK-7620 Lemvig Denmark.
2. Stock standards (1 mg/ml): Weigh 10 mg of each alkyl phosphate standard into separate 10 ml volumetric flasks. Dilute to volume with reagent grade water. Record the weights (+/- 0.1mg). Mix and transfer to amber bottles. Stable for 6 months at 2-8°C.
3. Intermediate standards (solutions 1 and 2), 10 mg/L

Using only Class A volumetric pipets transfer 0.5 ml of each analyte stock standard to a 50 ml volumetric flask and dilute to volume with water. Transfer the solution to a labeled amber glass bottle. Stable for 6 months at 2-8°C.
4. Calibration standard solutions (1 and 2), 400 µg/L

Prepare a calibration standard solution by pipetting 2 ml of the intermediate standard solution into a 50 ml volumetric flask and dilute to volume with a previously analyzed urine. Urine should be relatively free of alkyl phosphates (less than 100 µg/L DMP and all others not detected). Freeze the standard in 1.0 ml aliquots. Stable for 1 year at less than -10°C.
5. Linearity check standards

For linearity check, add 2.5, 5, 20, 40, 100 µl of each of the intermediate standard to a tube and add sufficient volume of urine to make a total volume of 1.0 ml. These standards correspond to nominal concentrations of 25, 50, 200, 400, and 1000 mg/L, respectively. Place extracts in tightly sealed vials and in the freezer for future linearity checks. Extracts are stable for 3 months at less than -10°C.

CONTROLS

1. Stock control standards (1 mg/ml). Proceed exactly the same as for the calibration standards, but prepare separately from the calibration standards.
2. Intermediate control standards (10 mg/L).
3. High and Low Controls:
Low Control: Add 2.0 ml of the intermediate control standard to a 100 ml volumetric flask and dilute to volume with the same urine used to prepare calibration standards.

High Control: Add 10.0 ml of the intermediate control standard to a 100 ml volumetric flask and dilute to volume with same urine used to prepare low control.

Freeze the controls in 1.0 ml aliquots. Stable for 1 year at less than -10°C.

EQUIPMENT AND MATERIALS

1. Gas Chromatograph, Perkin Elmer model 8500.
2. Virtis Bench Top 3 Model Freeze Dryer, Virtis Co., Inc., Gardiner, N.Y.
3. Analytical Balance, capable of weighing to 0.1 mg, Sartorius 1712 or equivalent.
Vortex mixer, Baxter Scientific Products #98220 or equivalent.
5. Heating Block (set at 70°C), Lab-line multiblock heater or equivalent.
6. Centrifuge, with head to accommodate 16 x 125 mm culture tubes, capable of speeds of 2,000 rpm. Damon/IEC Division Model HN/SII or equivalent.
7. 16 x 125 mm culture tubes with teflon lined screw caps, Kimble or equivalent.
8. Volumetric pipets, Class A, 0.5, 1.0, 2.0 and 10.0 ml. Baxter Scientific Products.
9. Serological pipets, 1 and 10 ml, disposable. Baxter Scientific Products.
10. Pasteur pipets, disposable, 5 3/4" long, VWR Scientific, or equivalent.
11. Auto sampler vials, 1.0 ml, crimp cap, teflon lined. Alltech Associates.
12. Volumetric flasks, Class A, 10 and 100 ml.
13. Syringes, 10, 100 and 500 ul, Hamilton or equivalent.
14. Repipet system, 1 ml, 1% accuracy. Oxford or equivalent.
15. Nitrogen slow-down vaporator concentration with 12 stations N-Evap Model III (Organomation Associates, Inc.) or equivalent.
NOTE: All pipets and volumetric flasks used in this method must be thoroughly washed and then rinsed with acetone followed by hexane and dried in a 100°C oven. All non-volumetric glassware is thoroughly washed, rinsed with deionized water, and baked for two hours at 350°C.

INSTRUMENTAL PARAMETERS

Perkin-Elmer Series 8500 Gas Chromatograph with a Flame Photometric Detector.

- a. Carrier gas, Nitrogen, 8.0 ml/min (instrument readout:80).
- b. Air flow: approx 90 ml/min at 28.5 psi.
- c. Hydrogen flow: 65 ml/min at 22 psi.
- d. Column: 30M DB-210, 0.53 i.d., 1.0 um film thickness
- e. Column Temperature. 160°C, hold for 6.0 min, 5°C/min to 210°C, 5 min hold.
- f. Injection Port temperature: 250°C.

- g. Data system: method 1: OPPS 2 and method 2: OPPS I.
- h. Detector temp: 300°C.
- i. Autosampler pressures: Aux 1: 45-55 psi, Aux 2: 35-45 psi

SAMPLE PREPARATION

1. Perform a creatinine determination on an aliquot of urine (See creatinine method).
2. Thaw controls, standards and blank. Pipet 1.0 ml of patient sample into an appropriately labeled 16 x 125 mm screw cap tube. Include an empty tube to be used for a reagent blank.
3. Prepare freeze dryer for use:
 - a. Remove drying chamber from unit and dry all inner surfaces.
 - b. Remove plug from black tubing in back of freeze dryer to drain water from refrigeration coil area.
 - c. Dry all surfaces of the coils and surrounding area.
 - d. Replace plug into black tubing. Replace drying chamber on to coil area.
 - e. Turn refrigeration power switch on (upper switch). When the temperature reaches -60°C, turn vacuum power switch on (lower switch). To generate a vacuum, push down on the top of the drying chamber to seal the chamber to the freeze-drying unit. When the vacuum gauge is less than 200 millitorr the freeze dryer is ready to use.

4. "Shell" freeze thin film of urine to the wall of each tube by quickly stirring the tube in a 400 ml beaker bath of acetone and dry ice. Place all the tubes (up to 20 samples) in the freeze-drying flask (previously placed in the freezer for 20 minutes).
5. Connect the freeze-drying flask to the drying chamber port. Open the valve to the port to generate a vacuum in the flask. Freeze dry overnight. When contents of tubes are completely dry, close the valve on the port and remove the flask.
6. To each tube add 1 ml of acetone, 3 carborundum chips and 300 μ l of 10% BTT reagent. Mix for 30 seconds.
7. Cap tightly and place in a heating block at 70°C for 2 hours. Shake the tube occasionally. Remove from the heating block and allow to continue incubating overnight at room temperature.
8. Add 5 drops of 6N HCl, mix.
9. Add 10 ml of saturated NaCl solution, mix.
10. Add 0.5 ml of cyclohexane containing 0.5 μ g/ml of fenthion.
11. Shake vigorously for 1 min.
12. Centrifuge at 2000 rpm for 3 min.

Transfer upper layer to a 15 ml graduated centrifuge tube.
14. Add 1.5 ml of cyclohexane (without fenthion) and repeat steps 11-13, combining extracts in the same centrifuge tube.
15. Concentrate extracts to 0.5 ml under a gentle stream of nitrogen at 30-40°C.
16. Transfer the concentrated extract to a 0.8 ml crimp cap autosampler vial for GC analysis.

GC PROCEDURE

First change the heat shield in the detector and change the injection port septum. Light the FPD flame and turn on the photomultiplier tube. Perform all necessary GC start-up checks and procedures. Load method 2: OPPS.

2. Calibration
 - a. Inject 3 μ l of the standard (DMP, DMTP, DMDTP) at 200 μ g/L.
 - b. Record the retention time, peak height, and response factor (see calculations) of DMP in the instrument performance log.

- c. Proceed if the DMP response factor is within limits (see Quality Assurance, Section 2: Instrument Performance Check). Make sure that the retention times and response factors for the alkyl phosphate analytes in OPPS are updated (replace if necessary).
 - d. Typical to gas chromatograms of the calibration standard is shown in Figure 1.
3. Sample Analysis
- a. Load method 1: OPPS 2.
 - b. Inject of 3 µl of each sample in the following sequence.
 1. 25 ppb Calibration Standard
 2. 50 ppb Calibration Standard
 3. 200 ppb Calibration Standard
 4. 400 ppb Calibration Standard 3. Urine blank
 5. 1000 ppb Calibration Standard Low Control II
 6. Reagent blank
 7. Low Control
 8. Samples 1-20
 9. Urine blank
 10. High Control

CALCULATIONS

1. Daily calibration is performed using Calibration Standard 200. The relative response factor (RF) for each analyte is determined using the following formula:

$$RF(std) = C(std)/H(std) \times H(is)/C(is)$$

Where

- C(std) = Known concentration of analyte
- C(is) = Concentration of internal standard
- H(std) = Peak height of analyte
- H(is) = Peak height of internal standard

2. The following formula is applied to each analyte to determine the concentration in the sample:

$$C(sample) = RF(std) \times \frac{H(s)}{H(is)} \times \frac{C(is)}{V(Sample)} \times S$$

Where $C(\text{sample})$ = concentration of analyte in sample.
 S = Scaling factor
 $V(\text{sample})$ = Volume of sample
 Both S and $V(\text{sample})$ normally equal 1.
 $H(s)$ = height of analyte peak in sample

3. Creatinine correction: The sample concentration in mg/L is corrected for creatinine concentration by the following equation:

$$C(\mu\text{g/l})/\text{Creat.} = C_{\text{corr.}} (\mu\text{g/g-creat})$$

4. Example Calculation

- a. Run the calibration standard (200 $\mu\text{g/l}$).

Retention Time, Min	Peak Name	Peak Height, H	Concentration, C $\mu\text{g/l}$
2.32	DMP	387.61	200.0
5.50	Fenthion	435.75	500.0

- b. Calculate the response factor for DMP.

$$\text{RF} = C(\text{std})/H(\text{std}) \times H(\text{is})/C(\text{is})$$

$$= \frac{(200.0 \mu\text{g/l})}{(387.61 \text{ counts})} \times \frac{(435.75 \text{ counts})}{(500.0 \mu\text{g/l})} = 0.4500 \text{ (unitless).}$$

- c. Run sample

Retention Time, Min	Peak Name	Peak Height, H	Concentration, C $\mu\text{g/l}$
2.25	DMP	268.58	unknown
5.42	Fenthion	526.16	500.0

$$C(s) = \text{RF}(\text{std}) \times \frac{H(s)}{H(\text{is})} \times \frac{C(\text{is})}{V(\text{sample})} \times S$$

$$= 0.4500 \times \frac{268.58 \text{ counts}}{526.16 \text{ counts}} \times \frac{500.0 \mu\text{g/l}}{1} \times 1$$

$$= 114.85 \mu\text{g/l (rounded off to 115 } \mu\text{g/l)}$$

- d. Correct DMP concentration for creatinine level.

$$C(\mu\text{g/L})/\text{Creat}(\text{g/L}) = C \text{ corr } (\mu\text{g/g-creat})$$

$$115 \mu\text{g/L} \div 0.96 \text{ g/L} = 120 \mu\text{g/g-creat.}$$

5. Blank measurements

With each set of samples or a maximum of 20 samples a reagent blank is analyzed (tube without urine that is carried through entire procedure). If the analysis produces a peak interfering with an analyte peak, its apparent concentration is subtracted from that of the urine blank controls and samples.

A urine blank is also analyzed with each set of samples. This is a pooled urine which was used to prepare the urine control. The apparent concentrations of any interfering peaks are subtracted from the concentrations of the controls only.

6. Interferences and Interpretation of Chromatographic Data.

Since the DMDTP interferes with DETP, careful interpretation of the chromatographic data is necessary to avoid misidentification. (See Method 3-OPPS-870216-4 for a discussion of identification of the diethylthiophosphate peaks)

- a. The monothiophosphates appear as two peaks. The two DMTP individual peak concentrations should be approximately equal (+/-20%).
- b. Exposure to methyl phosphate pesticides gives methyl phosphate metabolites. Metabolism tends to give the more highly oxygenated species. Therefore, the presence of DMP and DMTP would be expected with the presence of DMDTP.
- c. The retention times of each analyte peak are distinguishable from one another. Careful examination of retention times compared to standards will often help resolve identification questions.

7. Confirmation

When multiple phosphate pesticide (mixture of ethyl and methyl) exposure is evident, GC/MS confirmation may be necessary to resolve the interferences. (See organophosphate pesticide confirmation).

REPORTING RESULTS

The concentration of each analyte is reported in $\mu\text{g/L}$ and $\mu\text{g/g-creatinine}$ to a maximum of three significant figures. The following comment is included: "Please refer to Pacific Toxicology Organophosphate Pesticide Information Bulletin on this test procedure."

QUALITY ASSURANCE

Detector Linearity

Detector linearity is checked (1) with each new set of standards (2) when the controls have failed Westgard rules (3) when the daily instrument performance check is outside 15% limits (see below) and (4) when a new column is installed.

A five-point linearity check is performed using extracted standard which include the calibration standard (400 µg/L) and the 4 linearity check standards (see Calibration Standard Solutions, Linearity Check Standards). The standards spiked in urine are prepared as usual.

Analyze all standards, including the blank and record the retention time, peak height and response factor of each analyte. If the percent relative standard deviation of the response factors (RF) for the four standards is within 20% over the working range, linearity through the origin may be assumed.

2. Instrument Performance Check

The calibration standard solution #2 (400 µg/L) is injected prior to each run to ensure proper GC conditions, detector response and retention times. Record the retention time, peak area and response factor of DEP in the instrument performance log. If the daily response factor differs from the response factor established in the most recent linearity check by more than 15%, repeat the instrument performance check. If the response factor is still outside limits, a new linearity check is performed. The retention time and peak area are recorded to observe trends to aid in diagnosing instrumental problems. If a negative peak appears in the chromatogram just prior to the second DETP peak, the detector quartz liner should be exchanged with a clean silanized one.

3. Quality Control - Urine Controls

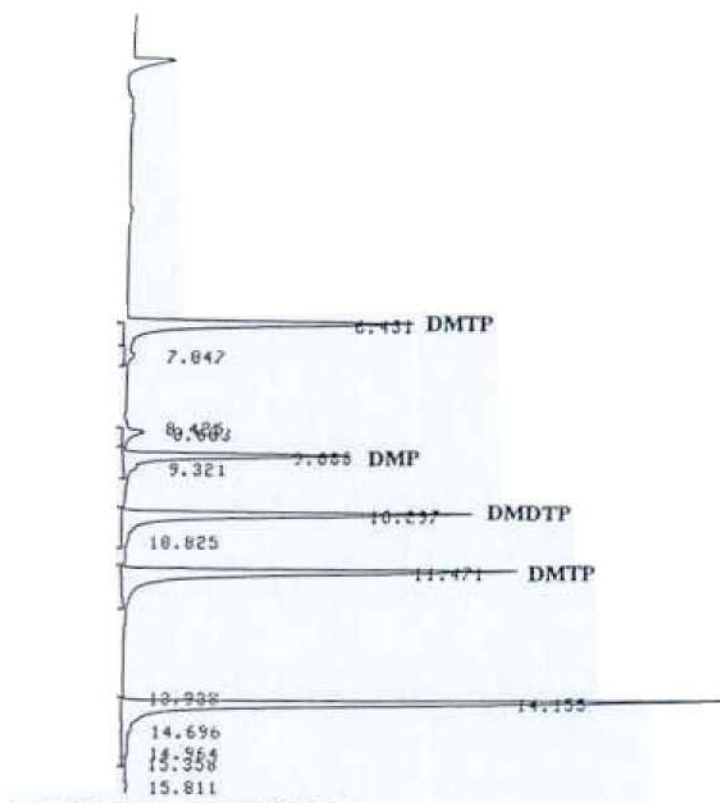
High and low urine controls are analyzed with each set of samples and the results are recorded on the sample work-sheet and under "Daily QC" in the lab computer system. Record "Out of Limits" controls in the "Out of Limits" log, describing the cause of the problem and actions taken to correct it. Bring any such condition to the attention of the supervisor or laboratory director. Acceptable ranges of the controls are listed in Table III.

Notes:

1. A negative deflection in the FPD chromatogram has been observed after a period of time when the FPD quartz heat shield has not been changed. This suppression of the baseline is due to the presence of a byproduct of the derivative reaction. If the heat shield is clean, the FPD background level is low enough that there is no signal for the interference to suppress, therefore, no negative peak appears. It is therefore important to replace the heat shield with a cleaned one daily to maintain a low background signal.
2. For studies only requiring a single or limited group of metabolites, standards and controls may be prepared for the measured analytes only. All other requirements for the preparation and storage remain the same as described in the method.

Figure 1

Typical chromatogram of 200 µg/l calibration standard.



CHROMATOGRAM 5 MEMORIZED

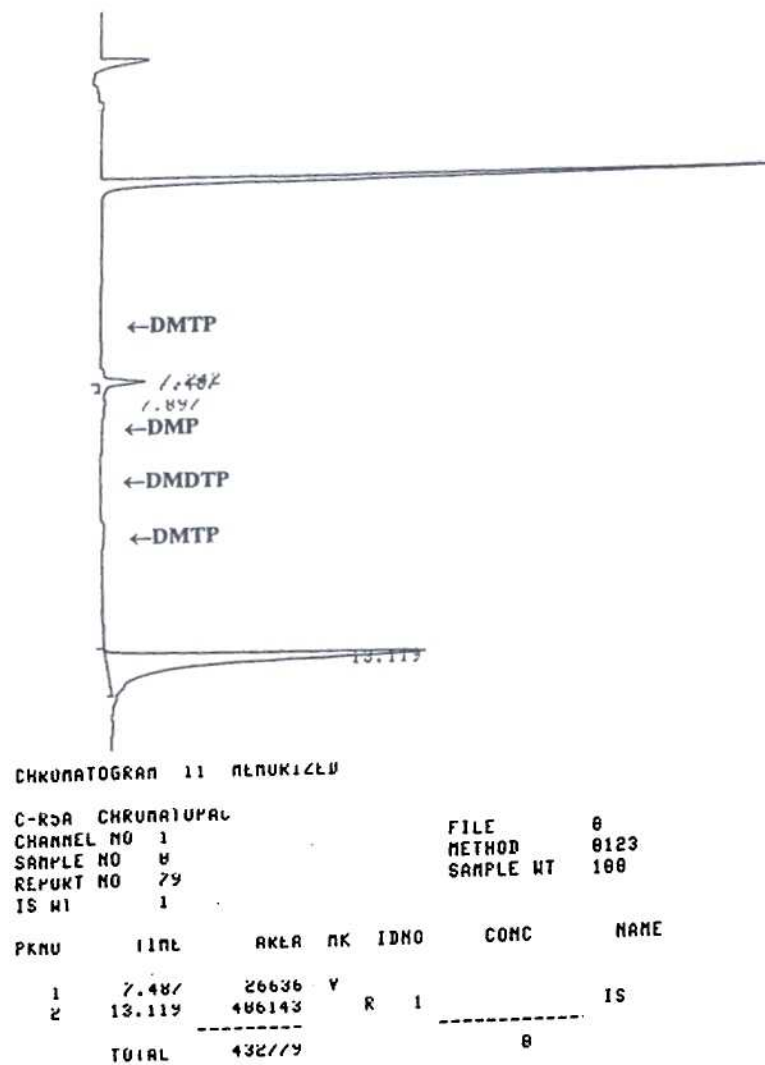
C-RSA CHROMATOPAC
CHANNEL NO 1
SAMPLE NO 8
REPORT NO 658
IS WT 1

FILE 8
METHOD 0123
SAMPLE WT 188

PKNO	TIME	AREA	NK	IDNO	CONC	NAME
------	------	------	----	------	------	------

1	6.431	212912		2	185.2462	DMTP
2	7.847	9225	V			
3	8.683	16562	V			
	9.888	146238	S	3	187.7378	DMP
	10.297	241281	S	4	189.7865	DMDTP
6	11.471	387988		5	196.9137	DMTP
7	14.155	419857	SV R	1		IS
TOTAL		1353894			759.6842	

Figure 2. Typical chromatogram of urine blank.



APPENDIX 4-

**QUALITY ASSURANCE PROGRAM
STANDARD OPERATING PROCEDURE:**

SOP: 2-QAP-891122-3 Section X

IX.

Instrument Calibration and Linearity

- A. The limits of an instrument's response linearity must be established prior to analysis and maintained at all times. Calibration standards may be external unextracted standards or extracted standards depending on the requirements of the method.

Instrument response linearity is checked under the following conditions:

- a. With each new set of standards (or a minimum of every six months)
- b. When controls fail the Westgard quality control rules and either the daily calibration factor (or instrument performance check) has shifted or there are other indications of a shift in the linearity response of the instrument
- c. The supervisor or lab director determines that the daily calibration factor (or instrument performance check) is falling either far outside of too often outside acceptable limits (the mean plus or minus 15% of this mean as determined by the last linearity check performed). This condition is meant to cover situations where the controls don't fail, but the instrument linearity has shifted. A failure of the daily calibration factor in conjunction with a developing trend (above or below the mean) in the daily quality control points may indicate that this has occurred. This condition may be due to changed in chromatographic conditions such as the column and may or may not be accompanied by a loss of linearity at the low and/or high ends of the expected curve.
- d. A critical part of the instrument is replaced (i.e. chromatographic column, a detector, UV lamp, PID lamp, photomultiplier tube, etc.)
- e. An exception to the above are those test for which complete standard curve are run daily with each sample batch. Currently all metals analysis performed by atomic absorption and ICP-MS fall into this category and standard curves are filed with each batch analysis.

(A) Quality Control (Clinical Division)

- A. Commercial controls are provided and used for quality control monitoring in the

- clinical division of the laboratory, including such testing as routine chemistry analysis, routine hematology (CBC), RIA, urinalysis, blood lead and ZPP
- B. Two, and if needed, three levels of controls will be included in each analytical batch. The number of patient samples and control samples in a given analytical batch is defined in each test procedure. Any deviation from the established protocol must be approved by a manager or higher level position.
 - C. Control ranges shall be easily accessible to all personnel in the clinical division and the control results shall be recorded and their acceptability verified before patient results are released.
 - D. Control ranges are provided with commercial quality control materials. These are the initial ranges used. At the end of each month, quality control charts are printed. Some instruments maintain their own quality control data and this data is not entered into the laboratory computer system. Monthly quality control charts are printed on the Beckman Synchron CX-7 terminal along with a monthly quality control summary report. Monthly quality control data for hematology are printed on the Technicon H-1 terminal as a cumulative report. All quality control data (laboratory computer system and instrument systems) are reviewed and control ranges are updated based on in-house performance when appropriate. All quality control data in the clinical division is reviewed and initialed by the manager. In addition, most quality control data is submitted monthly to an outside quality control service for peer group data comparison. This further ensures the reliability of our quality control program.
 - E. Suspected outliers, or measurements that do not belong in a population due to errors, instrument malfunctions, or unusual losses or contamination may be tested according to the Grubbs test (Appendix D)(9). If the results of this test show this data point to be an outlier this data point should be excluded from calculations made to determine new quality control ranges. If the results of this test show this data point to not be an outlier, this data point should be included in such calculations.
 - F. An "Out of Limits" or "Out of Control" log is used for recording all quality control results which do not fall within the expected range. Westgard rules are used to evaluate quality control data (see section X (B), items N & O in this manual). The log will document the data of each entry, the Westgard rule discrepancy observed, its cause (if known), the action taken, and the analyst's initials. Any other documentation deemed necessary by the supervisor will be documented and initialed.

(B) Quality Control (Environmental Division)

- A. On a day-to-day basis, it is the routine quality control program which ensures the accuracy and precision of the laboratory results. The program consists of the use of pure standard solution, spiked samples of the appropriate matrix and of known concentration (controls) and blanks (matrix and reagent).
- B. Controls and blanks are treated identically to unknown or patient samples and are therefore subject to the same analytical errors. The observed value of the control on a continuing basis reflects the precision, or reproducibility, of the test. Analyses of blanks demonstrate that possible interferences from the analytical system and glassware are monitored
- C. At least two controls and a blank should be included in each analytical batch. The low control should be at approximately five times the detection limit and the high control at a regulatory or clinical decision point or at a level approximately ten times the level of the low control.
- D. The U.S. E.P.A. has recognized that methods in which multiple, similar analytes are measured increases the probability that any one of the results will be out of control strictly due to random error (4,5). This is particularly burdensome when lengthy sample preparation schemes and limited sample amounts for repeat analysis are involved. Therefore, in methods where more than 5 analytes are measured, the control may consist of a number (30-60% of the total) of representative analytes which act as mode compounds for the entire suite of reported analytes.
- E. Controls ranges shall be easily accessible to all laboratory personnel performing the test and the control results shall be recorded and their acceptability verified before patient results are released.
- F. Initial control ranged for a new test procedure are established by analyzing 3 sets of 7 replicates of controls (and blanks). The mean and standard deviation are calculated and the ranges of plus or minus 2 standard deviations are set. Formulas for statistical calculations are presented in Appendix D. When 20 controls have been analyzed, re-evaluate the ranges and set new ranges, if necessary.
- G. Suspected outliers, or measurements that do not belong in a population due to errors, instrument malfunctions, or unusual losses of contamination may be tested according to the Grubbs test (Appendix D).
- H. Establishing in initial range for the new control lot will depend on the frequency with which the test is performed. All new control ranges must be approved by a supervisor, lab manager or director. All data representing the crossover period of the old and new control and the calculation of the initial new range should be recorded on a *Cross Log for New Controls* (see Pacific Toxicology Laboratories' *Approved Forms Manual CTL-4*) and this must be approved and entered into the laboratory computer quality control database. Approval is indicated on the "Crossover Log for New Controls" by the initials of a supervisor or lab director.
- I. Control solutions are not commercially available for many of the analyses performed at Pacific Toxicology Laboratories and many are made in-house. Consequently, the real stability of these solutions may initially only be arbitrarily assigned based on conservative estimates. Many solutions will be stable beyond the expiration date initially assigned to them. Under these circumstances the lab

director may periodically extend the expiration date of in-house control solutions providing the behavior of these controls are closely monitored. The quality control data associated with this material should be used to approve or disapprove continued use of this quality control material. All such extensions of expiration dates will be documented on an *Extension of Expiration Date of Control of Standard Material* (see Pacific Toxicology Laboratories' *Approved Forms Manual STD-6*) which is located in the reagent section of the calibration binder for each test.

- J. At the time of method development and if a quality control issue arises which indicates the need, reference material from an outside source (e.g. NIST standard reference material) is used to validate testing procedures.
- K. Quality Control Charts. Quality control charts are summary reports containing data collected during the time period requested and indicated at the top of the chart. In addition, the following information is included in the header a) name of the analyte, b) instrument of which the analysis is performed, c) control level, d) control lot number (for controls prepared in-house the lot number is the date prepared followed by the initials of the preparer), e) the material name (usually refers to the test procedure), f) the expiration date of the control and g) a summary of the Westgard rules in effect. Individual values are plotted on a bar graph in which the mean, one, two and three standard deviations from the mean are indicated. The following information is also included: the expected range, the date, the time and the technician's initials logged into the computer when the data point was entered and the date and the time the data was certified. The date of entry of QC data point(s) should coincide with the date on which the analysis was performed and/or the results were reported.
- L. When new controls are prepared all data concerning the crossover of the new control material with the current control material is entered on the "Crossover Log for New Controls". This log, along with the "Quality Control Range History" are kept in the quality control binder for that particular test. A listing of the Current QC Range is maintained in the calibration binder for each test (see Pacific Toxicology Laboratories' *Approved Forms Manual CTL-3* for examples of this form).
- M. Each analyte should have an Out of Limits Log Sheet (see Pacific Toxicology Laboratories' *Approved Forms Manual CTL-7*) showing the date of each entry, the Westgard rule discrepancy observed, its cause (if known), the action taken, and the analysts initials. Data points exceeding 3 S.D. of the mean will be entered on the Out of Limits Log, but not in the laboratory computer system's quality control database. The reason for this is to make the calculated mean and standard deviation information at the end of each quality control summary report more useful. It is not possible to exclude any points entered into the computer system from these calculations and points outside 3 S.D. would, under most circumstances, skew this data and make it useless. If the inclusion of any points outside 3 S.D. of the mean is desired for the re-evaluation of a mean and standard deviation the calculation will have to be performed manually using a calculator.
- N. The multi-rule quality control procedure developed by Westgard is followed to guide medical technologists in their decision making process (6,7). The "Westgard Rules" are used by the laboratory information system (computer) and

all rule violations are automatically flagged on the quality control summary charts. If a data point exceeds 3 S.D. of the mean it will be deleted before certification and entered on the "Out of Limits" log. If a data point does not exceed 3 S.D. of the mean but does cause a Westgard rule failure it will be left in the computer and certified. The appropriate corrective action statement will then be appended to the data point in the computer and listed at the bottom of the page of the summary report on which the data point is listed. The failing data points and the corrective action for these points which are within 3 S.D. of the mean will also be entered on the "out of Limits" log.

- O. The following describes the Westgard rules and how they are used:
If none of the control observations exceed the 2 S.D. limits, the analytical run is judged to be in control and patient data are reported. If any of the observations violate the 1-2s rule, the data point is further tested by applying the 1-3s, 2-2s, 2 of 3-2s, r-4s, 4-1s, and 12x rules. If none of these additional rules is violated the point flagged as low (L1) or high (H1), but the run is defined as out of control.

1-2s.

One control observation exceeds control limits set as the mean plus or minus two standard deviations. This is a warning rule and requires additional inspection of the control data.

2. 1-3s.

One control exceeds control limits set as the mean plus or minus three standard deviations.

3. 2-2s.

Two consecutive control observations exceed the same limit (the mean plus or minus two standard deviations). This rule can be applied across materials (levels) or for two consecutive runs of the same material (level).

4. R-4s.

Rule involving two observations of one control material in the same run in which the difference between the two observations is greater than four standard deviations. This rule should not be applied across runs.

5. 4-1s.

Four consecutive observations exceed the same limit defined as the mean plus or minus one standard deviation. This rule can be applied to different runs of the same material (level) or it can be used across control materials.

6. 12x.

Twelve consecutive control observations fall on the same side of the mean. This rule can be applied to the same material (levels). This is a warning that a trend has developed and the quality control range should be re-evaluated if appropriate or the problem causing this trend, if one can be identified, should be corrected.

Note: The experience of the medical technologist performing chromatographic analyses is invaluable to the success of the methods.

The chromatographic traces of blanks, calibration standards, controls and patient samples should be evaluated for peak shape, detector response, and peak integration baselines. For instance, if a patient sample chromatogram appears abnormal, even if the controls are in range, the sample should be reintegrated or reanalyzed (reinjected), if possible, to validate the result.

- P. When a run is considered out of control, notify your supervisor, lab director, or the medical director and review the data to determine the cause, if possible, such as:
1. Clerical or handling error.
 2. Reagent problem (incorrect pH, evaporation of solvent, bacterial growth, etc.)
 3. Instrument malfunction (leaky septum, clogged autosampler, dirty detector, etc.)
 4. Instrument calibration error (retention time shift, peak table not updated, calibration standards evaporated or degraded).
 5. Control degradation (labile constituent, evaporation, bacterial growth).
 6. Sample carry-over.
 7. Background contamination (dirty glassware, reagents or instrument)
 8. Random error (unknown).

Q. Determine the action to be taken, such as the following:

1. Repeat the entire analytical run, including calibration standards, controls and samples. This action would apply when a systematic error in sample preparation appears to be the cause.
2. Recalibrate the instrument, recalculate controls and patient samples. This would apply when the instrument was not sufficiently stabilized or equilibrated at the time of calibration.
3. Make instrument adjustments, replacements or repairs, and recalibrate the instrument. Reanalyze controls and patient samples. This would apply when the instrument appears to have malfunctioned requiring an adjustment before continuing.
4. Repeat the test on new vials of controls, new standards, and between 2 and 5 representative samples of the run. If the new controls are within acceptable limits, compare the patients' repeated values with their original values. If the compared values are within the acceptable limit of duplicate test values at that concentration, results may be released. If control values remain unacceptable, the problem must be investigated; consult your supervisor, or the medical director. This would apply to the situation where one control is causing what appears to be an anomalous out-of-control situation. Also, this action applies to the situation where there are samples with insufficient volumes to rerun the entire batch.
5. A special care may occur where all samples have an insufficient volume to rerun the analysis. If either the low or high controls is causing an out of control situation on the "high" side of the acceptable limits, all samples

which had not analytes detected (results = ND or none detected) may be reported. Samples with positive values cannot be reported. A problem sheet requesting additional sample from a referral laboratory or new samples from the patient should be immediately given to client services.

- R. Quality control charts should be printed out and reviewed by supervisors on a monthly basis to ensure that proper procedures have been followed and that complete and accurate documentation has been made. When the review is complete, the supervisor should initial the chart with the date.

**APPENDIX 5 - CERTIFICATES OF ANALYSIS FOR REFERENCE
STANDARDS**

BATCH ANALYTICAL CERTIFICATE
ARTICLE IDENTIFICATION

Article Name: **Malathion Monocarboxylic Acid** (α + β mixture) Reg. Dept. Code: -
 Manufacturer: **Cheminova Agro A/S** Batch No.: **275-MJH-82-1**
 Origin of Production: Commercial ☐ ; Pilot plant ☐ ; Laboratory ☒

PHYSICAL PROPERTIES

Technical Product ☐ ; Preparation of technical Product ☐ ; Analytical Standard ☒ ; Liquid ☒ ; Solid ☐ ; Colour: **Yellow**

Recommended Storage Conditions

Ambient temperature in the dark _____ Expiry Date: _____
 In refrigerator The article is stable at least 5 years from date
 In deep freezer X of analysis/last date of reanalysis when stored at
 recommended conditions.

Additional Comments:

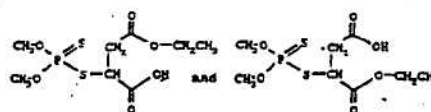
ACTIVE INGREDIENT IDENTIFICATION

Common Name/ISO-Name: - CAS-Name: **Butanedioic acid, ((dimethoxyphosphinothioyl)thio)-, monoethyl ester.**

 CAS No.: **35884-76-5**

 Empirical Formula: **C₉H₁₅O₆PS₂**

Structural Formula:

 Molecular Weight: **302.31 g/mol**


Identified by means of:

NMR ☒ ; IR ☒ ; UV ☒ ; MS ☒ ; Other Methods:

ANALYTICAL DATA

Certified Purity/Content of a.i.: **91.0% w/w**
 Analytical Method: **³¹P-NMR**
 Analytical Report (incl. amendments): **REF 119-01**

**VERIFIED COPY
OF ORIGINAL**

Date of analysis/ reanalysis (yy/mm/dd)	980622		
-for article stored at -	Cheminova Agro A/S		
		INITIALS	DATE

GLP-COMPLIANCE

The identification and determination of purity/content of active ingredient were performed at Cheminova Agro A/S and conducted in accordance with FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices. All raw data, documentation, records, protocols, test articles, reference samples, and report are retained in the GLP archives of Cheminova Agro A/S, Denmark.

 Date: **July 22, 1998**

Signature:

Bente C. Sandberg



Cheminova Agro A/S
P.O. Box 8
DK-7620 Lemvig
Denmark

Phone (+45) 97 83 41 00
Fax (+45) 97 83 45 55
Telex 86314 CHEMV DK
A/S reg.no. 177.122

BATCH ANALYTICAL CERTIFICATE

ARTICLE IDENTIFICATION

Article Name: Malathion Dicarboxylic Acid

Reg. Dept. Code: -

PHYSICAL PROPERTIES

Technical Product ☐ ; Preparation of technical Product ☐ ; Analytical Standard ☒ Liquid ☐ ; Solid ☒ ; Colour White

Recommended Storage Conditions

Ambient temperature in the dark

In refrigerator

In deep freezer

Expiry Date:

The article is stable at least 5 years from date of analysis/last date of reanalysis when stored at recommended conditions.

Additional Comments:

ACTIVE INGREDIENT IDENTIFICATION

Common Name/ISO-Name: -

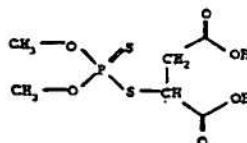
CAS-Name: Butane dioic acid,
[(dimethoxyphosphinothioyl)thio]

CAS No.: 1190-28-9

Empirical Formula: $C_6H_{12}O_6PS_2$

Molecular Weight: 274.26 g/mol

Structural Formula:



Identified by means of:

NMR ☒ ; IR ☒ ; UV ☒ ; MS ☒ ; Other Methods:

ANALYTICAL DATA

Certified Purity/Content of a.i.: 99.04 w/w

Analytical Method: ³¹P-NMR

Analytical Report (incl. amendments): REF 120-01

VERIFIED COPY
OF ORIGINAL

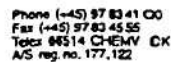
Date of analysis/ reanalysis (yy/mm/dd)	950630	980609	INITIALS	DATE
-for article stored at -	Cheminova Agro A/S	Cheminova Agro A/S		

GLP-COMPLIANCE

The identification and determination of purity/content of active ingredient were performed Cheminova Agro A/S and conducted in accordance with FIFRA Good Laboratory Practice Standard 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices. All raw data, documentation, records, protocols, test articles, reference samples, and reports are retained in the GLP archives of Cheminova Agro A/S, Denmark.

Date: June 24, 1998

Signature: Rente L. Sundberg



BATCH ANALYTICAL CERTIFICATE

maise 11-30-98 H
maise 1-11-99 M



Cheminova Agro A/S
P. O. Box 8
DK-7820 Lemvig
Denmark

Phone (+45) 97 83 41 00
Fax (+45) 97 83 45 55
Telex 86514 CHEMV DK
A/S reg. no. 177.122

Date:

BATCH ANALYTICAL CERTIFICATE

ARTICLE IDENTIFICATION

Article Name: O,O-dimethyl-thiophosphoric acid, dicyclohexylammonium salt. Reg. Dept. Code:
Manufacturer: Cheminova Agro A/S Batch No.: 267-05J-54B
Origin of Production: Commercial ☐ ; Pilot plant ☐ ; Laboratory ☒ ;

PHYSICAL PROPERTIES

Technical ☐ ; Preparation of ☐ ; Analytical ☒ ; Liquid ☐ ; Solid ☒ ; Colour: Colourless

Recommended Storage Conditions

Ambient temperature in the dark ☐ ; Expiry Date: The article is stable at least 5 years from date of analysis/last date of reanalysis when stored at recommended conditions.
In refrigerator ☐ ;
In deep freezer ☒ ;
Additional Comments:

ACTIVE INGREDIENT IDENTIFICATION

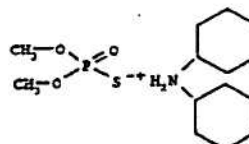
Common Name/ISO-Name : CAS-Name: Phosphorothioic acid, O,O-dimethyl ester (free acid)

CAS No : 1112-38-5

Structural Formula:

Empirical Formula : C₁₄H₃₀NO₃PS

Molecular Weight : 323.2



Identified by means of:

NMR ☒ ; IR ☒ ; UV ☒ ; MS ☒ ; Other Methods:

ANALYTICAL DATA

Certified Purity/Content of a.i.: 97.94 w/w Analytical Report (incl. amendments): RKP 083-01
Analytical Method: ³¹P-NMR

Date of analysis/ reanalysis (yy-mm-dd)	930609	960521	VERIFIED COPY OF ORIGINAL
-for article stored at -	Cheminova Agro A/S	Cheminova Agro A/S	
			INITIALS DATE

GLP-COMPLIANCE

The identification and determination of purity/content of active ingredient were performed at Cheminova Agro A/S and conducted in accordance with FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices. All raw data, documentation, records, protocols, test articles, reference samples, and report are retained in the GLP archives of Cheminova Agro A/S, Denmark.

Date: June 6, 1996

Signature: Elsa V. Sørensen
Elsa V. Sørensen



Chemnova Agro A/S
P.O. Box 9
DK-7620 Lemvig
Denmark

Phone (+45) 97 83 41 00
Fax (+45) 97 82 45 55
Telex 86514 CHEMV DK
A/S reg. no. 177.122

Date:

BATCH ANALYTICAL CERTIFICATE

ARTICLE IDENTIFICATION	
Article Name: MP-1-K-salt	Reg. Dept. Code: -
Manufacturer: Chemnova Agro A/S	Batch No.: 291-25e-62A
Origin of Production: Commercial <input type="checkbox"/> ; Pilot plant <input type="checkbox"/> ; Laboratory <input checked="" type="checkbox"/>	
PHYSICAL PROPERTIES	
Technical Product <input type="checkbox"/> ; Preparation of technical Product <input type="checkbox"/> ; Analytical Standard <input checked="" type="checkbox"/> ; Liquid <input type="checkbox"/> ; Solid <input checked="" type="checkbox"/> ; Colour: White	
Recommended Storage Conditions	
Ambient temperature in the dark _____	Expiry Date: _____
In refrigerator <input checked="" type="checkbox"/>	The article is stable at least 5 years from date of analysis/last date of reanalysis when stored at recommended conditions.
In deep freezer <input checked="" type="checkbox"/>	
Additional Comments: _____	
ACTIVE INGREDIENT IDENTIFICATION	
Common Name/ISO-Name: -	CAS-Name: Phosphorodithioic acid, 0,0-dimethyl ester, potassium salt
CAS No: 16001-66-6	
Empirical Formula: C ₂ H ₆ KO ₂ PS ₂	Structural Formula: $\begin{array}{c} \text{CH}_3-\text{O}-\text{P}(=\text{S})_2-\text{O}-\text{CH}_3 \\ \\ \text{K}^+ \end{array}$
Molecular Weight: 196.27 g/mol	
Identified by means of: NMR <input checked="" type="checkbox"/> ; IR <input checked="" type="checkbox"/> ; UV <input checked="" type="checkbox"/> ; MS <input type="checkbox"/> ; Other Methods: _____	
ANALYTICAL DATA	
Certified Purity/Content of a.i.: 99.14 w/w	
Analytical Method: ³¹ P-NMR Analytical Report (incl. amendments): REF 057-01	
Date of analysis/reanalysis (yy-mm-dd)	921006 950913 980903
-for article stored at.-	Chemnova Agro A/S Chemnova Agro A/S Chemnova Agro A/S
VERIFIED COPY OF ORIGINAL	
GLP-COMPLIANCE	
The identification and determination of purity/content of active ingredient were performed at Chemnova Agro A/S and conducted in accordance with FIFRA Good Laboratory Practices, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices. All raw data, documentation, records, protocols, test articles, reference samples, and report are retained in the GLP archives of Chemnova Agro A/S, Denmark.	
Date: September 14 1998	Signature: <u>Elsa V. Sorensen</u> Elsa V. Sorensen

performed 11.30.88 for
analysis 1.11.97 per

CERTIFICATE OF ANALYSIS

INVOICE #: CS195332
PO #: 104579

CATALOG #: PS-90

DESCRIPTION: Diazinon

LOT #: 214-64A

PURITY: 99.3%

EXPIRATION DATE: 10/01

CAS #: 333-41-5

VERIFIED COPY
OF ORIGINAL

INITIALS

DATE



Chem Service, Inc. guarantees the purity of this chemical $\pm 0.5\%$ deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

Susan J Fishman

Susan J Fishman
VPO/QAM

5-5 2001
M



660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599 • (610) 692-3026 • FAX (610) 692-8729

CERTIFICATE OF ANALYSIS

INVOICE #: CS191812
PO #: 151595

CATALOG #: PS-655

CAS #: 55-38-9

DESCRIPTION: Fenthion

LOT #: 208-52B

PURITY: 98.0%

EXPIRATION DATE: 07/01

VERIFIED COPY
OF ORIGINAL

JS
INITIALS 2/20/00
DATE



Chem Service, Inc. guarantees the purity of this chemical $\pm 0.5\%$ deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Two or more of the following methods of analysis are used to determine purity: Melting point, refractive index, titration, IR, TLC, GC/FID, GC/TCD, GC/ECD, GC/MS, HPLC or DSC.

Our standards are suitable for use with all EPA methods.

Certified By:

Susan J Fishman

Susan J Fishman
VPO/QAM

2-1-96-95
JS



660 Tower Lane • P.O. Box 599 • West Chester, PA 19381-0599 • (610) 692-3026 • FAX (610) 692-8729