

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 1 YEAR

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day															
		191- 294	113- 119	119- 122	130- 134	173- 182	406- 410	434- 438	469- 473	497- 501	532- 536	560- 564	588- 592	623- 627	631- 635	666- 670	714- 718
10	360	25	26	26	27	28	26	27	20	26	27	13	25	23	19	9 ^a	Dead
	361																20
	362	25	26	27	25	23	24	24	23	22	21	26	ND	21	24	ND	20
	363																
	364	23	26	27	23	27	26	27	19	25	25	25	23	28	28	13	25
	365																
	366	25	24	26	25	21	24	25	36	24	22	24	22	17		Dead	
	367																
	368	25	24	25	25	21	25	24	28	16	24	71 ^a	26	25	25	30	15
	369																
	370	26	24	27	26	ND	24	25	24	21	21						
	371																
	372	21	24	30	26	24	24	23	17	26	25	16	24	25	23	17 ^a	22
	373																
	374	25	28	27	29	ND	25	25	24	26	31	18					
	375																
	376	25	24	27	30	29	25	26	18	26	25	26	25	24		Dead	
	377																
	378	23	25	28	17	49 ^a	29	31	30	32	10				24	Dead	
	379																
	380																
	381												22	17	19	18	Dead
	382																
	383												27	26	23	23	23
	384																
	385																
	386																
	387																
	388															26	22
	389															20	14
	390																
	391																23
	392																
	393																
	394																
	395																
	396																
	397																
	398																
	399																
	900																Dead
	901																Dead
	902																
	903																
	904																
	905																
	906																
	907																
	908																
	909																
MEAN±SD		26±1	25±1	27±1	26±3	26±4	25±2	26±2	14±6	14±4	25±3	27±6	24±2	22±4 ^b	23±3	26±7	20±4

ND = No data available.

^aValues excluded from statistical analysis calculations.^bStatistically significant difference from control mean using Dunnett's test, $p < 0.05$.

APPENDIX 3 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 1,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day																							
		0- 3	4- 7	7- 11	11- 14	14- 18	18- 21	21- 25	25- 28	28- 31	31- 35	35- 38	38- 42	42- 46	46- 49	49- 53	53- 56	56- 60	60- 63	63- 67	67- 70	70- 74	74- 77		
10	310																								
	311	24	23	26	26	27	26	26	27	26	26	25	26	23	23	27	27	27	26	27	25	28	27		
	312																								
	313	22	26	25	24	25	25	25	25	24	25	23	23	27	25	25	25	26	23	25	24	25	24		
	314																								
	315	26	30	29	28	28	26	27	28	26	24	24	25	23	27	27	25	26	25	25	24	26	26		
	316																								
	317	24	26	22	26	27	25	25	27	26	26	20	25	27	26	25	24	25	24	ND	25	26	25		
	318																								
	319	26	28	26	25	27	25	24	26	25	26	ND	25	27	26	26	ND	26	ND	26	24	26	26		
	320																								
	321	24	29	27	28	29	28	27	28	26	27	27	26	29	27	28	27	26	ND	27	25	26	26		
	322																								
	323	24	26	25	25	25	25	23	24	24	24	24	23	25	25	24	23	23	22	26	22	24	23		
	324																								
	325	24	29	27	29	29	28	28	30	27	27	27	28	30	30	28	27	28	26	28	28	29	28		
	326																								
	327	24	26	26	26	25	25	24	26	25	24	25	26	27	25	25	25	25	ND	25	24	25	26		
	328																								
	329	24	25	25	25	26	25	24	26	24	24	25	25	27	25	25	24	24	24	25	24	24	25		
	330																								
	331																								
	332																								
	333																								
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	357																								
	358																								
	359																								

MEAN±SD 24±1 27±1 26±2 26±2 27±2 26±1 25±1 27±2 25±1 25±1 25±2 25±1 25±1 27±1 26±2 26±1 25±1 25±3 24±2 26±1 25±2 26±2 26±1

ND = No data available.

APPENDIX 8 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day															
		31- 36	34- 38	38- 41	41- 45	45- 49	49- 53	53- 57	57- 61	61- 65	65- 69	69- 73	73- 77	77- 81	81- 85	85- 89	89- 93
10	310	27	28	27	28	27	26	26	25	ND	25	26	29	29	ND	29	ND
	311																
	312	24	24	24	24	24	24	23	24	ND	25	24	26	25	27	25	25
	313																
	314	25	27	26	26	27	26	25	24	27	26	24	27	26	26	26	25
	315																
	316	26	25	25	25	25	26	25	24	28	26	25	28	27	27	26	25
	317																
	318	26	25	25	24	25	24	25	24	26	24	24	26	27	26	26	27
	319																
	320	26	26	26	26	26	26	25	24	27	24	24	26	26	25	25	27
	321																
	322	25	25	24	25	27	26	23	23	ND	17	23	27	28	27	25	24
	323																
	324	28	28	28	28	28	28	27	27	29	28	26	29	29	27	27	27
	325																
	326	24	25	25	25	24	24	24	22	26	24	23	25	24	24	24	24
	327																
	328	24	25	25	25	23	22	25	24	27	25	25	26	24	24	25	26
	329																
	330																
	331																
	332																
	333																
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	352																
	353																
	354																
	355																
	356																
	357																
	358																
	359																
MEAN±SD		26±1	26±1	26±1	25±1	26±2	25±2	25±1	24±1	27±1	24±3	24±1	27±1	26±2	26±1	26±1	25±1

ND = No data available.

APPENDIX 8 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day																
		291- 394	315- 319	319- 322	350- 354	378- 382	406- 410	434- 438	469- 473	497- 501	532- 536	560- 564	588- 592	623- 627	651- 655	688- 690	714- 718	
10	310	29	27	28	29	19	27	27	25	28	26	29	25	13 ^a	Dead			
	311														26	Dead		
	312	25	24	25	24	24	23	23					Dead					
	313								24	28	24	29	25	24	19	Dead		
	314	27	27	28	26	27	26	27	22	27	25	23	24	26	29	24	22	
	315																	
	316	27	26	27	25	25	20	14	12	24	24	20			Dead			
	317												Dead					
	318	27	25	27	33	28	26	25	28	25	26	33	25	25	20	13	15	
	319																	
	320	24	25	26	31	25	22	18					Dead					
	321								19	28	26	27	25	23	25	Dead		
	322	25	25	26	25	24	22	22					Dead					
	323								ND	26	24	45 ^a	24	23	17	20	ND	
	324	29	26	29	28	27	27	27	21	25	25	36	19		Dead			
	325													24	Dead			
	326	25	24	27	31	23	24	24	26	22	21			Dead				
	327											25	19	Dead				
	328	25	24	27	25	ND	24	25	23	31	24	35	20	23	Dead			
	329														Dead			
	330								Dead									
	331								Dead									
	332								Dead									
	333								Dead									
	334								Dead									
	335								Dead									
	336											25	24	Dead				
	337								Dead									
	338								Dead									
	339								Dead									
	340								Dead									
	341								Dead									
	342								Dead									
	343								Dead									
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	345								22 26 26									
	346								Dead									
	347								Dead									
	348								Dead									
	349								20									
	350								Dead									
	351								15 23 20									
	352								Dead									
	353								Dead									
	354								Dead									
	355								Dead									
	356								Dead									
	357								Dead									
	358								Dead									
	359								Dead									
MEAN±SD		26±2	25±1	27±1	28±3	27±5	24±2	23±4	22±5	26±3	24±1	29±6	23±3	24±1	21±5	22±3	21±5	

ND = No data available.

^avalues excluded from statistical analysis calculations.

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day																							
		0- 4	4- 7	7- 11	11- 14	14- 18	18- 21	21- 25	25- 29	29- 32	32- 35	35- 39	39- 42	42- 46	46- 49	49- 53	53- 56	56- 60	60- 63	63- 67	67- 70	70- 74	74- 77		
3	760																								
	761	25	26	26	26	27	27	26	25	25	26	26	25	27	25	25	24	25	24	25	24	25	24		
	762																								
	763	23	26	24	25	25	24	23	21	21	24	24	22	11 ^a	19	22	21	22	20	22	21	22	29		
	764																								
	765	22	25	24	24	26	23	24	23	24	23	23	24	25	25	24	24	24	23	24	22	23	23		
	766																								
	767	25	27	26	29	30	29	27	26	26	28	26	26	33	28	28	26	25	25	26	26	34	28		
	768																								
	769	25	31	30	28	30	29	28	30	27	27	28	28	31	30	29	ND	28	27	28	27	28	28		
	770																								
	771	23	25	24	24	25	25	25	26	25	24	25	24	26	28	24	23	23	22	24	22	23	24		
	772																								
	773	23	26	25	26	26	26	25	25	24	24	25	25	27	26	25	11 ^a	25	24	25	24	25	25		
	774																								
	775	23	25	25	26	27	26	26	25	25	24	23	23	25	25	25	23	24	23	25	23	24	24		
	776																								
	777	24	26	27	27	27	27	25	26	26	27	27	26	28	28	27	26	26	25	27	25	26	26		
	778																								
	779	26	28	28	28	28	29	28	28	27	27	27	27	30	28	28	26	27	27	29	27	28	28		
	780																								
	781																								
	782																								
	783																								
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	804																								
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	806																								
	807																								
	808																								
	809																								
MEAN±SD		24±1	26±2	26±2	26±2	27±2	26±2	26±2	25±2	25±2	25±2	25±2	25±2	28±3	26±3	26±2	24±2	25±2	24±2	26±2	24±3	26±4	26±2		

³Values excluded from statistical analysis calculations.

APPENDIX 3 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day																
		31- 34	34- 38	38- 41	41- 45	45- 49	49- 53	53- 57	57- 61	61- 65	65- 69	69- 73	73- 77	77- 81	81- 85	85- 89	89- 93	93- 97
3	760																	
	761	23	24	25	24	25	25	24	23	25	25	24	26	26	26	25	25	26
	762																	
	763	11 ^a	22	22	21	21	22	22	21	22	21	20	23	22	22	21	23	22
	764																	
	765	23	24	22	23	23	24	23	22	24	22	22	26	22	24	23	23	23
	766																	
	767	29	27	27	26	26	26	25	25	27	32	26	28	27	25	26	27	28
	768																	
	769	27	28	27	30	27	28	28	28	30	28	26	30	29	28	29	28	28
	770																	
	771	22	24	23	24	24	24	23	23	25	24	24	25	25	25	23	25	24
	772																	
	773	25	25	24	25	26	26	25	23	26	24	26	27	24	27	25	26	27
	774																	
	775	23	25	24	24	23	23	24	22	25	24	29	15	23	25	24	25	24
	776																	
	777	25	27	26	27	9 ^a	27	25	25	ND	19	25	28	27	27	27	27	26
	778																	
	779	27	28	27	27	26	27	26	26	29	27	20	33	28	27	28	29	27
	780																	
	781																	
	782																	
	783																	
	784																	
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	797																	
	798																	
	799																	
	800																	
	801																	
	802																	
	803																	
	804																	
	805																	
	806																	
	807																	
	808																	
	809																	
MEAN±SD		25±2	26±2	25±2	25±2	24±2	25±2	25±2	24±2	26±2	25±4	24±3	26±5	25±2	26±2	25±2	26±2	25±2

^aValues excluded from statistical analysis calculations.

APPENDIX 3 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF MALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day															
		191- 194	115- 119	119- 122	150- 154	178- 182	186- 190	194- 198	199- 203	197- 201	202- 206	200- 204	208- 212	213- 217	211- 215	216- 220	
J	760	27	24	15	25	25	26	24	44 ^a	25	25	24	24	23	9 ^a	Dead	
	761												Dead				
	762	22	22	22	21	23	22	18	10	3 ^a	22	19 ^a	23	23	21	19	
	763												Dead				
	764	24	24	25	24	24	24	24	21	23	22	23	25	26	26	18	
	765														Dead		
	766	27	28	29	28	31	26	25	25	24	20	31 ^a	9 ^a		Dead		
	767														Dead		
	768	30	27	30	25	19 ^a	25	27	ND	26	27	41 ^a	5 ^a		Dead		
	769														Dead		
	770	25	25	26	26	25	25	20	22	25	26	23	26	26	24	23	
	771																
	772	25	25	27	26	26	25	26	26	24	22	25			Dead		
	773																
	774	24	24	25	25	24	22	22	5 ^a	26	27	28	26	27	25	25	
	775														Dead		
	776	29	27	26	16										Dead		
	777					30	29	30	20						Dead		
	778	29	27	29	29	23	ND	28	24	28	28	29	19		Dead		
	779														Dead		
	780									26	27	25	22		Dead		
	781														Dead		
	782														Dead		
	783														Dead		
	784													25	24	24	
	785														Dead	23	
	786														Dead		
	787														Dead		
	788													21	29	27	
	789														Dead	19	
	790													20	18	Dead	
	791														Dead		
	792														Dead		
	793													25	11 ^a	21	
	794														34	Dead	
	795															21	
	796															12	
	797														Dead	Dead	
	798														Dead		
	799														Dead		
	800															26	
	801														Dead		
	802															Dead	
	803														Dead		
	804														Dead		
	805															14	
	806														Dead		
	807															23	
	808															Dead	
	809															Dead	
MEAN±SD		26±2	25±2	26±3	24±4	26±3	25±2	24±4	24±3	25±1	24±3	25±2	24±2	24±2	22±5	23±6	22±3

ND = No data available.

^aValues excluded from statistical analysis calculations.

[illegible]

13+2 19+2 13+2 19+1 21+2 21+3 20+3 20+2 18+2 19+2 19+1 19+1 21+3 19+2 19+2 18+1 18+1 13+1 19+2 13+2 19+3 13+1

21520

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day															
		31- 34	34- 38	38- 41	41- 45	45- 49	49- 53	53- 57	57- 61	61- 65	65- 69	69- 73	73- 77	77- 81	81- 85	85- 89	89- 93
0	910	19	21	17	17	19	20	19	18	21	19	17	20	21	21	19	20
	911																38 ^a
	912	19	16	18	17	21	20	17	17	20	17	15	19	19	16	15	16
	913																ND
	914	18	19	16	18	16	19	18	15	19	18	17	20	19	18	17	15
	915																19
	916	20	21	18	19	19	20	23	19	20	21	19	36 ^a	20	21	18	23
	917																22
	918	18	19	19	20	19	23	21	20	21	19	19	23	24	20	22	21
	919																21
	920	15	17	16	17	18	19	16	16	19	17	16	17	19	17	18	19
	921																16
	922	18	18	17	17	18	17	19	16	20	17	17	20	20	19	18	18
	923																18
	924	17	18	19	18	18	17	18	18	21	18	17	19	19	18	18	20
	925																31
	926	17	17	16	18	17	18	17	16	19	17	18	19	18	17	17	16
	927																
	928	21	24	25	19	20	21	19	19	20	20	19	23	19	20	20	20
	929																18
	930																
	931																
	932																
	933																
	934																
	935																
	936																
	937																
	938																
	939																
	940																
	941																
	942																
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	968																
	969																
	970																
	971																
	972																
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	974																
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	976																
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	978																
	979																
	980																
	981																
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	987																
	988																
	989																
	990																
	991																
	992																
	993																
	994																
	995																
MEAN±SD		18±2	19±2	18±3	18±1	19±1	20±2	19±2	17±2	20±1	18±1	17±1	20±2	20±2	19±2	18±2	19±2
																	20±5

ND = No data available.

^aValues excluded from statistical analysis calculations.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day																			
		3-	4-	7-	11-	14-	18-	21-	25-	28-	32-	35-	39-	42-	46-	49-	53-	56-	60-	63-	67-
	75-	4	7	11	14	18	21	25	28	32	35	39	42	46	49	53	56	60	63	67	70
		75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75
10	1096																				
	1097	21	20	19	18	19	21	17	17	17	16	17	17	18	16	18	16	16	16	17	14
	1098																				
	1099	19	21	20	19	19	15	19	20	21	21	19	18	19	18	18	16	16	19	19	17
	1100																				
	1101	21	20	20	19	19	20	18	18	21	18	18	21	ND	18	18	18	17	18	18	16
	1102																				
	1103	19	19	18	19	18	18	17	18	23	18	17	29	21	17	17	17	16	17	18	16
	1104																				
	1105	21	19	18	19	18	18	19	20	24	20	18	19	21	17	18	18	17	20	19	16
	1106																				
	1107	17	18	14	17	17	17	15	17	17	16	16	15	16	21	15	14	14	17	15	14
	1108																				
	1109	18	17	19	18	19	24	18	19	23	20	18	19	22	17	18	18	16	17	18	18
	1110																				
	1111	19	24	18	19	19	18	16	18	17	17	18	18	19	16	16	17	17	17	16	16
	1112																				
	1113	20	22	21	19	19	19	18	19	20	19	19	18	19	16	19	17	17	19	18	17
	1114																				
	1115	19	20	18	18	20	31	17	20	19	20	18	19	20	18	18	16	17	19	17	16
	1116																				
	1117																				
	1118																				
	1119																				
	1120																				
	1121																				
	1122																				
	1123																				
	1124																				
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	1139																				
	1140																				
	1141																				
	1142																				
	1143																				
	1144																				
	1145																				

MEAN±SD 19±1 20±2 18±2 18±1 18±1^b 20±5 17±1^b 19±1^b 20±3 19±2 18±1 18±1 19±2 18±2 17±1 17±1 16±1 18±1 18±1 16±1 18±3 18±1

ND = No data available.

^aValues excluded from statistical analysis-calculations.

^bStatistically significant deviation from control mean using Dunnett's Test, $p < 0.05$.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS CONTAINING 1,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day															
		31- 34	34- 38	38- 41	41- 45	45- 49	49- 53	53- 57	57- 61	61- 65	65- 69	69- 73	73- 77	77- 81	81- 85	85- 89	89- 93
10	1096	18	17	17	16	19	18	18	17	19	18	19	19	20	18	17	16
	1097																Dead 21
	1098	18	18	19	17	19	20	20	18	16	19	22	25	25	25	19	22
	1099																10
	1100	17	16	17	17	22	17	17	18	34 ^a	19	24	20	19	18	19	18
	1101																19
	1102	18	17	16	17	18	18	17	18	20	18	34 ^a	ND	22	16	19	17
	1103																17
	1104	19	19	18	17	19	17	19	18	19	19	17	20	19	17	18	22
	1105																
	1106	16	17	15	14	17	16	16	16 ^a	14	17	19	24	19	20	20	19
	1107																17
	1108	17	19	20	19	22	23	20	22	22	20	20	32	29	27	25	26
	1109																20
	1110	15	18	16	17	20	19	17	16	18	17	17	20	19	19	18	18
	1111																
	1112	17	19	19	19	20	20	19	17	ND	17	24	23	20	20	18	14
	1113																18
	1114	18	19	18	17	18	18	19	15	22	17	20	22	20	21	21	20
	1115																21
	1116																
	1117																
	1118																
	1119																
	1120																
	1121																
	1122																
	1123																
	1124																
	1125																
	1126																
	1127																
	1128																
	1129																
	1130																
	1131																
	1132																
	1133																
	1134																
	1135																
	1136																
	1137																
	1138																
	1139																
	1140																
	1141																
	1142																
	1143																
	1144																
	1145																

MEAN±SD 17±1 18±1 17±2 17±1 19±2 19±2 18±1 17±2 19±3 19±1 20±3 23±4 21±3 20±3 19±2 19±3 19±2

ND = No data available.

^aValues excluded from statistical analysis calculations.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Grams/Rat/Day															
		291- 294	313- 319	319- 322	350- 354	373- 382	406- 410	434- 438	469- 473	497- 501	532- 536	560- 564	588- 592	623- 627	651- 655	686- 690	714- 718
30	1096																
	1097	ND	22	22	26	ND	19	20	25	21	18	22	22	22	20	22	22
	1098	20	19	24	20	22	21	21	18	21	20	23	15	21	17	17	20
	1099																
	1100	20	18	20	17	18	18	19	19	21	17	23	18	20	17	30	18
	1101													Dead			
	1102	19	17	17	20	17	19	17	18	12	17	14	20	23	18	30 ^a	28
	1103																
	1104	19	18	19	19	19	19	19	11	17	19	33	19	21	23	40 ^a	22
	1105																
	1106	17	18	19	23	23	17	17	9 ^a	15	17	57 ^a	20	20	17		Dead
	1107													Dead			
	1108	25	23	25	22	25	20	20	25	21	22	21	21	21	16	ND	20
	1109																
	1110	19	19	18	17	16	19	17	16	17	18	24	17	20	17	13	18
	1111																
	1112	19	18	20	20	17	19	19	9 ^a	20	19	29	19	22	22	2 ^a	23
	1113																
	1114	17	18	21	21	28	23	18	23	20	18	20	17	15			
	1115														23	18	21
	1116															18	19
	1117																
	1118																
	1119																
	1120																
	1121																
	1122																
	1123																
	1124																
	1125																
	1126																
	1127																
	1128																
	1129																
	1130																
	1131																
	1132																
	1133																
	1134																
	1135																
	1136																
	1137																
	1138																
	1139																
	1140																
	1141																
	1142																
	1143																
	1144																
	1145																
MEAN±SD		19±2	19±2	20±2	21±3	21±4	19±2	19±1	19±3	21±5	18±2	24±4	19±3	20±2	19±3	20±6	21±3

ND = No data available.

^aValues excluded from statistical analysis calculations.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 7 YEARS

Animal Number	Days on Test and Means/Day													
	0- 7- 75-	1- 7- 75-	2- 7- 75-	3- 7- 75-	4- 7- 75-	5- 7- 75-	6- 7- 75-	7- 7- 75-	8- 7- 75-	9- 7- 75-	10- 7- 75-	11- 7- 75-	12- 7- 75-	13- 7- 75-
10														
1046	1.9	1.8	1.9	1.8	1.8	1.9	1.7	1.7	1.9	1.9	1.7	1.7	1.6	1.9
1047														
1048	1.9	2.3	1.9	2.2	2.2	2.6	1.9	2.0	2.6	2.0	1.9	2.6	2.2	2.0
1049														
1050	2.1	2.1	2.1	2.0	2.0	2.0	1.8	1.7	1.9	2.7	1.7	1.9	2.0	1.7
1051														
1052	2.2	2.5	2.0	2.0	2.0	1.9	1.8	1.8	2.1	2.2	1.7	2.0	1.9	1.6
1053														
1054	1.6	1.8	1.9	1.8	1.9	1.8	1.9	2.1	2.0	2.1	1.9	1.9	1.7	2.0
1055														
1056	2.2	1.9	2.0	2.0	2.8	2.1	1.8	1.7	3.2	2.1	1.9	2.1	2.4	2.5
1057														
1058	1.8	2.0	2.0	1.8	1.9	1.8	1.8	1.9	2.1	1.8	2.0	2.1	1.6	1.8
1059														
1060	2.1	1.8	1.9	1.9	2.0	2.0	1.8	1.8	2.0	1.8	1.7	1.6	1.7	1.7
1061														
1062	1.8	1.8	1.9	2.0	2.0	2.2	1.8	1.6	2.0	1.8	1.8	2.1	1.7	1.8
1063														
1064	1.9	2.0	1.9	2.0	2.0	1.9	1.9	1.9	2.0	2.1	1.8	1.8	2.1	1.9
1065														
1066														
1067														
1068														
1069														
1070														
1071														
1072														
1073														
1074														
1075														
1076														
1077														
1078														
1079														
1080														
1081														
1082														
1083														
1084														
1085														
1086														
1087														
1088														
1089														
1090														
1091														
1092														
1093														
1094														
1095														
MEANS	20±2	20±2	19±1	19±1	21±3	20±2	18±1	18±1	22±4	21±3	18±1	21±5	19±4	18±3
SD	20±2	20±2	19±1	19±1	21±3	20±2	18±1	18±1	22±4	21±3	18±1	21±5	19±4	18±3
SE	20±2	20±2	19±1	19±1	21±3	20±2	18±1	18±1	22±4	21±3	18±1	21±5	19±4	18±3
95%	20±2	20±2	19±1	19±1	21±3	20±2	18±1	18±1	22±4	21±3	18±1	21±5	19±4	18±3
99%	20±2	20±2	19±1	19±1	21±3	20±2	18±1	18±1	22±4	21±3	18±1	21±5	19±4	18±3

ND = No data available.
* Statistically significant difference from control mean using Dunnett's test, $p < 0.05$.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Dose mg/kg/day	Animal Number	Days on Test and Days/Rat/Day																			
		31- 34	34- 38	38- 41	41- 45	45- 49	49- 53	53- 57	57- 61	61- 65	65- 69	69- 73	73- 77	77- 81	81- 85	85- 89	89- 93	93- 97	97- 101	101- 105	105- 109
10	1047	16	20	17	17	19	18	18	18	19	16	22	18	20	18	19	15	21	25	33	23
	1048	20	24	29	21	29	29	23	21	ND	15	21	ND	25	33	23	23	ND	21	21	19
	1049	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067
	1050	16	18	17	18	20	18	18	18	18	26	19	18	20	21	21	16	20	20	22	19
	1051	19	20	18	18	6 ^a	13	38 ^a	ND	20	16	16	26	20	22	22	20	31	36 ^a	26	27
	1052	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1053	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1054	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1055	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1056	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
10	1057	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1058	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1059	22	26	19	19	19	20	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1060	16	19	18	17	17	18	17	16	16	24	22	22	20	31	36 ^a	26	27	27	18	18
	1061	16	19	18	17	17	18	17	16	16	24	22	22	20	31	36 ^a	26	27	27	18	18
	1062	18	19	18	18	18	19	18	17	18	18	19	24	21	21	22	22	22	22	22	18
	1063	18	19	18	18	18	19	18	17	18	18	19	24	21	21	22	22	22	22	22	18
	1064	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1065	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1066	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
10	1067	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1068	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1069	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1070	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1071	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1072	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1073	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1074	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1075	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1076	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
10	1077	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1078	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1079	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1080	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1081	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1082	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1083	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1084	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1085	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1086	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
10	1087	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1088	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1089	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1090	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1091	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1092	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1093	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1094	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1095	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
	1096	19	19	18	18	18	19	19	19	18	18	19	24	21	21	22	22	22	22	22	18
MEAN±SD		19±2	20±3	19±3	18±1	19±4	19±4	19±2	18±2	21±5	19±2	19±2	20±2	22±4	22±5	20±3	21±2	19±2			

ND = No data available.

^aValues excluded from statistical analysis calculations.

INDIVIDUAL AND MEAN 5000 CONCEPTION OF FEMALE RATS MAINTAINED ON DIETS CONTAINING 2,4,5-T FOR 2 YEARS

³ values excluded from statistical analysis calculations.

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APPENDIX 9 (Continued).

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS CONTAINING 2,4,5-F FOR 2 YEARS

Animal No.	Day g/k/day	Days on Test and Grams/Rat/Day																							
		1-75	7-75	11-75	14-75	18-75	22-75	25-75	28-75	31-75	3-76	6-76	9-76	12-76	15-76	18-76	21-76	24-76	27-76	30-76	31-76	3-77	6-77	9-77	
3		4	7	11	14	18	22	25	28	31	3-76	6-76	9-76	12-76	15-76	18-76	21-76	24-76	27-76	30-76	31-76	3-77	6-77	9-77	
996	17	19	17	19	20	18	18	18	18	18	17	19	20	20	18	22	18	19	19	20	19	19	17	18	
997																									
998	21	20	19	20	19	24	18	18	19	13	19	13	20	19	18	22	18	19	19	20	19	19	17	18	
999																									
1000	20	19	19	21	20	16	17	19	20	20	20	18	22	18	19	19	20	19	19	19	16	16	18		
1001																									
1002	18	20	18	19	18	19	18	13	21	19	18	19	20	17	19	18	18	16	ND	15	18	18	18		
1003																									
1004	20	19	17	18	18	18	18	19	18	18	19	19	20	18	17	15	17	17	18	17	19	19	19		
1005																									
1006	19	19	20	20	14	19	19	19	21	18	20	21	22	19	28	25	17	16	ND	18	25	19	19		
1007																									
1008	23	21	18	18	21	18	17	19	20	17	17	18	18	16	16	14	16	15	19	13	20	17	17		
1009																									
1010	23	20	21	18	18	19	18	19	19	18	19	18	19	18	19	17	19	18	20	18	19	20	20		
1011																									
1012	20	21	21	19	ND	20	20	19	20	20	20	20	24	19	20	18	20	18	20	19	24	20	20		
1013																									
1014	20	21	21	21	20	22	21	21	21	21	20	21	21	19	21	22	22	19	22	20	21	21	21		
1015																									
1016																									
1017																									
1018																									
1019																									
1020																									
1021																									
1022																									
1023																									
1024																									
1025																									
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1037																									
1038																									
1039																									
1040																									
1041																									
1042																									
1043																									
1044																									
1045																									
MEAN±SD	20±2	20±1	19±1	19±1	19±2	19±2	18±1	18±2 ^a	20±1	19±1	19±1	19±2	20±2	18±1	19±3	18±3	19±2	17±1	19±1	17±2	20±3	19±1	19±1	19±1	

ND = No data available.

^aStatistically significant deviation from control mean using Dunnett's test, p<0.05.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2.4,3-T FOR 2 YEARS

Animal Number	Dose mg/kg/day	Days on Test and Grams/Rat/Day																								
		31- 34	34- 38	38- 41	41- 45	45- 49	49- 53	53- 57	57- 61	61- 65	65- 69	69- 73	73- 77	77- 81	81- 85	85- 89	89- 93	93- 97	97- 101	101- 105	105- 109	109- 113	113- 117	117- 121	121- 125	
3	996	18	19	17	16	17	17	18	14	20	18	18	22	24	22	18	18	18	18	18	18	18	18	18	18	18
	997																									
	998	16	18	18	19	18	18	18	17	22	20	18	24	20	18	18	18	18	18	18	18	18	18	18	18	18
	999																									
	1000	18	20	19	18	13	13	13	20	18	17	20	19	23	22	20	18	18	18	18	18	18	18	18	18	18
	1001																									
	1002	17	18	17	19	22	19	14	16	19	18	18	20	17	18	20	20	20	20	20	20	20	20	20	20	20
	1003																									
	1004	18	19	18	19	18	20	19	17	20	19	18	23	21	21	19	20	20	20	20	20	20	20	20	20	20
	1005																									
	1006	17	19	17	20	19	18	18	18	20	20	20	21	24	20	18	19	22	19	22	19	22	19	22	19	19
	1007																									
	1008	15	18	16	14	18	19	16	13	18	16	16	ND	15	17	16	15	16	15	16	15	16	16	16	16	16
	1009																									
	1010	20	20	17	19	19	20	19	19	22	22	19	25	20	21	20	22	20	22	20	22	20	22	20	22	20
	1011																									
	1012	19	19	19	20	21	22	20	18	24	19	22	22	19	20	18	22	21	22	21	22	21	22	21	21	21
	1013																									
	1014	19	21	20	20	21	20	19	20	21	19	21	20	21	22	21	21	22	21	21	21	21	21	21	21	20
	1015																									
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	1045																									

MEANS: 13±1 18±1 18±1 18±2 19±2 19±1 13±2 17±2 20±2 19±1 19±2 22±3 20±2 20±2 19±2 20±2 19±2

ND = No data available.

APPENDIX 9 (Continued)

INDIVIDUAL AND MEAN FOOD CONSUMPTION OF FEMALE RATS MAINTAINED ON DIETS
CONTAINING 2,4,5-T FOR 2 YEARS

Animal Number	Days on Test and Gains/Rat/Day	Days on Test and Gains/Rat/Day															
		291-394	313-319	319-322	350-354	378-382	406-410	414-418	477-479	497-501	512-516	546-548	592-598	623-627	631-635	646-650	714-719
1																	
996	13	19	19	19	21	21	21	20	11	23	20	22 ^a	22	24	18	13	19
997	21	19	20	19	20	20	19	20	16	19	19	21	22	20	21	23	21
998	21	19	20	19	20	20	19	20	16	19	19	21	22	20	21	23	21
999	17	20	22	21	21	21	21	23	20	24	18	23	23	Dead	Dead	Dead	Dead
1000	19	18	21	19	19	19	20	19	19	17	20	16	22	26	29	Dead	Dead
1001	21	18	21	19	21	21	22	21	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
1002	21	18	21	19	21	21	22	21	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
1003	20	22	22	19	20	20	19	20	22	18	19	28	20	26	21	23	21
1004	16	16	18	18	18	16	16	16	15	19	16	19	16	19	16	19	19
1005	22	20	23	21	21	21	20	22	12	19	20 ^a	16	16	30	21	18	15
1006	26	18	22	24	22	22	22	23	20	ND	25	25	25	26	28	40 ^a	20
1007	23	20	22	21	15	15	21	22	17	21	21	33	25	25	22	20	32
1008	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1009	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1010	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1011	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1012	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1013	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1014	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1015	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1016	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1017	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1018	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1019	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1020	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1021	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1022	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1023	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1024	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1025	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1026	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1027	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1028	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1029	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1030	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1031	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1032	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1033	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1034	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1035	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1036	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1037	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1038	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1039	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1040	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1041	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1042	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1043	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1044	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
1045	19	19	19	19	20	20	20	20	22	19	17	20	20	20	20	20	20
MEAN±SD	20±2	19±2	21±2	20±2	19±2	20±2	20±2	21±2	17±4	20±2	19±3	24±6	21±2	23±4	21±4	20±2	20±4

ND = No data available.
*Values excluded from statistical analysis calculations.

APPENDIX 10

HEMATOLOGICAL VALUES OF MALE RATS ON DAY 82 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^3/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $\times 10^6/\text{mm}^3$	Reticulo- cytes %
						Neut	Lymph	Mono	Eosin	Baso		
0	75-394	33.0	3.31	16.4	12.5	20	78	2	0	0	1.285	0.5
	75-395	33.5	9.13	16.0	11.5	12	32	5	0	0	1.330	1.3
	75-397	33.5	9.32	16.6	12.2	4	39	3	3	1	1.505	1.1
	75-398	33.0	9.45	17.5	14.5	8	35	4	2	1	0.855	1.3
	75-399	31.5	3.32	11.9	12.9	14	31	3	3	3	1.320	2.0
	75-400	32.0	3.39	16.7	15.3	14	34	2	0	0	1.315	1.0
	75-401	35.0	9.71	17.4	12.3	3	94	3	0	3	1.325	1.0
	75-402	36.0	9.51	18.4	18.3	19	75	3	3	0	1.710	1.0
	MEAN	34.2	9.21	17.0	13.7	12	34	3	1	0	1.393	1.2
	S.D.	1.3	0.14	0.7	1.2						0.253	
30	75-624	33.0	9.21	16.3	12.1	12	37	1	0	0	1.380	1.3
	75-625	32.5	9.34	16.9	12.9	10	35	4	1	0	1.425	1.1
	75-626	34.0	3.89	16.3	16.2	10	90	0	0	0	1.080	2.0
	75-628	35.5	9.33	17.8	11.5	15	34	1	0	0	1.225	0.7
	75-629	34.5	8.93	17.1	16.7	15	34	0	1	0	1.190	1.6
	75-630	31.5	9.14	17.2	14.7	3	93	2	0	0	1.445	1.8
	75-631	32.5	9.44	17.3	13.6	10	38	2	0	0	1.365	0.5
	75-632	36.5	9.44	17.5	15.0	4	92	2	1	1	1.275	0.4
	MEAN	34.0	9.22	17.1	14.1	10	38	2	0	0	1.298	1.2
	S.D.	1.4	0.20	0.5	1.3						0.128	
10	75-614	33.0	8.88	17.0	15.4	8	88	3	1	0	1.010	2.4
	75-617	32.5	8.52	17.0	16.3	16	82	0	2	0	1.075	2.4
	75-618	30.5	8.38	16.5	16.8	8	86	4	2	0	1.360	1.9
	75-619	34.5	9.66	17.3	12.3	6	92	1	0	1	1.000	0.7
	75-620	33.5	9.48	17.5	14.5	5	93	1	1	0	1.480	0.4
	75-621	36.5	9.77	18.3	13.4	11	33	1	5	0	1.280	0.3
	75-622	32.0	9.30	17.4	18.6	20	77	2	1	0	1.325	1.2
	75-623	33.0	9.42	17.5	15.3	10	34	4	2	0	1.085	0.2
	MEAN	33.2	9.18	17.3	15.3	11	36	2	1	0	1.202	1.2
	S.D.	1.8	0.52	0.5	2.0						0.132	
3	75-604	36.0	3.62	17.1	14.1	17	30	2	1	0	1.240	0.6
	75-607	30.0	3.74	16.1	15.9	6	90	2	1	1	0.845	0.3
	75-608	49.5	3.71	17.1	16.3	7	39	1	2	1	1.080	0.8
	75-609	34.5	9.00	17.3	9.9	13	35	1	1	0	1.245	0.6
	75-610	32.0	8.93	17.1	13.9	16	33	0	0	1	1.200	0.5
	75-611	32.0	9.57	19.1	17.5	15	34	1	0	0	1.480	0.4
	75-612	33.5	9.22	17.4	13.6	7	93	0	0	0	1.560	0.6
	75-613	32.5	8.49	16.9	15.1	6	91	0	2	1	1.710	1.2
	MEAN	32.5	8.91	17.3	14.5	11	37	1	1	0	1.295	0.6
	S.D.	2.2	0.35	0.8	1.3						0.278	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 11

HEMATOLOGICAL VALUES OF FEMALE RATS ON DAY 33 OF A 113-119 DAY DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $\times 10^3/\text{mm}^3$	Reticulo- cytes %
						Neut	Lymph	Mono	Eosin	Baso		
0	75-634	52.5	3.44	16.7	12.7	9	39	1	1	0	1.165	0.7
	75-635	50.5	7.38	13.3	11.0	6	91	2	1	0	1.325	1.5
	75-636	51.0	9.10	17.3	12.5	15	85	0	0	0	1.310	0.9
	75-637	49.5	7.79	15.7	12.0	7	90	1	2	0	0.965	2.1
	75-638	47.0	8.05	16.4	14.5	5	90	2	2	1	1.005	1.7
	75-639	51.0	8.12	15.8	13.0	5	94	0	1	0	1.290	1.7
	75-640	51.0	3.27	16.0	9.4	14	81	2	2	1	1.070	1.2
	75-641	48.0	7.34	15.5	14.0	16	82	0	2	0	1.275	2.7
	MEAN	50.1	3.19	16.2	12.4	10	38	1	1	0	1.175	1.6
	±S.D.	1.3	0.43	0.5	1.6						0.145	
30	75-664	44.5	7.64	14.5	8.6	10	87	1	2	0	1.350	0.9
	75-665	51.5	3.34	15.8	13.5	10	87	2	1	0	0.990	0.7
	75-666	49.0	8.38	15.7	15.6	18	80	0	2	0	1.420	1.4
	75-667	48.0	8.11	15.7	9.9	11	88	0	1	0	1.100	1.5
	75-668	53.0	3.49	16.8	10.5	2	97	0	1	0	1.255	1.7
	75-670	55.0	8.78	16.3	12.7	8	88	2	2	0	1.335	1.4
	75-672	58.0	8.97	17.7	9.5	17	81	2	0	0	1.195	1.2
	75-673	61.0	9.77	18.4	15.7	9	89	0	2	0	0.820	1.6
	MEAN	52.5	8.36	16.4	12.0	11	87	1	1	0	1.183	1.3
	±S.D.	5.4	0.53	1.2	2.8						0.203	
10	75-654	48.5	8.05	15.9	11.5	9	90	0	1	0	1.020	2.2
	75-655	51.0	8.42	16.6	13.4	13	36	0	1	0	0.950	1.6
	75-656	48.5	7.70	15.3	15.2	7	89	2	2	0	1.080	1.4
	75-657	49.0	8.11	15.8	12.3	8	89	2	1	0	1.010	1.5
	75-658	49.5	7.38	15.6	7.8	3	95	1	1	0	1.055	1.3
	75-659	50.0	7.97	15.8	11.9	8	91	0	0	1	0.805	1.3
	75-660	55.0	8.38	16.1	9.5	7	89	1	3	0	0.915	1.5
	75-661	53.0	3.48	16.4	10.5	3	97	0	0	0	0.990	1.0
	MEAN	50.6	8.12	15.9	11.5	7	91	1	1	0	0.978	1.4
	±S.D.	2.1	0.28	0.4	2.3						0.088	
3	75-644	48.5	7.70	15.0	7.0	12	38	0	0	0	1.430	1.6
	75-645	49.0	8.25	15.7	10.5	10	87	0	3	0	1.030	1.2
	75-646	47.0	7.77	15.4	11.5	7	92	1	0	0	1.085	1.2
	75-648	47.5	8.05	15.4	16.5	4	94	0	1	1	1.115	0.8
	75-649	51.0	8.27	16.0	13.7	10	89	0	1	0	1.025	1.6
	75-651	50.5	8.24	16.7	11.0	1	98	0	0	1	1.015	0.5
	75-652	56.0	9.11	17.9	14.4	14	85	1	0	0	1.430	0.4
	75-653	51.5	7.79	15.8	11.8	9	91	0	0	0	1.000	1.4
	MEAN	50.1	8.15	16.0	11.4	8	91	0	1	0	1.144	1.1
	±S.D.	2.9	0.45	0.9	2.2						0.187	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 12

HEMATOLOGICAL VALUES OF MALE RATS ON DAY 89 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $10^3/\text{mm}^3$	Reticulo- cytes %
						Neut	Lymph	Mono	Eosin	Baso		
0	75-674	55.5	9.21	17.5	9.5	11	86	2	2	1	1.080	1.4
	75-675	57.0	9.71	18.2	11.8	15	84	1	0	0	1.100	1.4
	75-676	55.0	9.10	17.2	10.3	10	88	0	2	0	1.040	1.4
	75-679	53.0	9.16	16.9	14.6	12	86	2	0	0	0.990	2.0
	75-680	57.0	9.32	18.5	3.9	13	85	2	0	0	1.275	1.1
	75-681	57.0	9.04	17.6	15.0	8	87	4	1	0	1.295	1.6
	75-683	55.5	8.55	16.1	17.0	10	87	3	0	0	1.260	1.0
	75-686	55.5	9.00	17.1	10.3	7	90	2	1	0	1.590	1.1
	MEAN	55.7	9.22	17.4	12.3	11	87	2	0	0	1.204	1.1
	$\pm$ S.D.	± 1.4	$\pm .41$	$\pm .3$	± 2.9						$\pm .194$	
30	75-861	54.0	9.26	17.5	12.8	8	90	2	0	0	1.385	.9
	75-863	56.5	9.40	17.9	13.0	8	88	2	2	0	1.200	1.0
	75-864	55.0	9.29	17.4	7.5	9	90	0	0	1	1.130	1.5
	75-865	55.0	9.63	17.9	12.5	13	85	0	1	1	1.000	1.3
	75-866	54.5	9.37	17.5	12.4	20	78	0	2	0	1.250	1.6
	75-867	55.0	9.25	17.2	14.8	4	94	1	1	0	1.285	1.2
	75-868	54.0	8.56	17.2	14.3	9	88	2	0	1	1.485	1.4
	75-870	54.0	9.51	17.6	15.0	13	85	0	2	0	1.475	.9
	MEAN	54.8	9.29	17.5	12.8	11	87	1	1	0	1.276	.9
	$\pm$ S.D.	$\pm .3$	$\pm .32$	$\pm .3$	± 2.4						$\pm .169$	
10	75-810	53.5	9.79	17.3	12.8	13	81	0	1	0	1.660	.7
	75-811	58.0	9.30	18.6	12.8	16	83	1	0	0	1.445	1.4
	75-812	57.5	9.53	18.9	12.9	12	86	2	0	0	1.535	.7
	75-814	54.0	9.14	17.5	12.9	10	87	1	2	0	1.070	1.4
	75-815	49.5	8.93	16.1	11.0	13	87	0	0	0	0.820	2.3
	75-817	59.5	9.51	18.7	15.3	6	92	0	2	0	1.325	1.6
	75-818	57.0	9.67	17.5	13.9	6	92	0	2	0	1.040	1.5
	75-820	54.0	8.93	17.0	11.3	15	81	1	1	2	1.440	1.6
	MEAN	55.0	9.41	17.7	12.9	12	86	1	1	0	1.292	1.1
	$\pm$ S.D.	± 3.1	$\pm .36$	± 1.0	± 1.3						$\pm .287$	
3	75-760	55.0	9.57	17.8	12.0	11	89	0	0	0	1.165	1.0
	75-762	52.0	9.02	17.2	7.9	16	81	1	2	0	1.130	1.4
	75-763	55.5	9.82	17.8	12.3	9	90	0	0	1	0.805	.9
	75-764	54.0	9.00	17.0	9.9	9	88	1	1	1	1.055	1.6
	75-766	58.0	9.55	18.2	17.9	11	87	1	1	0	1.010	1.3
	75-767	59.5	9.86	19.5	12.6	16	84	0	0	0	1.135	1.7
	75-768	53.5	9.95	17.0	16.4	10	87	2	1	0	1.225	1.7
	75-770	56.0	9.73	17.8	12.7	9	91	0	0	0	1.150	1.7
	MEAN	55.4	9.44	17.8	12.7	11	87	1	1	0	1.084	1.1
	$\pm$ S.D.	± 2.4	$\pm .39$	$\pm .3$	± 3.2						$\pm .131$	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 13

HEMATOLOGICAL VALUES OF FEMALE RATS ON DAY 90 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^5/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $\times 10^3/\text{mm}^3$	Reticulo- cytes %
						Neut	Lymph	Mono	Eosin	Baso		
0	75-910	48.0	7.55	15.0	9.9	12	37	0	1	0	1.230	1.4
	75-912	47.5	8.23	15.3	9.0	7	92	0	1	0	1.130	1.0
	75-913	50.5	3.52	16.1	9.4	3	95	2	0	0	1.395	1.2
	75-916	50.5	7.77	15.8	10.3	11	87	1	0	1	1.045	1.7
	75-917	49.0	3.23	15.8	7.8	10	90	0	0	0	0.965	2.2
	75-919	49.0	7.88	15.5	7.7	15	85	0	0	0	1.210	1.3
	75-920	50.0	8.70	16.7	7.3	3	92	0	0	0	1.370	1.4
	75-921	50.0	8.24	15.9	13.0	9	90	0	1	0	1.745	1.4
	MEAN	49.3	8.17	15.8	9.4	9	90	1	0	0	1.258	1.4
	$\pm$ S.D.	± 1.1	$\pm .38$	$\pm .6$	± 1.8						$\pm .241$	
30	75-1096	47.0	8.30	16.6	14.0	9	91	0	0	0	1.290	1.2
	75-1097	47.0	7.81	15.8	11.3	8	85	4	3	0	1.135	2.1
	75-1098	50.0	8.56	16.0	10.6	8	91	0	1	0	0.905	1.3
	75-1100	50.0	8.62	16.9	14.0	7	90	1	2	0	1.010	2.1
	75-1101	48.0	8.12	16.3	16.0	13	80	3	3	1	0.925	1.2
	75-1102	53.0	8.34	16.9	9.9	8	91	0	1	0	1.010	1.6
	75-1103	51.5	8.58	16.5	10.9	9	91	0	0	0	1.110	1.2
	75-1104	44.0	7.55	16.7	9.4	6	92	1	1	0	1.150	2.5
	MEAN	48.8	8.24	16.2	12.0	9	89	1	1	0	1.073	1.7
	$\pm$ S.D.	± 2.9	$\pm .39$	$\pm .7$	± 2.4						$\pm .134$	
10	75-1046	48.0	7.51	14.9	8.1	12	86	0	2	0	1.120	1.2
	75-1047	51.5	8.45	16.3	15.2	5	94	0	1	0	1.255	1.4
	75-1048	50.0	8.08	15.9	16.1	15	85	0	0	0	1.315	1.7
	75-1049	50.0	3.27	15.7	15.1	15	84	0	1	0	1.505	2.2
	75-1050	52.0	8.35	16.8	7.7	16	78	1	5	0	1.200	1.0
	75-1051	51.0	3.53	15.8	10.1	9	91	0	0	0	1.220	1.0
	75-1052	46.5	7.60	15.6	9.0	12	80	2	6	0	1.140	1.9
	75-1054	53.5	8.89	17.7	10.3	9	87	2	0	2	1.260	1.6
	MEAN	50.3	8.27	16.2	11.5	12	86	1	1	0	1.253	1.1
	$\pm$ S.D.	± 2.2	$\pm .52$	$\pm .9$	± 3.5						$\pm .121$	
1	75-998	49.0	7.60	15.5	10.5	9	90	1	0	0	1.425	1.2
	75-999	51.0	8.20	16.4	14.9	10	87	1	2	0	1.090	2.0
	75-1000	49.0	7.47	15.6	11.2	3	93	2	2	0	1.120	1.7
	75-1003	53.0	8.52	16.9	10.1	6	89	1	4	0	1.210	1.3
	75-1004	48.5	7.30	15.5	9.1	6	91	1	1	1	1.145	1.9
	75-1005	53.5	8.30	17.4	12.5	6	92	1	1	0	0.995	1.6
	75-1009	52.0	8.79	17.0	12.3	10	89	0	1	0	1.165	1.3
	75-1010	49.5	8.23	16.1	10.1	13	86	1	0	0	1.215	1.6
	MEAN	50.7	8.11	16.3	11.3	8	90	1	1	0	1.171	1.4
	$\pm$ S.D.	± 2.0	$\pm .46$	$\pm .7$	± 1.8						$\pm .125$	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

73-760	53.0	3.79	17.1	11.1	1.6	34	1	1	0	1.038	1.4
73-762	39.5	6.78	13.1	11.2	24	71	3	0	0	0.888	1.0
73-763	53.0	9.39	17.4	9.7	17	79	1	3	0	0.883	1.9
73-764	48.0	7.83	15.4	13.0	13	87	0	0	0	0.908	1.9
73-766	50.5	8.15	16.7	18.6	27	68	3	2	0	1.153	1.0
73-767	53.0	9.06	19.4	13.3	10	96	0	4	0	0.905	2.3
73-768	43.0	7.38	15.1	20.3	19	75	5	1	0	1.165	2.3
73-770	53.0	9.04	17.1	16.1	18	77	2	1	0	1.285	1.5
MEAN	49.6	3.30	16.4	14.3	18	78	2	2	0	1.028	1.2
S.D.	5.2	.92	4.6	3.9						1.56	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 14

HEMATOLOGICAL VALUES OF VALE RATS ON DAY 366 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose	ANIMAL	PCV	RBC	Hgb	MCV	RBC DIFFERENTIAL COUNT (%)	THROMBOCYTES	RETICULO-				
mg/kg/day	NUMBER	PERCENT	$\times 10^9/\text{mm}^3$	g/100 ml	$\times 10^3/\text{mm}^3$	NEUT	LYMPH	CYTES				
						MONO	EOSIN	%				
0	75-674	50.0	8.71	16.3	7.0	14	33	2	1	0	0.790	1.3
	75-675	57.0	9.51	18.6	11.3	22	73	2	2	0	1.278	1.9
	75-676	51.0	8.33	17.0	10.2	11	35	2	2	0	1.033	1.6
	75-679	46.0	7.35	15.3	8.6	12	34	4	0	0	1.130	1.5
	75-680	43.5	7.11	16.3	3.9	16	32	2	0	0	1.315	2.1
	75-681	53.0	8.33	17.1	11.7	14	36	1	1	0	0.848	2.4
	75-683	51.0	8.05	16.3	19.3	26	67	5	2	0	1.283	1.9
	75-686	52.0	7.34	15.9	11.5	20	73	5	2	0	1.238	2.1
	MEAN	50.4	8.36	16.4	12.4	17	79	3	1	0	1.121	1.4
	$\pm$ S.D.	$\pm$ 4.2	$\pm$.71	$\pm$ 1.3	$\pm$ 5.2						$\pm$.206	
30	75-661	47.5	7.90	15.7	11.4	3	39	2	1	0	1.196	1.6
	75-663	50.3	8.11	16.6	16.3	16	81	1	2	0	0.805	1.6
	75-664	46.0	8.23	15.2	8.9	21	72	3	3	1	1.045	1.7
	75-665	32.0	8.45	16.5	13.5	16	36	0	0	0	1.250	1.8
	75-666	50.0	8.41	16.4	15.3	22	73	3	2	0	1.025	1.1
	75-667	49.5	8.32	15.5	13.7	9	89	2	0	0	1.063	1.6
	75-668	52.5	8.56	17.5	13.7	3	95	2	3	0	1.075	1.0
	75-670	51.5	7.93	16.7	13.4	22	72	1	5	0	1.825	1.9
	MEAN	49.9	8.26	16.4	13.5	15	81	2	2	0	1.161	1.9
	$\pm$ S.D.	$\pm$ 2.2	$\pm$.26	$\pm$.7	$\pm$ 2.3						$\pm$.299	
10	75-610	50.5	8.79	16.7	13.0	20	77	0	3	0	1.513	1.0
	75-611	55.5	8.99	17.4	11.2	19	80	1	1	0	1.058	1.5
	75-612	47.0	7.46	15.6	11.8	20	76	1	1	0	1.040	1.7
	75-614	53.0	8.39	16.7	13.1	13	34	2	2	0	0.985	1.2
	75-615	50.0	8.65	16.6	12.8	12	86	0	2	0	1.055	1.2
	75-617	46.0	7.36	15.4	14.1	22	75	2	1	0	0.998	1.0
	75-618	51.5	7.31	15.3	12.5	21	33	4	4	2	0.900	1.9
	75-620	45.5	7.36	16.0	12.1	12	73	1	4	0	1.255	1.2
	MEAN	49.9	8.10	16.2	12.5	17	79	2	2	0	1.101	1.1
	$\pm$ S.D.	$\pm$ 3.5	$\pm$.68	$\pm$.3	$\pm$ 1.5						$\pm$.195	

APPENDIX 15

HEMATOLOGICAL VALUES OF FEMALE RATS ON DAY 365 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $10^6/\text{mm}^3$	Reticulo- cytes %
						Neut	Lymph	Mono	Eosin	Baso		
0	75-910	47.0	7.44	14.9	8.3	20	78	2	0	0	1.028	1.5
	75-912	48.5	8.20	15.9	7.8	16	80	2	1	1	0.875	1.2
	75-913	45.0	7.72	15.0	7.5	17	78	1	4	0	1.080	1.0
	75-916	47.0	7.10	15.2	15.9	21	75	2	2	0	0.825	1.6
	75-917	46.0	7.36	14.8	7.7	13	84	1	2	0	0.833	.5
	75-919	49.5	7.90	15.5	10.0	20	75	1	4	0	0.953	.4
	75-920	47.0	7.43	15.0	13.4	9	86	2	3	0	1.025	.9
	75-921	47.0	7.51	15.0	15.0	9	89	0	1	0	0.883	1.4
	MEAN	47.1	7.58	15.2	10.7	16	81	1	2	0	0.938	1.1
	$\pm$ S.D.	± 1.4	$\pm .34$	$\pm .4$	± 3.5						$\pm .098$	
30	75-1097	48.0	7.58	15.6	11.7	12	82	3	3	0	0.818	.5
	75-1098	45.0	7.18	14.6	10.3	10	87	0	3	0	0.955	2.0
	75-1099	47.0	7.48	14.8	13.3	28	72	0	0	0	0.968	1.0
	75-1100	50.5	8.62	17.0	10.1	9	89	1	1	0	0.915	1.3
	75-1101	48.0	7.93	16.6	17.0	8	89	1	2	0	1.123	.7
	75-1102	45.0	7.24	15.1	10.3	14	82	2	1	1	0.923	.9
	75-1103	51.0	8.19	16.3	7.5	7	87	2	3	1	0.710	1.7
	75-1104	46.5	7.55	15.5	9.8	15	82	2	1	0	0.813	1.3
	MEAN	47.6	7.72	15.7	11.3	13	84	1	2	0	0.903	1.2
	$\pm$ S.D.	± 2.2	$\pm .49$	$\pm .9$	± 2.8						$\pm .125$	
10	75-1046	44.0	7.11	14.3	8.9	15	79	4	2	0	1.175	1.1
	75-1047	50.5	8.29	16.4	10.7	12	84	2	2	0	1.183	2.1
	75-1048	46.5	7.35	14.9	7.3	14	81	1	4	0	1.135	1.4
	75-1049	46.0	7.70	15.4	11.6	17	81	0	2	0	1.158	1.0
	75-1050	46.5	8.41	15.8	6.3	25	72	1	2	0	1.118	.8
	75-1051	47.5	7.99	15.9	5.6	18	80	2	0	0	0.738	.4
	75-1053	46.0	7.93	15.0	10.4	11	88	0	1	0	0.923	.4
	75-1054	44.0	7.51	15.0	8.3	20	76	1	3	0	0.915	1.4
	MEAN	46.4	7.79	15.3	8.6	17	80	1	2	0	1.052	1.1
	$\pm$ S.D.	± 2.1	$\pm .45$	$\pm .7$	± 2.2						$\pm .170$	
3	75-998	44.5	7.56	15.0	3.3	17	79	1	3	0	1.058	.9
	75-999	48.5	8.46	16.3	13.2	25	71	2	2	0	0.968	.6
	75-1000	45.0	7.04	14.4	7.3	5	85	5	5	0	0.810	1.4
	75-1003	46.5	7.68	15.2	9.1	11	83	3	3	0	1.050	.8
	75-1004	44.0	7.18	14.1	8.7	15	81	2	2	0	0.963	.7
	75-1005	52.0	8.24	17.1	9.3	13	86	1	0	0	0.908	1.7
	75-1009	44.0	8.02	15.0	9.1	25	68	2	5	0	0.995	.7
	75-1010	46.0	8.17	15.6	11.4	12	87	1	0	0	0.853	1.2
	MEAN	46.3	7.79	15.3	8.9	15	80	2	3	0	0.951	1.0
	$\pm$ S.D.	± 2.7	$\pm .51$	± 1.0	± 2.9						$\pm .089$	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 16

HEMATOLOGICAL VALUES OF MALE RATS ON DAY 726 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $10^3/\text{mm}^3$	Reticulo- cytes %
						Neuc	Lymph	Mono	Eosin	Baso		
0	75-678	45.0	6.38	14.6	13.2	33	60	7	0	0	0.955	2.3
	75-679	46.0	7.71	16.4	13.2	35	38	6	1	0	1.350	2.2
	75-680	48.5	7.50	15.3	10.6	39	50	9	2	0	0.950	2.1
	75-681	50.0	7.42	16.3	20.4	17	72	10	1	0	1.115	1.4
	75-684	45.5	7.11	15.1	10.9	39	53	8	0	0	1.235	1.3
	75-729	33.5	5.13	11.7	6.1	83	12	5	0	0	0.555	1.3
	75-732	39.0	5.47	12.7	26.4	39	49	12	0	0	1.350	1.7
	75-748	46.5	7.48	15.6	12.4	50	40	8	2	0	1.015	1.3
	MEAN	44.3	6.79	14.8	14.8	42	49	8	1	0	1.066	1.7
	$\pm$ S.D.	± 3.4	± 1.01	± 1.7	± 6.5						± 0.262	
30	75-861	43.0	7.40	15.5	23.4	50	41	8	1	0	1.000	1.2
	75-862	50.5	8.04	16.3	10.8	31	62	4	3	0	1.005	2.8
	75-864	49.0	7.49	15.4	10.4	26	64	9	1	0	0.885	1.6
	75-871	52.0	7.92	16.2	10.4	11	86	3	0	0	0.755	1.2
	75-884	50.0	7.28	15.1	9.9	21	76	3	0	0	0.830	1.8
	75-885	46.0	7.75	14.9	17.2	53	35	11	1	0	0.800	2.3
	75-889	48.0	8.46	16.4	15.8	24	67	9	0	0	0.750	1.4
	75-891	43.0	6.65	13.5	17.2	47	45	6	2	0	0.985	5.4
	MEAN	47.7	7.62	15.4	14.4	33	59	7	1	0	0.878	2.1
	$\pm$ S.D.	± 3.4	± 0.55	± 1.0	± 4.8						± 0.107	
10	75-814	48.5	8.05	16.3	18.0	43	45	10	2	0	0.795	1.7
	75-819	51.0	7.60	16.4	13.5	43	49	7	1	0	0.395	1.6
	75-844	46.5	7.26	14.3	24.8	43	45	8	4	0	0.790	1.8
	75-850	43.0	7.08	14.6	14.2	37	52	9	2	0	0.880	2.0
	75-815			No blood could be obtained								
	MEAN	47.3	7.50	15.6	17.6	42	48	8	2	0	0.840	1.8
	$\pm$ S.D.	± 3.4	± 0.43	± 0.9	± 5.2						± 0.055	
3	75-762	48.0	7.16	15.1	17.7	22	67	9	2	0	1.340	1.5
	75-764	45.0	7.33	15.0	21.3	52	34	12	2	0	0.940	1.6
	75-771	48.0	7.73	16.1	12.0	25	70	4	1	0	0.820	1.9
	75-773	43.0	7.08	14.3	10.6	31	62	7	0	0	0.805	1.8
	75-774	40.0	6.44	13.9	9.8	23	68	9	0	0	1.025	1.6
	75-788	38.0	5.88	12.9	9.7	40	55	4	1	0	0.875	1.2
	75-794	41.5	6.85	15.2	10.8	28	64	6	2	0	0.950	1.3
	75-795	50.0	7.91	16.8	11.5	25	67	7	1	0	0.700	1.1
	MEAN	44.2	7.05	14.9	12.9	31	61	7	1	0	0.932	1.1
	$\pm$ S.D.	± 4.3	± 0.66	± 1.2	± 4.2						± 0.193	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 17

HEMATOLOGICAL VALUES OF FEMALE RATS ON DAY 727 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)					Throm- bocytes $10^6/\text{mm}^3$	Reticulo- cytes %
						Neut	Lymph	Mono	Eosin	Baso		
0	75-912	42.0	6.38	13.5	6.9	24	71	4	1	0	1.092	2.0
	75-914	45.0	7.04	15.0	9.6	41	47	11	1	0	1.000	1.1
	75-918	50.0	7.18	16.1	5.1	32	59	7	2	0	1.100	.9
	75-926	49.5	7.11	14.9	7.1	31	58	7	4	0	1.025	1.0
	75-927	50.0	7.46	16.6	6.8	14	79	4	3	0	1.025	1.8
	75-929	53.0	7.63	15.0	10.8	14	76	8	2	0	1.115	1.7
	75-935	44.0	6.28	13.8	8.3	29	63	8	0	0	0.975	1.8
	75-936	45.0	6.20	14.3	9.0	13	78	8	1	0	1.465	3.6
	MEAN	47.3	6.91	14.9	8.0	25	66	7	2	0	1.200	1.6
	$\pm$ S.D.	± 3.8	± 0.55	± 1.1	± 1.8						± 0.156	
30	75-1098	47.0	6.03	15.2	9.1	46	49	4	1	0	1.285	1.6
	75-1100	43.5	5.65	14.2	9.2	37	55	7	1	0	0.645	1.4
	75-1102	48.0	6.58	15.5	7.1	18	75	5	2	0	0.822	1.8
	75-1108	48.0	7.33	15.4	5.8	15	77	6	2	0	1.365	1.2
	75-1110	31.5	3.34	9.8	9.2	21	78	1	0	0	1.310	18.5
	75-1112	46.0	6.55	15.0	8.3	19	69	11	1	0	1.408	1.6
	75-1116	43.0	6.09	14.1	10.6	35	59	5	1	0	1.005	1.8
	75-1119	48.0	6.72	15.3	5.6	--	--	--	--	--	1.235	2.0
	MEAN	44.4	6.04	14.3	8.1	27	66	6	1	0	1.134	3.7
	$\pm$ S.D.	± 5.6	± 1.20	± 1.9	± 1.8						± 0.279	
10	75-1046	47.0	6.66	14.7	6.8	22	69	6	3	0	1.192	2.8
	75-1056	45.0	6.69	16.0	4.6	36	53	9	2	0	0.978	1.9
	75-1059	35.0	3.89	10.7	7.2	29	66	4	1	0	1.082	5.4
	75-1070	49.0	7.12	16.3	6.3	12	75	9	4	0	0.950	2.0
	75-1072	45.0	6.69	15.1	8.5	28	61	11	0	0	0.878	1.0
	75-1086	46.0	6.80	15.5	6.7	19	71	5	5	0	0.962	2.1
	75-1091	44.0	6.65	15.4	12.2	26	66	6	2	0	1.005	1.0
	75-1092	46.5	6.68	15.2	13.0	40	57	3	0	0	0.878	2.2
	MEAN	44.7	6.40	14.9	8.2	26	65	7	2	0	0.991	2.3
	$\pm$ S.D.	± 4.2	± 1.03	± 1.8	± 2.9						± 0.105	
3	75-997	50.0	6.74	16.7	6.4	12	77	7	4	0	1.152	1.9
	75-999	49.0	7.26	16.9	8.3	24	73	3	0	0	1.245	.9
	75-1007	45.0	5.99	15.1	7.4	17	74	8	1	0	1.148	1.8
	75-1008	50.5	6.88	16.3	11.1	25	68	7	0	0	1.189	1.2
	75-1010	47.0	6.66	16.3	9.2	53	40	6	1	0	1.635	1.7
	75-1011	37.0	5.44	12.1	12.3	32	59	9	0	0	1.135	1.3
	75-1012	39.0	5.65	13.1	8.9	60	30	9	1	0	1.390	2.5
	75-1017	44.0	6.03	14.4	10.3	35	58	7	0	0	0.885	0.5
	MEAN	45.2	6.33	15.1	9.3	32	60	7	1	0	1.222	1.5
	$\pm$ S.D.	± 5.0	± 0.64	± 1.8	± 2.0						± 0.218	

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 18

RESULTS OF URINALYSES OF MALE RATS ON DAY 82 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

<u>Dose</u> <u>mg/kg/day</u>	<u>Animal</u> <u>Number</u>	<u>Specific</u> <u>Gravity</u> ^a	<u>pH</u>	<u>Sugar</u>	<u>Protein</u>	<u>Ketones</u>	<u>Occult</u> <u>Blood</u>	<u>Bilirubin</u>
0	75-594	1.039	7.0	neg.	+	neg.	neg.	neg.
	75-595	1.052	6.5	neg.	+++	neg.	neg.	neg.
	75-597	1.043	7.0	neg.	++	neg.	moderate	neg.
	75-598	1.041	6.5	neg.	+++	neg.	neg.	neg.
	75-599	1.039	7.0	neg.	+++	neg.	neg.	neg.
	75-600	1.032	6.5	neg.	++	neg.	neg.	neg.
	75-601	1.050	7.0	neg.	++	neg.	neg.	neg.
	75-602	1.044	6.5	neg.	+++	neg.	neg.	neg.
	MEAN±S.D.	1.030±.006						
30	75-624	1.030	7.0	neg.	++	neg.	neg.	neg.
	75-625	1.046	6.5	neg.	++	neg.	neg.	neg.
	75-626	1.038	7.0	neg.	+	neg.	neg.	neg.
	75-628	1.056	7.0	neg.	+++	neg.	neg.	neg.
	75-629	1.041	6.5	neg.	+++	neg.	neg.	neg.
	75-630	1.038	6.5	neg.	+++	neg.	neg.	neg.
	75-631	1.050	6.5	neg.	++	neg.	neg.	neg.
	75-632	1.036	6.5	neg.	++	neg.	neg.	neg.
	MEAN±S.D.	1.027±.007						
10	75-614	1.063	6.0	neg.	+++	neg.	neg.	neg.
	75-617	1.052	6.0	neg.	+++	neg.	neg.	neg.
	75-618	1.029	7.0	neg.	++	neg.	small	neg.
	75-619	1.028	7.0	neg.	+	neg.	neg.	neg.
	75-620	1.044	6.5	neg.	+++	neg.	neg.	neg.
	75-621	1.043	6.0	neg.	+++	neg.	moderate	neg.
	75-622	1.052	6.0	neg.	+++	neg.	neg.	neg.
	75-623	1.038	6.0	neg.	+	neg.	neg.	neg.
	MEAN±S.D.	1.035±.005						
3	75-604	1.039	6.5	neg.	+++	neg.	neg.	neg.
	75-607	1.034	7.0	neg.	+	neg.	neg.	neg.
	75-608	1.063	6.5	neg.	++	neg.	neg.	neg.
	75-609	1.050	7.0	neg.	+++	neg.	small	neg.
	75-610	1.057	6.0	neg.	++	neg.	neg.	neg.
	75-611	1.059	6.5	neg.	+++	neg.	neg.	neg.
	75-612	1.048	7.0	neg.	+++	neg.	neg.	neg.
	75-613	1.048	6.0	neg.	+++	neg.	neg.	neg.
	MEAN±S.D.	1.038±.010						

^aNo statistically significant differences from control mean using analysis of variance and Dunnett's test $p < 0.05$.

APPENDIX 19

RESULTS OF URINALYSES OF FEMALE RATS ON DAY 83 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

<u>Dose</u> <u>mg/kg/day</u>	<u>Animal</u> <u>Number</u>	<u>Specific</u> <u>Gravity</u> ^a	<u>pH</u>	<u>Sugar</u>	<u>Protein</u>	<u>Ketones</u>	<u>Occult</u> <u>Blood</u>	<u>Bilirubin</u>
0	75-634	1.038	6.5	neg.	trace	neg.	neg.	neg.
	75-635	1.021	6.5	neg.	trace	neg.	neg.	neg.
	75-636	1.032	7.0	neg.	+	neg.	neg.	neg.
	75-637	1.028	6.5	neg.	+	neg.	neg.	neg.
	75-638	1.036	7.0	neg.	+++	neg.	neg.	neg.
	75-639	1.033	7.0	neg.	++	neg.	neg.	neg.
	75-640	1.025	7.0	neg.	trace	neg.	neg.	neg.
	75-641	1.024	6.5	neg.	+	neg.	neg.	neg.
	MEAN±S.D.	1.043±.006						
30	75-664	1.039	6.0	neg.	trace	neg.	neg.	neg.
	75-665	1.021	6.5	neg.	trace	neg.	neg.	neg.
	75-666	1.026	7.0	neg.	trace	neg.	neg.	neg.
	75-667	1.026	7.0	neg.	trace	neg.	neg.	neg.
	75-668	1.017	7.5	neg.	trace	neg.	neg.	neg.
	75-670	1.025	6.5	neg.	trace	neg.	neg.	neg.
	75-672	1.033	6.5	neg.	+	neg.	neg.	neg.
	75-673	1.030	6.0	neg.	++	neg.	neg.	neg.
	MEAN±S.D.	1.042±.008						
10	75-654	1.038	7.5	neg.	+	neg.	neg.	neg.
	75-655	1.032	6.5	neg.	+	neg.	neg.	neg.
	75-656	1.028	7.5	neg.	+++	neg.	neg.	neg.
	75-657	1.034	7.0	neg.	++	neg.	neg.	neg.
	75-658	1.029	6.5	neg.	+	neg.	neg.	neg.
	75-659	1.039	6.0	neg.	+	neg.	neg.	neg.
	75-660	1.035	6.5	neg.	+	neg.	neg.	neg.
	75-661	1.044	6.0	neg.	++	neg.	neg.	neg.
	MEAN±S.D.	1.044±.013						
3	75-644	1.056	8.0	neg.	+++	neg.	neg.	neg.
	75-645	1.038	7.0	neg.	++	neg.	neg.	neg.
	75-646	1.027	6.5	neg.	trace	neg.	neg.	neg.
	75-648	1.036	6.5	neg.	+	neg.	neg.	neg.
	75-649	1.038	6.5	neg.	+	neg.	neg.	neg.
	75-651	1.031	6.0	neg.	++	neg.	neg.	neg.
	75-652	1.047	6.0	neg.	+++	neg.	neg.	neg.
	75-653	1.031	6.0	neg.	+++	neg.	neg.	neg.
	MEAN±S.D.	1.050±.010						

^aNo statistically significant differences from control mean using analysis of variance and Dunnett's test, p<0.05.

APPENDIX 20

RESULTS OF URINALYSES OF MALE RATS ON DAY 89 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

<u>Dose</u> <u>mg/kg/day</u>	<u>Animal</u> <u>Number</u>	<u>Specific</u> <u>Gravity</u> ^a	<u>pH</u>	<u>Glucose</u>	<u>Protein</u>	<u>Ketones</u>	<u>Occult</u> <u>Blood</u>	<u>Bilirubin</u>
0	75-674	1.050	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-675	1.044	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-676	1.045	7.0	Neg.	++	Neg.	Neg.	Neg.
	75-679	1.054	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-680	1.042	7.5	Neg.	+	Neg.	Neg.	Neg.
	75-681	1.047	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-683	1.038	6.0	Neg.	++	Neg.	Neg.	Neg.
	75-686	1.036	7.0	Neg.	+++	Neg.	Neg.	Neg.
	MEAN±S.D.	1.044±.006						
30	75-861	1.054	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-863	1.047	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-864	1.050	7.0	Neg.	+	Neg.	Neg.	Neg.
	75-865	1.047	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-866	1.030	7.0	Neg.	++	Neg.	Neg.	Neg.
	75-867	1.043	6.5	Neg.	+	Neg.	Neg.	Neg.
	75-868	1.039	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-870	1.039	7.0	Neg.	++	Neg.	Neg.	Neg.
	MEAN±S.D.	1.044±.008						
10	75-810	1.038	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-811	1.028	6.5	Neg.	+	Neg.	Neg.	Neg.
	75-812	1.049	6.0	Neg.	+++	Neg.	Neg.	Neg.
	75-814	1.038	6.0	Neg.	+	Neg.	Neg.	Neg.
	75-815	1.031	6.0	Neg.	++	Neg.	Neg.	Neg.
	75-817	1.048	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-818	1.042	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-820	1.055	6.5	Neg.	+++	Neg.	Neg.	Neg.
	MEAN±S.D.	1.041±.009						
3	75-760	1.045	7.0	Neg.	++	Neg.	Neg.	Neg.
	75-762	1.046	7.0	Neg.	+	Neg.	Neg.	Neg.
	75-763	1.032	6.5	Neg.	+	Neg.	Neg.	Neg.
	75-764	1.038	6.5	Neg.	+	Neg.	Neg.	Neg.
	75-766	1.047	6.0	Neg.	+++	Neg.	Neg.	Neg.
	75-767	1.052	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-768	1.034	7.5	Neg.	++	Neg.	Neg.	Neg.
	75-770	1.044	6.5	Neg.	+	Neg.	Neg.	Neg.
	MEAN±S.D.	1.042±.007						

^aNo statistically significant differences from control means using analysis of variance and Dunnett's test, p<0.05.

APPENDIX 21

RESULTS OF URINALYSES OF FEMALE RATS ON DAY 90 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

<u>Dose</u> <u>mg/kg/day</u>	<u>Animal</u> <u>Number</u>	<u>Specific</u> <u>Gravity</u> ^a	<u>pH</u>	<u>Glucose</u>	<u>Protein</u>	<u>Ketones</u>	<u>Occult</u> <u>Blood</u>	<u>Bilirubin</u>
0	75-910	1.035	7.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-912	1.019	7.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-913	1.044	6.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-916	1.039	6.0	Neg.	+++	Neg.	Neg.	Neg.
	75-917	1.023	7.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-919	1.025	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-920	1.028	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-921	1.031	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	MEAN±S.D.	1.030±.008						
30	75-1096	1.021	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-1097	1.033	6.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-1098	1.029	7.0	Neg.	++	Neg.	Neg.	Neg.
	75-1100	1.021	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-1101	1.029	6.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-1102	1.028	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-1103	1.024	7.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-1104	1.025	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	MEAN±S.D.	1.026±.004						
10	75-1046	1.036	6.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-1047	1.021	6.5	Neg.	+	Neg.	Small	Neg.
	75-1048	1.030	7.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-1049	1.017	7.0	Neg.	Trace	Neg.	Neg.	Neg.
	75-1050	1.023	6.5	Neg.	+	Neg.	Neg.	Neg.
	75-1051	1.030	7.0	Neg.	++	Neg.	Neg.	Neg.
	75-1052	1.017	7.5	Neg.	+	Neg.	Neg.	Neg.
	75-1054	1.024	6.5	Neg.	+++	Neg.	Neg.	Neg.
	MEAN±S.D.	1.025±.007						
3	75-998	1.027	6.5	Neg.	++	Neg.	Neg.	Neg.
	75-999	1.040	6.0	Neg.	+	Neg.	Neg.	Neg.
	75-1000	1.030	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-1003	1.031	6.5	Neg.	+	Neg.	Neg.	Neg.
	75-1004	1.034	7.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-1005	1.026	6.5	Neg.	+++	Neg.	Neg.	Neg.
	75-1009	1.026	6.5	Neg.	Trace	Neg.	Neg.	Neg.
	75-1010	1.021	7.5	Neg.	++	Neg.	Neg.	Neg.
	MEAN±S.D.	1.029±.006						

^aNo statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 22

RESULTS OF URINALYSES OF MALE RATS ON DAY 364 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Specific Gravity ^a	pH	Glucose	Protein	Ketones	Occult Blood	Bilirubin	Urobilinogen Ehrlich Units/dl
0	75-674	1.049	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-675	1.039	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-676	1.039	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-679	1.052	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-680	1.034	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-681	1.049	5.5	Neg.	+++	Neg.	Small	Neg.	0.1
	75-684	1.036	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-686	1.032	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN $\pm$ S.D.	1.041 $\pm$.008							
30	75-861	1.039	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-863	1.035	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-864	1.050	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-865	1.050	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-866	1.036	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-867	1.023	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-868	1.043	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-870	1.040	7.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN $\pm$ S.D.	1.039 $\pm$.009							
10	75-810	1.033	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-811	1.027	6.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-812	1.023	5.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-814	1.027	5.5	Neg.	+++	Neg.	Small	Neg.	0.1
	75-815	1.048	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-817	1.023	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-818	1.039	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-820	1.025	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN $\pm$ S.D.	1.031 $\pm$.009							
3	75-760	1.046	6.5	Neg.	++++	Neg.	Neg.	Neg.	0.1
	75-762	1.047	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-775	1.029	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-764	1.045	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-766	1.035	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-767	1.044	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-768	1.036	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-773	1.043	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN $\pm$ S.D.	1.041 $\pm$.007							

^aStatistically significant difference from control mean using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 23

RESULTS OF URINALYSES OF FEMALE RATS ON DAY 365 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Specific Gravity ^a	pH	Glucose	Protein	Ketones	Occult Blood	Bilirubin	Urobilinogen Ehrlich Units/dl
0	75-910	1.034	6.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-911	1.017	6.0	Neg.	++	Neg.	Neg.	Neg.	0.0
	75-915	1.015	6.5	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-916	1.018	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-917	1.023	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-919	1.027	6.0	Neg.	+	Neg.	Neg.	Neg.	0.1
	75-920	1.039	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-918	1.023	5.5	Neg.	++	Neg.	Neg.	Neg.	0.1
	MEAN±S.D.	1.025±.009							
30	75-1105	1.019	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1098	1.027	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1099	1.022	7.0	Neg.	+++	Neg.	Neg.	Neg.	0.0
	75-1107	1.023	6.0	Neg.	+	Neg.	Neg.	Neg.	0.1
	75-1108	1.033	5.5	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1102	1.015	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1103	1.019	6.0	Neg.	+	Neg.	Neg.	Neg.	0.1
	75-1104	1.015	5.5	Neg.	+	Neg.	Neg.	Neg.	0.1
	MEAN±S.D.	1.022±.006							
10	75-1046	1.031	6.0	Neg.	++	Neg.	Small	Neg.	0.1
	75-1056	1.023	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1057	1.022	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1058	1.049	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1050	1.022	6.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1051	1.031	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1053	1.026	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1054	1.024	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN±S.D.	1.029±.009							
3	75-998	1.041	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-999	1.040	5.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1000	1.015	5.5	Neg.	+	Neg.	Neg.	Neg.	0.1
	75-1003	1.023	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1004	1.028	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1005	1.044	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1009	1.030	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1010	1.030	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN±S.D.	1.031±.010							

^aNo statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

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APPENDIX 24

RESULTS OF URINALYSES OF MALE RATS ON DAY 726 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

<u>Dose</u> <u>mg/kg/day</u>	<u>Animal</u> <u>Number</u>	<u>Specific</u> <u>Gravity^a</u>	<u>pH</u>	<u>Glucose</u>	<u>Protein</u>	<u>Ketones</u>	<u>Occult</u> <u>Blood</u>	<u>Bilirubin</u>	<u>Urobilinogen</u> <u>Ehrlich Units/dl</u>
0	75-678				No urine obtained				
	75-679	1.025	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-680	1.033	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-681	1.019	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-684	1.030	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-729	1.014	6.0	Neg.	+++	Neg.	Moderate	Neg.	0.1
	75-732	1.014	7.0	Neg.	+++	Neg.	Small	Neg.	0.1
	75-748	1.029	6.0	Neg.	+++	Neg.	Neg.	Neg.	1.0
	MEANS.D.	1.023±.008							
30	75-861	1.015	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-862	1.030	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-864	1.029	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-871	1.043	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-884	1.033	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-885	1.021	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-889	1.017	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-891	1.030	6.5	Neg.	+++	Neg.	Neg.	Neg.	1.0
	MEANS.D.	1.027±.009							
10	75-814	1.022	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-817	1.025	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-844				No urine obtained				
	75-850	1.019	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-815	1.020	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEANS.D.	1.022±.003							
3	75-762	1.026	6.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-764	1.014	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-771	1.018	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-773	1.018	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-774	1.021	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-788	1.031	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-794	1.033	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-795	1.032	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEANS.D.	1.024±.007							

^aNo statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 25

RESULTS OF URINALYSES OF FEMALE RATS ON DAY 727 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

<u>Dose</u> <u>mg/kg/day</u>	<u>Animal</u> <u>Number</u>	<u>Specific</u> <u>Gravity</u> ^a	<u>pH</u>	<u>Glucose</u>	<u>Protein</u>	<u>Ketones</u>	<u>Occult</u> <u>Blood</u>	<u>Bilirubin</u>	<u>Urobilinogen</u> <u>Ehrlich Units/dl</u>
0	75-912	1.036	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-914	1.032	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-918	1.019	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-926	1.015	6.0	Neg.	++	Neg.	Small	Neg.	0.1
	75-927	1.032	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-929	1.042	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-935	1.035	6.0	Neg.	+++	Neg.	Trace	Neg.	0.1
	75-936	1.024	7.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN±S.D.	1.029±.009							
30	75-1098	1.030	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1100	1.025	6.0	Neg.	+++	Neg.	Small	Neg.	0.1
	75-1102	1.025	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1108	1.023	6.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1110	1.015	6.0	Neg.	++	Neg.	Large	Neg.	0.1
	75-1112	1.023	7.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1116	1.039	6.0	Neg.	+	Neg.	Trace	Neg.	0.1
	75-1119	1.023	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	MEAN±S.D.	1.025±.007							
10	75-1046	1.011	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1056	1.054	6.0	Neg.	++++	Neg.	Neg.	Neg.	0.1
	75-1059	1.024	7.0	Neg.	+++	Neg.	Large	Neg.	0.1
	75-1070	1.031	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1072	1.022	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1086	1.039	6.0	Neg.	+++	Neg.	Trace	Neg.	0.1
	75-1091	1.031	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1092				No urine obtained				
	MEAN±S.D.	1.030±.014							
3	75-997	1.042	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-999	1.030	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1007	1.044	6.0	Neg.	++	Neg.	Neg.	Neg.	0.1
	75-1008	1.040	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1010	1.036	6.0	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1011	1.023	7.0	Neg.	+++	Neg.	Small	Neg.	0.1
	75-1012	1.020	6.5	Neg.	+++	Neg.	Neg.	Neg.	0.1
	75-1017	1.021	6.0	Neg.	+++	Neg.	Small	Neg.	0.1
	MEAN±S.D.	1.032±.010							

^aNo statistically significant differences from control means using analysis of variance and Dunnett's test, p<0.05.

75-597	1	30	15.6	9.1	0.58	5.8	0.37	100	6.4
	2	42	26.9	28.0	1.04	5.2	0.19	160	6.0
	3	38	30.4	19.0	0.62	2.1	0.07	114	3.8
75-598	1	52	31.7	21.0	0.66	6.8	0.21	130	4.1
	2	48	31.7	15.0	0.47	4.8	0.15	104	3.3
	3	49	38.2	22.0	0.58	2.6	0.07	101	2.6
MEAN		38	29.4	17.5	0.60	4.1	0.15	116	4.1
	±S.D.	±8	±5.4	±4.9	±0.16	±1.8	±0.09	±22	±1.1

^aSample Period -

#1 = days 74-76 of study

#2 = days 99-101 of study

#3 = days 104-106 of study

No statistically significant difference from control mean using analysis of variance and Dunnett's Test, $p < 0.05$.

APPENDIX 26

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN MALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T
COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day		Animal Number		Sample Period		Total Urine Vol. 48 hr. (ml)		Creatinine mg/48 hr.		Coproporphyrin µg/48 hr.		<u>µg Coproporphyrin</u> mg Creatinine		Uroporphyrin µg/48 hr.		<u>µg Uroporphyrin</u> mg Creatinine		delta-ALA µg/48 hr.		<u>µg delta-ALA</u> mg Creatinine	
0		75-594		1	36	22.3	17.0	0.76	6.5	0.29	90	4.0									
				2	22	24.0	13.0	0.54	3.0	0.12	100	4.2									
				3	34	29.2	16.0	0.55	1.4	0.05	102	3.5									
		75-595		1	32	30.4	11.0	0.36	4.6	0.15	90	3.0									
				2	34	30.6	13.0	0.42	4.6	0.15	130	4.2									
				3	38	32.7	18.0	0.55	1.9	0.06	121	3.7									
		75-596		1	40	33.2	19.0	0.57	5.9	0.18	120	3.6									
				2	34	29.9	18.0	0.60	3.7	0.12	162	5.4									
				3	39	33.9	23.0	0.68	2.2	0.06	117	3.4									

APPENDIX 26 (Continued)

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN MALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day	Animal Number	Sample Period	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin µg/48 hr.	µg Coproporphyrin mg Creatinine	Uroporphyrin µg/48 hr.	µg Uroporphyrin mg Creatinine	delta-ALA µg/48 hr.	µg delta-ALA mg Creatinine
30	75-624	1	53	28.1	46.0	1.64	8.4	0.30	50	1.8
		2	54	21.6	39.0	1.80	9.4	0.44	115	5.3
		3	68	30.6	52.0	1.70	1.9	0.06	143	4.7
	75-625	1	44	28.2	33.0	0.72	7.4	0.26	60	2.1
		2	56	29.7	39.0	1.31	13.0	0.44	73	2.5
		3	59	34.8	57.0	1.64	3.9	0.11	94	2.7
	75-626	1	50	32.0	56.0	1.75	12.0	0.38	110	3.4
		2	54	30.2	44.0	1.46	16.0	0.53	84	2.8
		3	48	29.8	54.0	1.81	2.7	0.09	130	4.4
	75-627	1	43	25.8	34.0	1.32	7.1	0.28	60	2.3
		2	51	27.0	31.0	1.15	10.0	0.37	62	2.3
		3	55	27.0	41.0	1.52	2.2	0.08	99	3.7
	75-628	1	42	28.1	45.0	1.60	6.9	0.25	60	2.1
		2	23	26.4	30.0	1.14	14.0	0.53	156	5.9
		3	32	33.0	53.0	1.61	1.2	0.04	113	3.4
	MEAN		49 ^b	28.8	43.6 ^b	1.48 ^b	7.7 ^b	0.28 ^b	94	3.3
	±S.D.		±11	±3.2	±9.3	±0.30	±4.7	±0.17	±33	±1.3

^aSample Period - #1 = days 74-76 of study
#2 = days 99-101 of study
#3 = days 104-106 of study

^bStatistically significant difference from control mean using analysis of variance and Dunnett's Test, $p < 0.05$.

75-617	1	37	30.0	22.0	0.73	4.2	0.14	80	2.7
	2	33	30.0	16.0	0.53	6.9	0.23	140	4.7
	3	39	34.7	24.0	0.69	1.7	0.05	125	3.6
75-618	1	52	36.4	45.0	1.24	6.7	0.18	100	2.7
	2	51	29.6	36.0	1.22	10.0	0.34	106	3.6
	3	60	39.0	40.0	1.02	1.4	0.04	132	3.4
MEAN		36	29.1	24.6 ^b	0.84 ^b	5.0	0.19	102	3.7
±S.D.		±14	±7.6	±9.6	±0.22	±3.1	±0.16	±36	±1.9

^a Sample Period - #1 = days 74-76 of study

#2 = days 99-101 of study

#3 = days 104-106 of study

^b Statistically significant difference from control mean using analysis of variance and Dunnett's Test, $p < 0.05$.

COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day		10		75-614		75-615		75-616	
		Animal Number		Sample Period		Total Urine Vol. 48 hr. (ml)		Creatinine mg/48 hr.	
		1		1		1		1	
		2		2		2		2	
		3		3		3		3	
		32		32		14		42	
		28		28		10		34	
		31		31		26		45	
		28.8		28.8		15.7		33.6	
		30.0		30.0		10.9		30.6	
		31.9		31.9		21.8		34.2	
		20.0		20.0		10.0		25.0	
		22.0		22.0		12.0		20.0	
		28.0		28.0		23.0		26.0	
		0.69		0.69		0.64		0.74	
		0.73		0.73		1.10		0.65	
		0.88		0.88		1.06		0.76	
		5.0		5.0		11.0		4.8	
		6.4		6.4		1.3		7.5	
		4.6		4.6		1.9		1.3	
		0.17		0.17		0.70		0.14	
		0.21		0.21		0.12		0.24	
		0.14		0.14		0.09		0.04	
		100		100		10		170	
		99		99		104		70	
		107		107		95		86	
		3.5		3.5		0.6		5.0	
		3.3		3.3		9.5		2.3	
		3.4		3.4		4.4		2.5	

APPENDIX 26 (Continued)

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN MALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T
COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day	Animal Number	Sample Period	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin µg/48 hr.	µg Coproporphyrin mg Creatinine	Uroporphyrin µg/48 hr.	µg Uroporphyrin mg Creatinine	delta-ALA µg/48 hr.	µg delta-ALA mg Creatinine
3	75-604	1	56	34.7	25.0	0.72	5.2	0.15	110	3.2
		2	46	30.8	24.0	0.78	6.9	0.22	99	3.2
		3	47	30.6	23.0	0.75	1.7	0.06	122	4.0
	75-605	1	44	31.7	21.0	0.66	4.8	0.15	110	3.5
		2	24	25.4	20.0	0.79	6.0	0.24	100	3.9
		3	31	32.6	30.0	0.92	1.9	0.06	117	3.6
	75-606	1	56	33.6	19.0	0.56	5.9	0.18	100	3.0
		2	43	31.0	23.0	0.74	8.5	0.27	220	7.1
		3	38	33.8	34.0	1.01	2.9	0.08	144	4.3
	75-607	1	42	29.4	24.0	0.82	6.5	0.22	110	3.7
		2	36	28.1	21.0	0.75	3.7	0.13	59	2.1
		3	45	30.2	22.0	0.73	3.8	0.12	77	2.6
	75-608	1	29	26.7	19.0	0.71	3.6	0.14	100	3.7
		2	28	26.9	19.0	0.71	4.4	0.16	109	4.0
		3	34	32.3	22.0	0.68	2.2	0.07	120	3.7
	MEAN		40	30.5	23.1	0.76	4.5	0.15	113	3.7
	±S.D.		±10	±2.8	±4.2	±0.11	±2.0	±0.07	±35	±1.1

^aSample Period - #1 = days 74-76 of study
#2 = days 99-101 of study
#3 = days 104-106 of study

No statistical difference from control mean using analysis of variance
and Dunnett's Test, $p < 0.05$.

APPENDIX 27

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN FEMALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day	Animal Number	Sample Period	Total Urine Vol. 48 Hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin µg/48 hr.	µg Coproporphyrin mg Creatinine	Uroporphyrin µg/48 hr.	µg Uroporphyrin mg Creatinine	delta-ALA µg/48 hr.	µg delta-ALA mg Creatinine
0	75-634	1	42	18.5	6.2	0.34	2.2	0.12	40	2.2
		2	14	13.7	3.4	0.25	0.9	0.07	36	2.6
		3	54	22.7	4.1	0.18	1.4	0.06	92	4.0
	75-635	1	34	16.3	10.0	0.61	3.6	0.22	30	1.8
		2	62	19.8	11.0	0.56	1.4	0.07	94	4.8
		3	56	20.2	9.6	0.48	1.5	0.07	106	5.2
	75-636	1	39	20.7	7.1	0.34	2.2	0.11	40	1.9
		2	24	20.2	8.1	0.40	0.9	0.04	33	1.6
		3	40	23.6	8.5	0.36	1.1	0.05	88	3.7
	75-637	1	28	17.6	7.5	0.43	3.6	0.20	60	3.4
		2	39	23.0	8.3	0.36	1.2	0.05	142	6.2
		3	50	25.0	7.7	0.31	1.5	0.06	105	4.2
	75-638	1	34	16.3	7.6	0.47	2.8	0.17	40	2.4
		2	42	20.2	7.8	0.39	1.3	0.06	102	5.0
		3	42	22.3	9.3	0.42	1.4	0.06	105	4.7
	MEAN		40	20.0	7.7	0.39	1.8	0.09	74	3.6
	±S.D.		±12	±3.1	±2.0	±0.11	±0.9	±0.06	±36	±1.4

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

^aSample Period - #1 = days 74-76 of study
#2 = days 99-101 of study
#3 = days 104-106 of study

APPENDIX 27 (Continued)

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN FEMALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day	Animal Number	Sample Period	Total Urine Vol. 48 Hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin µg/48 hr.	<u>µg Coproporphyrin</u> <u>mg Creatinine</u>	Uroporphyrin µg/48 hr.	<u>µg Uroporphyrin</u> <u>mg Creatinine</u>	delta-ALA µg/48 hr.	<u>µg delta-ALA</u> <u>mg Creatinine</u>
30	75-664	1	15	14.3	5.4	0.38	2.1	0.15	10	0.7
		2	51	19.9	12.0	0.60	0.8	0.04	93	4.3
		3	43	18.9	8.6	0.46	1.5	0.08	-	-
	75-665	1	59	16.5	8.7	0.53	2.7	0.16	40	2.4
		2	56	16.8	13.0	0.77	1.1	0.06	122	7.3
		3	50	21.5	10.0	0.46	0.7	0.03	80	3.7
	75-666	1	46	14.3	8.5	0.59	3.0	0.21	50	3.5
		2	43	14.6	8.8	0.60	1.4	0.10	88	6.0
		3	37	16.3	4.1	0.25	1.4	0.08	220	13.5
	75-667	1	54	20.0	7.2	0.36	2.9	0.14	50	2.5
		2	50	18.0	7.8	0.43	1.2	0.07	107	5.9
		3	67	-	11.6	-	1.5	-	130	-
	75-668	1	18	10.3	4.6	0.45	1.5	0.15	10	1.0
		2	50	19.0	9.4	0.49	1.9	0.10	119	6.3
		3	86	20.6	-	-	-	-	120	5.8
	MEAN		48	17.2	8.6	0.49	1.7	0.10	88	4.8
	±S.D.		±17	±3.1	±2.6	±0.13	±0.7	±0.05	±56	±3.3

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

^aSample Period - #1 = days 74-76 of study
#2 = days 99-101 of study
#3 = days 104-106 of study

75-657	1	39	16.4	10.0	0.61	5.0	0.30	50	3.0
	2	65	20.8	13.0	0.62	1.7	0.08	130	6.2
	3	77	24.6	13.0	0.53	1.5	0.06	116	4.7
75-658	1	-	-	-	-	-	-	-	-
	2	64	25.0	6.8	0.27	1.3	0.05	62	2.5
	3	69	20.7	7.8	0.38	1.5	0.07	76	3.7
MEAN		50	20.3	9.1	0.45	1.9	0.10	86	4.3
	±S.D.	±16	±2.7	±2.2	±0.11	±1.2	±0.08	±27	±1.4

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

^a Sample Period -

- #1 = days 74-76 of study
- #2 = days 99-101 of study
- #3 = days 104-106 of study

APPENDIX 27 (Continued)

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN FEMALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day		Animal Number		Sample Period		Total Urine Vol. 48 Hr. (ml)		Creatinine mg/48 hr.		Coproporphyrin µg/48 hr.		<u>µg Coproporphyrin</u> mg Creatinine		Uroporphyrin µg/48 hr.		<u>µg Uroporphyrin</u> mg Creatinine		delta-ALA µg/48 hr.		<u>µg delta-ALA</u> mg Creatinine	
10	75-654			1	49	19.6	5.9	0.30	1.9	0.10	60	3.1									
				2	46	17.5	6.8	0.39	1.4	0.08	98	5.6									
				3	44	21.1	7.2	0.34	0.9	0.04	88	4.2									
	75-655			1	46	17.0	8.3	0.49	3.9	0.23	50	2.9									
				2	52	17.7	8.5	0.48	1.7	0.10	130	7.3									
				3	59	21.2	9.3	0.44	1.2	0.06	94	4.4									
	75-656			1	36	22.7	11.0	0.48	2.1	0.09	80	3.5									
				2	24	18.0	9.5	0.53	2.1	0.12	101	5.6									
				3	25	21.5	10.0	0.46	0.7	0.03	68	3.2									

APPENDIX 27 (Continued)

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN
AND DELTA-ALA (DELTA-AMINOLEVULINIC ACID) IN FEMALE RATS FOR UP TO
DAY 106 OF A 118-119 DAY DIETARY STUDY OF 2,4,5-T

COMBINED RESULTS OF THREE SAMPLE PERIODS^a

Dose 2,4,5-T mg/kg/day	Animal Number	Sample Period	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin µg/48 hr.	µg Coproporphyrin mg Creatinine	Uroporphyrin µg/48 hr.	µg Uroporphyrin mg Creatinine	delta-ALA µg/48 hr.	µg delta-ALA mg Creatinine
3	75-644	1	61	16.5	5.3	0.32	2.8	0.17	40	2.4
		2	35	17.2	9.4	0.55	1.3	0.08	61	3.6
		3	58	20.9	8.1	0.39	1.1	0.05	87	4.2
	75-645	1	35	16.5	8.0	0.48	3.1	0.19	20	1.2
		2	14	14.3	5.8	0.40	1.0	0.07	5	0.4
		3	62	21.9	7.0	0.32	1.5	0.07	112	5.1
	75-646	1	29	20.6	4.9	0.24	5.7	0.28	40	1.9
		2	30	17.7	8.5	0.48	1.7	0.10	42	2.4
		3	45	23.4	9.4	0.40	1.2	0.05	86	3.7
	75-647	1	14	12.0	9.0	0.75	1.7	0.14	10	0.8
		2	25	16.5	11.0	0.67	2.7	0.16	98	5.9
		3	38	17.9	8.4	0.47	1.3	0.07	72	4.0
	75-648	1	49	21.1	9.5	0.45	3.2	0.15	70	3.3
		2	45	19.4	11.0	0.57	1.4	0.07	75	3.9
		3	46	23.5	8.4	0.35	1.0	0.04	87	3.7
	MEAN		39	18.6	8.3	0.46	2.0	0.11	60	3.1
	±S.D.		±15	±3.3	±1.8	±0.14	±1.3	±0.07	±33	±1.6

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

^aSample Period - #1 = days 74-76 of study
#2 = days 99-101 of study
#3 = days 104-106 of study

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30	75-629	16.5	11.9	24.0	2.02	0.5	0.04
	75-630	17.0	14.6	31.0	2.12	1.9	0.13
	75-631	5.5	7.3	15.0	2.05	1.3	0.18
	75-632	6.5	7.1	13.0	1.83	1.0	0.14
	75-633	11.0	12.1	19.0	1.57	1.1	0.09
	MEAN	11.3	10.6	20.4	1.92 ^a	1.2	0.12
	±S.D.	±5.4	±3.3	±7.2	±0.22	±0.5	±0.05
10	75-619	23.0	13.3	26.0	1.95	1.0	0.08
	75-620	14.0	11.8	19.0	1.61	0.4	0.03
	75-621	14.5	15.2	16.0	1.05	0.7	0.05
	75-622	27.0	15.7	13.0	0.83	0.6	0.04
	75-623	10.5	10.5	20.0	1.90	1.6	0.15
	MEAN	17.8	13.3	18.8	1.47	0.9	0.07
	±S.D.	±6.9	±2.2	±4.9	±0.50	±0.5	±0.05
3	75-609	13.0	13.3	14.0	1.05	0.5	0.04
	75-610	5.5	2.7	2.2	0.81	0.2	0.07
	75-611	11.5	12.4	11.0	0.96	0.6	0.05
	75-612	22.5	13.3	24.0	1.80	1.8	0.14
	75-613	22.0	10.6	12.0	1.13	0.8	0.08
	MEAN	14.9	10.5	12.6	1.15	0.8	0.08
	±S.D.	±7.3	±4.5	±7.8	±0.38	±0.6	±0.04

^aStatistically significant difference from control mean using analysis of variance and Dunnett's Test $p < 0.05$.

APPENDIX 28

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN AND UROPORPHYRIN
FOR MALE RATS ON DAYS 117-118 OF A 118-119 DAY STUDY OF 2,4,5-T

Dose 2,4,5-T mg/kg/day		Animal Number	Total Urine Vol. 24 hr. (ml)	Creatinine mg 24 hr.	Coproporphyrin µg/24 hr.	<u>µg Coproporphyrin</u> mg Creatinine	Uroporphyrin µg/24 hr.	µg Uroporphyrin mg Creatinine
0								
		75-599	11.0	10.9	9.8	0.90	1.2	0.11
		75-600	13.0	10.1	9.7	0.96	0.6	0.06
		75-601	10.5	14.4	16.0	1.11	0.8	0.06
		75-602	16.0	12.5	16.0	1.28	0.8	0.06
		75-603	13.5	12.2	17.0	1.39	0.9	0.07
MEAN			12.8	12.0	13.7	1.13	0.9	0.07
±S.D.			±2.2	±1.6	±3.6	±0.21	±0.2	±0.02

APPENDIX 29

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN AND UROPORPHYRIN
FOR FEMALE RATS ON DAYS 118-119 OF A 118-119 DAY STUDY OF 2,4,5-T

Dose 2,4,5-T mg/kg/day	Animal Number	Total Urine Vol. 24 hr. (ml)	Creatinine mg/24 hr.	Coproporphyrin µg/24 hr.	µg Coproporphyrin mg Creatinine	Uroporphyrin µg/24 hr.	µg Uroporphyrin mg Creatinine
0	75-639	18.0	9.2	4.0	0.43	0.5	0.05
	75-640	22.0	7.5	4.0	0.53	0.4	0.05
	75-641	11.0	7.7	3.6	0.47	0.2	0.02
	75-642	19.0	8.6	6.1	0.71	0.4	0.05
	75-643	22.0	9.5	4.4	0.46	0.6	0.06
	MEAN	18.4	8.5	4.4	0.52	0.4	0.05
	±S.D.	±4.5	±0.9	±1.0	±0.11	±0.1	±0.01
30	75-669	25.5	8.9	7.9	0.89	0.1	0.01
	75-670	17.5	5.6	2.9	0.52	0.2	0.04
	75-671	34.0	8.2	4.6	0.56	0.3	0.04
	75-672	10.0	7.5	9.9	1.32	0.02	0.00
	75-673	18.5	6.1	7.2	1.18	0.1	0.02
	MEAN	21.1	7.3	6.5	0.89 ^a	0.1 ^a	0.02
	±S.D.	±9.1	±1.4	±2.8	±0.36	±0.1	±0.02
10	75-659	14.0	8.7	4.5	0.52	0.3	0.03
	75-660	21.0	7.6	5.3	0.70	0.2	0.03
	75-661	10.0	7.1	5.4	0.76	0.1	0.01
	75-662	13.0	8.6	5.7	0.66	0.2	0.02
	75-663	17.0	8.5	4.6	0.54	0.3	0.04
	MEAN	15.0	8.1	5.1	0.64	0.2	0.03
	±S.D.	±4.2	±0.7	±0.5	±0.10	±0.1	±0.01
3	75-649	32.5	7.8	5.1	0.65	0.5	0.06
	75-650	13.5	9.0	4.2	0.47	0.4	0.04
	75-651	13.5	9.5	4.6	0.48	0.5	0.05
	75-652	14.0	6.2	3.5	0.56	0.3	0.05
	75-653	15.0	8.6	4.3	0.50	0.2	0.02
	MEAN	17.7	8.2	4.3	0.53	0.4	0.04
	±S.D.	±8.3	±1.3	±0.6	±0.07	±0.1	±0.02

^aStatistically significant difference from control mean using analysis of variance and Dunnett's Test, $p < 0.05$.

APPENDIX 10

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND delta-ALA (delta-aminolevulinic acid)
FOR MALE AND FEMALE RATS ON DAYS 95-97 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Sex	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin ug/48 hr.	ug Coproporphyrin/ mg Creatinine	Uroporphyrin ug/48 hr.	ug Uroporphyrin/ mg Creatinine	delta-ALA ug/48 hr.	ug delta-ALA/ mg Creatinine
0	75-674	M	22	23.5	14.0	.60	2.1	.09	130	5.5
	75-675	M	12	30.1	22.0	.73	2.7	.09	180	5.0
	75-676	M	20	22.2	27.0	1.22	4.8	.22	130	5.3
	75-679	M	38	23.3	17.0	.59	2.1	.07	180	6.2
	75-680	M	44	33.0	20.0	.61	2.7	.08	140	4.2
	MEAN		30	27.5	20.0	.75	2.9	.11	152	5.5
	±S.D.		±10	±4.5	±4.9	±.27	±1.1	±.06	±26	±0.8
30	75-861	M	37	30.7	36.0	1.17	2.6	.08	90	2.9
	75-863	M	31	27.8	41.0	1.47	1.9	.07	110	3.5
	75-864	M	24	31.0	34.0	1.10	2.5	.08	160	5.2
	75-865	M	33	28.0	44.0	1.57	4.4	.16	80	2.8
	75-866	M	49	27.9	43.0	1.54	3.2	.11	140	5.0
	MEAN		35	29.1	39.6 ^a	1.37 ^a	2.9	.10	116	3.9
	±S.D.		±9	±1.6	±4.4	±.22	±0.9	±.04	±34	±1.1
10	75-810	M	22	28.2	35.0	1.24	3.4	.12	90	3.2
	75-811	M	30	21.0	22.0	1.05	2.9	.14	160	7.6
	75-812	M	26	23.7	26.0	1.10	1.6	.07	100	4.2
	75-814	M	58	37.7	44.0	1.17	2.6	.07	250	6.6
	75-815	M	35	24.3	28.0	1.13	1.8	.07	110	4.4
	MEAN		34	27.1	31.0 ^a	1.14 ^a	2.5	.09	142	5.2
	±S.D.		±14	±6.5	±3.7	±.07	±0.8	±.03	±66	±1.3
3	75-760	M	34	30.6	30.0	.98	2.9	.09	90	2.9
	75-762	M	31	27.3	27.0	.99	2.0	.07	210	7.7
	75-763	M	42	24.8	20.0	.81	2.4	.10	160	6.4
	75-764	M	37	28.1	18.0	.64	3.0	.11	240	3.5
	75-766	M	37	26.3	17.0	.65	2.2	.08	130	4.9
	MEAN		36	27.4	22.4	.81	2.5	.09	166	6.1
	±S.D.		±4	±2.2	±5.8	±.17	±0.4	±.02	±60	±2.2
0	75-910	F	37	20.0	6.5	.32	1.5	.08	210	10.5
	75-912	F	68	17.0	8.1	.48	1.6	.09	230	13.5
	75-913	F	10	7.5	2.1	.28	1.4	.19	20	2.7
	75-916	F	45	22.0	7.6	.34	1.3	.06	150	6.8
	75-917	F	64	21.8	8.9	.41	1.6	.07	170	7.8
	MEAN		45	17.7	6.6	.37	1.5	.10	156	8.3
	±S.D.		±21	±6.0	±2.7	±.08	±0.1	±.03	±32	±4.0
30	75-1096	F	46	17.5	12.0	.68	1.2	.07	140	8.0
	75-1097	F	17	14.8	8.2	.55	1.3	.09	170	11.5
	75-1098	F	40	15.6	8.2	.52	0.8	.05	0	0
	75-1100	F	33	13.2	3.2	.62	1.2	.09	130	9.8
	75-1101	F	17	13.6	12.0	.88	1.2	.09	140	10.3
	MEAN		31	14.9	9.7	.65 ^a	1.1	.08	116	7.9
	±S.D.		±13	±1.7	±2.1	±.14	±0.2	±.02	±66	±4.6
10	75-1046	F	51	21.4	8.7	.41	1.5	.07	150	7.0
	75-1047	F	14	8.0	3.0	.38	0.8	.10	130	16.2
	75-1048	F	28	17.6	10.8	.61	0.8	.04	130	7.4
	75-1049	F	60	20.4	12.0	.59	2.7	.13	200	9.8
	75-1050	F	50	20.0	7.1	.36	1.1	.06	150	7.5
	MEAN		41	17.5	8.3	.47	1.4	.08	152	9.6
	±S.D.		±19	±5.5	±3.5	±.12	±0.3	±.04	±29	±3.9
3	75-998	F	44	20.7	7.8	.38	2.1	.10	150	7.2
	75-999	F	36	20.2	9.0	.44	1.0	.05	100	5.0
	75-1000	F	67	20.1	16.0	.80	3.4	.17	180	9.0
	75-1003	F	50	21.0	9.4	.45	1.2	.06	120	5.7
	75-1004	F	47	15.0	6.2	.41	1.2	.08	150	10.0
	MEAN		49	19.4	9.7	.50	1.8	.09	140	7.4
	±S.D.		±11	±2.5	±3.7	±.17	±1.0	±.03	±31	±2.1

^a Statistically significant increase from control mean using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 31

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND delta-ALA (delta-aminolevulinic acid)
FOR MALE AND FEMALE RATS ON DAYS 188-190 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Sex	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin ug/48 hr.	ug Coproporphyrin/ mg Creatinine	Uroporphyrin ug/48 hr.	ug Uroporphyrin/ mg Creatinine	delta-ALA ug/48 hr.	ug delta-ALA/ mg Creatinine
0	75-674	M	24.0	26.0	17.0	.65	1.6	.062	70	2.7
	75-675	M	44.5	37.0	29.0	.78	3.2	.086	110	3.0
	75-676	M	45.0	31.0	13.0	.42	2.5	.081	110	3.5
	75-679	M	27.0	26.0	15.0	.58	1.9	.073	70	2.7
	75-680	M	27.0	31.0	22.0	.71	1.8	.058	60	1.9
	MEAN		33.5	30.2	19.2	.63	2.2	.072	84	2.8
	±S.D.		±10.3	±4.5	±6.4	±.14	±0.7	±.012	±24	±0.6
30	75-861	M	30.0	29.0	39.0	1.34	2.0	.069	120	4.1
	75-863	M	35.0	30.0	53.0	1.77	2.6	.087	90	3.0
	75-864	M	37.0	33.0	46.0	1.39	3.0	.091	100	3.0
	75-865	M	56.0	30.0	62.0	2.07	3.5	.117	130	4.3
	75-866	M	40.0	31.0	37.0	1.19	5.9	.190	110	3.5
	MEAN		39.6	30.6	47.4 ^a	1.55 ^a	3.4	.111	110	3.6
	±S.D.		±9.9	±1.5	±10.3	±.36	±1.5	±.048	±16	±0.6
10	75-810	M	31.0	29.0	31.0	1.07	1.3	.045	160	5.5
	75-811	M	42.0	25.0	26.0	1.04	1.6	.064	80	3.2
	75-812	M	52.0	32.0	36.0	1.13	2.2	.069	130	4.1
	75-814	M	47.0	36.0	32.0	.89	4.0	.111	140	3.9
	75-815	M	38.0	34.0	24.0	.71	3.0	.088	90	2.6
	MEAN		42.0	31.2	29.8	.97	2.4	.075	120	3.9
	±S.D.		±8.1	±4.3	±4.8	±.17	±1.1	±.025	±34	±1.1
3	75-760	M	45.0	36.0	29.0	.81	2.7	.075	110	3.0
	75-762	M	21.0	24.0	28.0	1.17	1.2	.050	70	2.9
	75-763	M	46.0	31.0	25.0	.81	2.8	.090	120	3.9
	75-764	M	38.0	32.0	21.0	.65	2.2	.069	110	3.4
	75-766	M	44.0	28.0	25.0	.89	2.5	.089	140	5.0
	MEAN		38.8	30.2	25.6	.87	2.3	.075	110	3.6
	±S.D.		±10.4	±4.5	±3.1	±.19	±0.6	±.016	±26	±0.8
0	75-910	F	20.0	15.0	9.3	.62	1.3	.087	50	3.3
	75-912	F	28.0	20.0	11.0	.55	1.5	.075	80	4.0
	75-913	F	46.0	17.0	7.4	.44	1.6	.094	90	5.3
	75-916	F	45.0	23.0	12.0	.52	1.7	.074	100	4.3
	75-917	F	66.0	25.0	8.2	.33	1.4	.056	130	5.2
	MEAN		41.0	20.0	9.6	.49	1.5	.077	90	4.4
	±S.D.		±17.9	±4.1	±1.9	±.11	±.2	±.015	±30	±0.8
30	75-1096	F	54.0	18.0	17.0	.94	2.0	.111	70	3.9
	75-1097	F	55.0	22.0	15.0	.68	1.7	.077	100	4.5
	75-1098	F	33.0	15.0	10.0	.67	1.5	.100	60	4.0
	75-1100	F	40.0	18.0	12.0	.67	1.7	.094	80	4.4
	75-1101	F	44.0	22.0	28.0	1.27	1.4	.064	90	4.1
	MEAN		45.2	19.0	16.4 ^a	.85 ^a	1.7	.089	80	4.2
	±S.D.		±9.4	±3.0	±7.0	±.26	±.2	±.019	±20	±0.2
10	75-1046	F	38.0	20.0	9.1	.46	1.6	.080	90	4.5
	75-1047	F	48.0	20.0	9.8	.49	1.1	.055	90	4.5
	75-1048	F	36.0	15.0	9.2	.61	1.6	.107	90	6.0
	75-1049	F	51.0	17.0	10.0	.59	1.2	.071	70	4.1
	75-1050	F	38.0	17.0	8.0	.47	1.1	.065	80	4.7
	MEAN		42.2	17.8	9.2	.52	1.3	.076	80	4.8
	±S.D.		±6.8	±2.2	±.8	±.07	±.3	±.020	±10	±0.7
3	75-998	F	44.0	20.0	12.0	.60	1.3	.065	80	4.0
	75-999	F	24.0	14.0	7.1	.51	1.1	.079	60	4.3
	75-1000	F	59.0	18.0	15.0	.83	1.1	.061	80	4.4
	75-1003	F	76.0	23.0	11.0	.48	1.4	.061	130	5.6
	75-1004	F	54.0	18.0	6.6	.37	1.0	.056	100	5.6
	MEAN		51.4	18.6	10.3	.56	1.2	.064	90	4.8
	±S.D.		±19.2	±3.3	±3.5	±.17	±.2	±.009	±30	±0.8

^aStatistically significant increase from control mean using analysis of variance and Dunnett's test, $p < 0.05$.

0	75-910 F	55.0	30.0	18.0	.60	1.3	.060	80	2.7
	75-912 F	37.0	18.0	11.0	.61	1.2	.067	80	2.7
	75-913 F	37.0	15.0	7.4	.49	1.1	.073	40	3.7
	75-916 F	80.0	25.0	16.0	.64	2.3	.092	30	3.2
	75-917 F	95.0	31.0	15.0	.48	2.6	.084	90	2.9
	MEAN	60.3	23.8	13.5	.56	1.8	.075	70	3.0
	± 5.0	± 26.0	± 7.1	± 4.2	$\pm .07$	$\pm .7$	$\pm .013$	± 20	± 0.3
30	75-1097 F	36.0	25.0	19.0	.76	.7	.028	50	2.4
	75-1098 F	52.0	21.0	20.0	.95	.9	.043	60	2.8
	75-1099 F	70.0	23.0	21.0	.84	2.2	.088	90	3.6
	75-1100 F	51.0	23.0	16.0	.70	1.6	.070	70	3.0
	75-1101 F	83.0	22.0	22.0	1.05	1.6	.073	120	5.4
	MEAN	58.9	23.2	19.8 ^a	.86 ^a	1.4	.060	80	3.4
	± 5.0	± 11.9	± 4.8	± 3.6	$\pm .14$	$\pm .6$	$\pm .024$	± 10	± 1.2
10	75-1066 F	52.0	26.0	16.0	.62	2.0	.077	30	3.1
	75-1067 F	83.0	25.0	16.0	.58	1.3	.054	80	3.3
	75-1068 F	74.0	23.0	18.0	.72	3.3	.140	30	3.7
	75-1069 F	66.0	19.0	15.0	.72	2.2	.116	50	3.2
	75-1080 F	53.0	21.0	13.0	.71	2.1	.100	80	3.3
	MEAN	63.0	23.0	15.4	.67	2.2	.097	30	3.3
	± 5.0	± 12.1	± 4.9	± 1.7	$\pm .07$	$\pm .3$	$\pm .033$	± 10	± 0.3
3	75-998 F	46.0	26.0	19.0	.73	1.5	.058	50	2.3
	75-999 F	38.0	20.0	10.0	.50	1.7	.065	60	3.0
	75-1000 F	35.0	23.0	14.0	.61	1.4	.061	50	2.6
	75-1003 F	30.0	27.0	15.0	.56	1.8	.067	90	3.3
	75-1004 F	63.0	22.0	11.0	.50	1.5	.068	70	3.2
	MEAN	36.4	23.6	13.3	.58	1.5	.064	70	2.9
	± 5.0	± 16.2	± 2.9	± 3.6	$\pm .09$	$\pm .2$	$\pm .004$	± 10	± 0.4

^a Statistically significant increase from control means by analysis of variance and Dunnett's test, p<0.05.

^b Bloody sample or insufficient volume; not used for statistical analysis.

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APPENDIX 32

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND DELTA-ALA (delta-aminolevulinic acid)
FOR MALE AND FEMALE RATS ON DAYS 361-363 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Sex	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin ug/48 hr.	ug Coproporphyrin/ mg Creatinine	Uroporphyrin mg/48 hr.	ug Uroporphyrin/ mg Creatinine	delta-ALA ug/48 hr.	ug delta-ALA/ mg Creatinine	
0	75-674	M	41.0	37.0	18.0	.49	3.7	.100	120	3.2	
	75-675	M	46.0	42.0	23.0	.55	1.8	.043	120	2.6	
	75-676	M	43.0	34.0	19.0	.56	2.4	.071	90	2.6	
	75-679	M	23.0	33.0	17.0	.52	1.0	.030	100	3.0	
	75-680	M	5.0	ONS	.1	---	.01	---	ONS	---	
	MEAN		39.5	36.5	19.3	.53	2.2	.061	110	2.8	
	±S.D.		±7.9	±4.0	±2.6	±.03	±1.1	±.031	±20	±0.3	
	30	75-861	M	34.0	32.0	38.0	1.19	1.2	.038	90	2.3
		75-863	M	54.0	37.0	49.0	1.32	3.0	.081	80	2.2
		75-864	M	25.0	36.0	41.0	1.14	2.3	.064	70	1.9
75-865		M	46.0	37.0	56.0	1.31	2.8	.076	30	1.9	
75-866		M	46.0	32.0	36.0	1.13	1.1	.034	80	2.3	
MEAN			41.0	34.8	44.0	1.26	2.1	.059	80	2.3	
±S.D.			±11.4	±2.6	±8.3	±.16	±.8	±.022	±10	±0.4	
10		75-810	M	26.0	33.0	22.0	.67	1.5	.045	80	2.5
		75-811	M	59.0	33.0	26.0	.79	2.4	.073	110	3.0
		75-812	M	90.0	36.0	25.0	.69	4.7	.131	110	3.0
	75-814	M	54.0	40.0	38.0	.95	3.4	.085	170	4.2	
	75-815	M	36.0	40.0	10.0	.25	3.3	.083	100	2.5	
	MEAN		53.0	36.4	24.2	.67	3.1	.083	110	3.1	
	±S.D.		±24.6	±3.5	±10.0	±.26	±1.2	±.031	±30	±0.7	
	3	75-760	M	50.0	43.0	31.0	.89	1.6	.046	120	2.8
		75-762	M	37.0	35.0	11.0	.64	2.5	.069	100	2.8
		75-763	M	37.0	36.0	23.0	.70	3.7	.100	100	2.7
75-764		M	44.0	29.0	17.0	.59	1.7	.059	110	3.8	
75-766		M	41.0	36.0	24.3	.71	2.4	.069	110	3.0	
MEAN			41.8	36.0	24.3	.71	2.4	.069	110	3.0	
±S.D.			±5.4	±5.0	±5.9	±.13	±1.0	±.023	±10	±0.5	

No 4-8 vital was sent, therefore unable to do porphyrins on this

APPENDIX 33

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, AND UROPORPHYRIN FOR MALE AND FEMALE RATS
ON DAYS 564-566 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Sex	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin ug/48 hr.	ug Coproporphyrin/ mg Creatinine	Uroporphyrin ug/48 hr.	ug Uroporphyrin/ mg Creatinine
0	75-674	M	47.0	36.0	24.0	.67	11.4	.32
	75-675	M	53.0	24.0	22.0	.92	10.2	.43
	75-678	M	46.0	25.0	34.0	1.36	10.8	.43
	75-679	M	50.0	—	—	—	—	—
	75-680	M	45.0	35.0	34.0	.97	11.4	.33
	MEAN		48.2	30.0	28.5	.98	11.0	.38
	±S.D.		±3.3	±6.4	±6.4	±.29	±.6	±.06
30	75-861	M	63.0	25.0	22.0	.88	8.0	.32
	75-863	M	110.0	44.0	36.0	.82	9.0	.20
	75-864	M	40.0	28.0	62.0	2.21	6.0	.21
	75-865	M	127.0	25.0	24.0	.96	6.2	.25
	75-866	M	129.0	17.0	38.0	2.24	9.6	.56
	MEAN		93.8 ^a	27.8	36.4	1.42	7.8	.31
	±S.D.		±40.1	±9.9	±16.0	±.73	±1.6	±.15
10	75-810	M	103.0	31.0	30.0	.97	14.6	.47
	75-811	M	78.0	25.0	24.0	.96	10.4	.42
	75-813	M	69.0	29.0	22.0	.76	9.8	.34
	75-814	M	50.0	31.0	58.0	1.87	16.8	.54
	75-815	M	57.0	34.0	30.0	.88	12.6	.37
	MEAN		71.4	30.0	32.8	1.09	12.8	.43
	±S.D.		±20.7	±3.3	±14.5	±.45	±2.9	±.08
3	75-760	M	71.0	28.0	54.0	1.93	14.8	.53
	75-762	M	40.0	27.0	52.0	1.93	16.6	.61
	75-764	M	44.0	35.0	44.0	1.26	11.8	.34
	75-765	M	71.0	18.0	28.0	1.56	7.8	.43
	75-766	M	112.0	28.0	19.0	.68	11.6	.41
	MEAN		67.6	27.2	39.4	1.47	12.5	.46
	±S.D.		±23.8	±6.1	±15.3	±.52	±3.4	±.11
0	75-911	F	94.0	12.0	7.6	.63	4.6	.38
	75-912	F	61.0	23.0	14.8	.64	6.6	.29
	75-913	F	27.0	14.0	6.4	.46	4.0	.29
	75-914	F	17.0	16.0	13.6	.85	4.0	.25
	75-917	F	69.0	26.0	11.2	.43	7.4	.28
	MEAN		53.6	18.2	10.7	.60	5.3	.30
	±S.D.		±31.5	±6.0	±3.7	±.17	±1.6	±.05
30	75-1097	F	60.0	17.0	20.0	1.18	7.4	.44
	75-1098	F	58.0	22.0	28.0	1.27	11.2	.51
	75-1099	F	96.0	22.0	18.2	.83	12.4	.56
	75-1100	F	52.0	12.0	12.0	1.00	4.2	.35
	75-1102	F	129.0	13.0	18.4	1.42	9.4	.72
	MEAN		79.0	17.2	19.3 ^a	1.14 ^a	8.9	.52 ^a
	±S.D.		±32.9	±4.8	±5.7	±.23	±3.2	±.14
10	75-1046	F	82.0	25.0	19.8	.79	7.0	.28
	75-1047	F	40.0	20.0	12.2	.61	6.8	.34
	75-1049	F	75.0	21.0	12.6	.60	4.6	.22
	75-1050	F	68.0	24.0	17.2	.72	10.2	.43
	75-1053	F	64.0	16.0	20.0	1.25	10.4	.65
	MEAN		65.8	21.2	16.4	.79	7.8	.38
	±S.D.		±16.0	±3.6	±3.8	±.27	±2.5	±.17
3	75-999	F	70.0	21.0	16.0	.76	5.4	.26
	75-1000	F	95.0	24.0	18.2	.76	6.6	.28
	75-1003	F	91.0	18.0	14.6	.81	7.6	.42
	75-1007	F	50.0	15.0	17.4	1.16	7.4	.49
	75-1008	F	56.0	28.0	15.2	.54	7.0	.25
	MEAN		72.4	21.2	16.3	.81	6.8	.34
	±S.D.		±20.2	±5.1	±1.5	±.22	±.9	±.11

^aStatistically significant increase from control mean by analysis of variance and Dunnett's test, $p < 0.05$.

^bSample not analyzed; sample was bloody due to rat having scratched his side against cage.

APPENDIX 34

URINARY EXCRETION VALUES OF CREATININE, COPROPORPHYRIN, UROPORPHYRIN, AND delta-ALA (delta-aminolevulinic acid)
FOR MALE AND FEMALE RATS ON DAYS 698-700 OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Sex	Total Urine Vol. 48 hr. (ml)	Creatinine mg/48 hr.	Coproporphyrin mg/48 hr.	ug Coproporphyrin/ mg Creatinine	Uroporphyrin mg/48 hr.	ug Uroporphyrin/ mg Creatinine	delta-ALA mg/48 hr.	ug delta-ALA/ mg Creatinine
0	75-678	M	71.0	18.0	17.0	.94	6.9	.38	130	7.2
	75-679	M	83.0	22.0	10.0	.45	3.3	.15	100	4.5
	75-680	M	35.0	32.0	24.0	.75	6.5	.20	130	4.7
	75-684	M	74.0	28.0	12.0	.43	3.9	.14	100	3.6
	75-732	M	138.0	24.0	9.9	.41	3.1	.13	130	5.4
	MEAN		90.2	24.8	14.6	.50	4.7	.20	120	5.1
	±S.D.		±27.4	±5.4	±6.0	±.24	±1.3	±.10	±20	±1.3
30	75-861	M	129.0	12.0	15.0	1.25	4.0	.33	130	10.8
	75-863	M	154.0	7.0	7.8	1.11	4.2	.50	170	24.3
	75-864	M	79.0	26.0	32.0	1.23	6.4	.25	120	4.6
	75-867	M	83.0	22.0	19.0	.86	2.6	.12	110	5.0
	75-871	M	53.0	31.0	40.0	1.29	2.7	.09	110	3.5
	MEAN		99.6	19.6	22.3	1.15 ^a	4.0	.28	130	9.6
	±S.D.		±40.9	±9.9	±13.0	±.17	±1.5	±.20	±20	±8.7
10	75-814	M	28.0	24.0	13.0	.54	4.7	.20	90	3.8
	75-815	M	76.0	27.0	12.0	.44	2.4	.09	110	4.1
	75-818	M	127.0	19.0	2.3	.12	1.4	.07	100	3.3
	75-819	M	38.0	23.0	9.4	.41	2.8	.12	70	3.0
	75-844	M	53.0	31.0	12.0	.39	3.8	.12	120	3.9
	MEAN		64.4	24.8	9.7	.38	3.0	.12	100	4.0
	±S.D.		±39.4	±4.5	±4.4	±.16	±1.3	±.05	±20	±0.8
3	75-762	M	75.0	17.0	15.0	.88	2.4	.14	130	7.6
	75-764	M	66.0	30.0	10.0	.33	1.6	.05	110	3.7
	75-770	M	114.0	26.0	16.0	.62	3.7	.14	230	9.6
	75-771	M	114.0	22.0	20.0	.91	3.7	.17	210	9.3
	75-773	M	107.0	26.0	15.0	.58	3.4	.13	220	8.5
	MEAN		95.2	24.2	15.2	.66	3.0	.13	180	7.3
	±S.D.		±22.9	±4.9	±3.6	±.24	±.9	±.05	±60	±2.4
0	75-912	F	53.0	15.0	3.8	.25	2.2	.15	130	8.7
	75-913	F	59.0	20.0	4.9	.25	1.1	.06	140	7.0
	75-914	F	66.0	19.0	8.5	.45	2.4	.13	120	6.3
	75-915	F	52.0	22.0	6.9	.31	1.5	.07	130	5.9
	75-918	F	58.0	19.0	4.9	.26	1.1	.06	140	7.3
	MEAN		57.6	19.0	5.8	.30	1.7	.09	130	7.0
	±S.D.		±5.6	±2.5	±1.9	±.09	±.6	±.04	±10	±1.1
30	75-1097	F	28.0	11.0	1.2	.11	1.1	.10	70	5.4
	75-1098	F	61.0	15.0	9.0	.60	5.4	.46	140	9.3
	75-1100	F	41.0	18.0	7.4	.41	1.0	.06	100	5.6
	75-1104	F	85.0	14.0	14.0	1.00	3.1	.22	200	14.3
	75-1108	F	59.0	22.0	8.5	.39	1.7	.08	140	6.4
	MEAN		54.8	16.0	8.0	.50	2.5	.16	130	8.4
	±S.D.		±21.7	±4.2	±9.8	±.33	±1.8	±.13	±50	±3.6
10	75-1046	F	58.0	26.0	13.0	.50	1.6	.06	130	5.0
	75-1047	F	43.0	20.0	7.6	.38	2.8	.14	120	6.0
	75-1049	F	65.0	9.0	8.6	.96	1.9	.21	150	16.6
	75-1056	F	77.0	19.0	10.0	.53	3.3	.20	170	3.9
	75-1059	F	65.0 ^a	16.0	11.0	.59	2.3	.13	140	3.3
	MEAN		60.8	18.5	9.3	.59	2.5	.15	140	9.1
	±S.D.		±14.2	±7.0	±2.3	±.25	±1.0	±.07	±20	±4.5
3	75-999	F	91.0	14.0	7.9	.56	3.1	.22	190	13.6
	75-1007	F	64.0	21.0	7.5	.36	3.8	.13	190	9.0
	75-1008	F	72.0	21.0	5.3	.31	5.6	.27	130	3.4
	75-1009	F	39.0	18.0	4.5	.23	1.6	.09	130	10.0
	75-1011	F	39.0	19.0	5.9	.31	2.3	.15	120	5.3
	MEAN		71.0	18.6	6.5	.36	3.4	.13	170	9.4
	±S.D.		±21.2	±2.9	±2.3	±.12	±1.5	±.07	±30	±2.3

No statistically significant difference from control means by analysis of variance and Dunnett's test, $p < 0.05$.

^aAnimal not included in statistics because of blood in urine sample due to a cut toenail.

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APPENDIX 35

CLINICAL CHEMISTRY VALUES OF MALE RATS AT TERMINATION OF A 118 DAY DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	mg/100 ml BUN	mU/ml SGPT	mU/ml AP	mg/100 ml Bilirubin Total	mg/100 ml Bilirubin Direct	mg/100 ml Bilirubin Indirect	gm/100 ml Total Protein	gm/100 ml Albumin	gm/100 ml Globulin
0	75-594	22	53	115	.2	.1	.1	6.5	2.1	4.4
	75-595	16	50	92	.1	0	.1	6.2	2.1	4.1
	75-596	16	62	155	.1	0	.1	6.0	2.1	3.9
	75-597	32	68	102	.1	0	.1	6.6	2.1	4.5
	75-598	21	52	84	.1	0	.1	6.2	2.1	4.1
	75-599	21	64	122	.1	0	.1	6.6	2.3	4.3
	75-600	16	42	101	.1	0	.1	6.2	2.3	3.9
	75-601	18	54	149	.1	-	.1	6.5	2.6	3.9
	75-602	23	42	85	.1	0	.1	6.1	1.6	4.5
	75-603	19	48	103	.1	0	.1	6.7	2.3	4.4
	MEAN	21	54	111	.1	0	.1	6.4	2.2	4.2
	±S.D.	5	9	25	0	0	0	0.2	0.2	0.2
30	75-624	18	68	144	.1	0	.1	6.1	2.0	4.1
	75-625	17	56	109	.1	0	.1	6.6	1.9	4.7
	75-626	18	53	94	.1	0	.1	6.1	2.1	4.0
	75-627	17	73	199	.1	0	.1	6.2	2.6	3.6
	75-628	18	42	90	.1	0	.1	6.2	1.8	4.4
	75-629	19	59	137	.1	0	.1	6.3	2.2	4.1
	75-630	17	59	72	.1	0	.1	6.3	2.2	4.1
	75-631	20	69	114	.1	0	.1	6.4	2.4	4.0
	75-632	21	47	105	.1	0	.1	5.9	2.2	3.7
	75-633	21	46	105	.1	0	.1	6.2	2.4	3.8
	MEAN	19	57	117	.1	0	.1	6.2	2.2	4.0
	±S.D.	2	10	36	0	0	0	0.2	0.2	0.3
10	75-614	21	58	90	.1	0	.1	6.3	2.3	4.0
	75-615	16	74	115	.1	0	.1	6.3	2.2	4.1
	75-616	23	49	79	.1	0	.1	6.1	1.7	4.4
	75-617	18	55	107	.1	0	.1	6.5	2.0	4.5
	75-618	19	46	79	.1	0	.1	6.1	2.1	4.0
	75-619	18	60	112	.1	0	.1	6.7	2.7	4.0
	75-620	20	40	125	.1	0	.1	5.9	2.2	3.7
	75-621	21	51	122	.1	0	.1	6.6	2.2	4.4
	75-622	20	44	92	.1	0	.1	5.7	1.9	3.8
	75-623	20	53	79	.1	0	.1	6.3	2.4	3.9
	MEAN	20	53	100	.1	0	.1	6.2	2.2	4.1
	±S.D.	2	10	18	0	0	0	0.3	0.3	0.3
3	75-604	22	45	97	.1	0	.1	5.9	2.1	3.8
	75-605	21	47	89	.1	0	.1	6.2	1.7	4.5
	75-606	17	49	72	.1	0	.1	6.0	1.8	4.2
	75-607	21	53	138	.1	0	.1	6.5	2.2	4.3
	75-608	18	57	95	.1	0	.1	5.9	2.4	3.5
	75-609	17	55	115	.1	0	.1	6.6	2.4	4.2
	75-610	16	46	133	.1	0	.1	6.1	2.2	3.9
	75-611	25	56	139	.1	0	.1	6.6	2.0	4.6
	75-612	23	42	125	.1	0	.1	6.1	2.3	3.8
	75-613	18	49	138	.1	0	.1	6.5	2.4	4.1
	MEAN	20	50	114	.1	0	.1	6.2	2.2	4.1
	±S.D.	3	5	24	0	0	0	0.3	0.2	0.3

No statistically significant differences from control means using analysis of variance and Dunnett's Test, $p < 0.05$.

APPENDIX 16

CLINICAL CHEMISTRY VALUES OF FEMALE RATS AT TERMINATION OF A 119 DAY DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	mg/100 ml BUN	mU/ml SGPT	mU/ml AP	mg/100 ml Bilirubin Total	mg/100 ml Bilirubin Direct	mg/100 ml Bilirubin Indirect	gm/100 ml Total Protein	gm/100 ml Albumin	gm/100 ml Globulin
0	75-634	18	47	101	.1	.1	0	6.6	2.7	3.9
	75-635	17	45	73	.1	.1	0	6.3	2.7	3.8
	75-636	19	50	109	.1	.1	0	6.2	2.5	3.7
	75-637	22	40	125	.1	.1	0	6.7	2.4	4.3
	75-638	20	43	94	.1	.1	0	6.8	2.7	4.1
	75-639	18	42	84	.1	.1	0	6.5	2.5	4.0
	75-640	16	42	135	.1	.1	0	6.4	2.7	3.7
	75-641	22	46	101	.1	.1	0	7.1	3.0	4.1
	75-642	18	43	125	.1	0	.1	6.2	2.3	3.9
	75-643	22	43	76	.1	0	.1	6.8	2.5	4.3
	MEAN	19	44	102	.1	.1	0	6.6	2.6	4.0
	±S.D.	2	3	21	0	0	0	0.3	0.2	0.2
30	75-664	21	46	96	.1	0	.1	6.5	2.6	3.9
	75-665	20	42	132	.1	0	.1	6.8	2.8	4.0
	75-666	21	51	129	.1	0	.1	7.0	2.6	4.4
	75-667	19	56	127	.1	0	.1	7.6	2.8	4.8
	75-668	21	42	90	.1	0	.1	6.6	2.7	3.9
	75-669	19	46	114	.1	0	.1	6.8	2.9	3.9
	75-670	17	38	86	.1	0	.1	6.2	2.6	3.6
	75-671	28	42	116	.1	0	.1	7.0	2.8	4.2
	75-672	19	51	125	.1	0	.1	6.9	3.0	3.9
	75-673	21	48	134	.1	0	.1	7.0	2.7	4.3
	MEAN	21	46	115	.1	0	.1	6.8	2.3	4.1
	±S.D.	3	5	18	0	0	0	0.4	0.1	0.3
10	75-654	22	53	161	.1	.1	0	6.3	2.5	3.8
	75-655	27	66	124	.1	0	.1	7.0	2.9	4.1
	75-656	23	49	86	.1	.1	0	6.3	2.4	3.9
	75-657	23	61	92	.1	.1	0	7.0	2.6	4.4
	75-658	21	37	99	.1	.1	0	6.6	2.2	4.4
	75-659	23	54	91	.1	.1	0	7.2	2.7	4.5
	75-660	17	44	88	.1	.1	0	6.6	2.5	4.1
	75-661	26	80	184	.1	.1	0	7.2	3.3	3.9
	75-662	23	55	86	.1	0	.1	6.9	2.9	4.0
	75-663	18	58	95	.1	0	.1	7.1	2.3	4.8
	MEAN	22	56 ^a	111	.1	.1	0	6.8	2.6	4.2
	±S.D.	3	12	35	0	0	0	0.3	0.3	0.3
3	75-644	21	55	89	.1	0	.1	6.4	2.8	3.6
	75-645	19	49	115	.1	.1	0	7.0	3.0	4.0
	75-646	27	49	100	.1	0	.1	7.1	2.8	4.3
	75-647	16	49	94	.1	.1	0	6.9	2.7	4.2
	75-648	19	54	117	.1	.1	0	6.5	2.8	3.7
	75-649	22	55	76	.1	.1	0	6.8	2.9	3.9
	75-650	17	52	84	.1	.1	0	6.9	2.6	4.3
	75-651	22	50	82	.1	0	.1	7.2	3.1	4.1
	75-652	19	60	74	.1	0	.1	6.6	2.6	4.0
	75-653	18	45	74	.1	.1	0	7.1	2.7	4.4
	MEAN	20	52	90	.1	.1	0	6.8	2.8	4.0
	±S.D.	3	4	16	0	0	0	0.3	0.2	0.3

^aStatistically significant difference from control mean using analysis of variance and Dunnett's Test, $p < 0.05$.

APPENDIX 37

CLINICAL CHEMISTRY VALUES OF MALE RATS AT TERMINATION OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	mg/100 ml BUN	mU/ml SGPT	mU/ml AP	mg/100 ml Bilirubin Total	mg/100 ml Bilirubin Direct	mg/100 ml Bilirubin Indirect	gm/100 ml Total Protein	gm/100 ml Albumin	gm/100 ml Globulin
0	75-678	40	35	105	.3	.1	.2	5.2	1.4	3.8
	75-769	45	55	92	.2	.1	.1	5.5	1.6	3.9
	75-680	18	37	71	.2	.1	.1	5.7	1.9	3.8
	75-681	43	32	73	.2	.1	.1	5.6	1.6	4.0
	75-684	Dead	--	--	--	--	--	--	--	--
	75-729	Dead	--	--	--	--	--	--	--	--
	75-732	171	38	114	.2	.1	.1	6.5	1.6	3.9
	75-748	16	40	83	.2	.1	.1	5.9	1.6	4.3
	MEAN	56	40	90	.2	.1	.1	5.7	1.6	4.0
	±S.D.	58	8	17	0	0	0	0.4	0.2	0.2
30	75-861	42	48	263	.2	.1	.1	6.1	1.5	4.6
	75-862	20	49	93	.2	.1	.1	5.8	1.9	3.9
	75-863	171	33	133	.1	0	.1	5.4	1.5	3.9
	75-864	18	51	67	.2	.1	.1	6.2	2.1	4.1
	75-867	36	69	150	.2	.1	.1	5.9	1.7	4.2
	75-871	15	49	72	.2	.1	.1	5.9	2.4	3.5
	75-882	21	40	86	.2	.1	.1	6.1	2.2	3.9
	75-884	12	59	81	.2	.1	.1	6.0	2.4	3.6
	75-885	36	37	68	.3	.1	.2	6.4	1.8	4.6
	75-889	26	42	72	.2	.1	.1	6.0	1.6	4.4
	MEAN	40	48	108	.2	.1	.1	6.0	1.9	4.1
	±S.D.	47	11	61	0	0	0	0.3	0.3	0.4
10	75-814	12	42	103	.2	.1	.1	5.7	1.6	4.1
	75-819	53	37	95	.2	.1	.1	5.5	1.5	4.0
	75-844	20	30	89	.2	.1	.1	5.7	1.6	4.1
	75-850	61	30	97	.2	.1	.1	5.5	1.5	4.0
	MEAN	36	35	96	.2	.1	.1	5.6	1.6	4.0
	±S.D.	24	6	6	0	0	0	0.1	0.0	0.6
3	75-762	13	45	61	.2	.1	.1	6.0	1.9	4.1
	75-764	Dead	--	--	--	--	--	--	--	--
	75-771	18	35	50	.2	.1	.1	6.2	1.9	4.3
	75-773	33	38	64	.2	.1	.1	5.9	1.7	4.2
	75-774	77	38	106	.2	.1	.1	5.8	2.0	3.8
	75-784	16	38	117	.1	0	.1	6.3	1.9	4.4
	75-788	Dead	--	--	--	--	--	--	--	--
	75-794	19	40	74	.2	.1	.1	6.0	1.4	4.6
	75-795	17	48	80	.2	.1	.1	6.0	1.9	4.1
	75-800	17	30	66	.2	.1	.1	5.1	2.3	2.8
	75-807	17	35	49	.2	.1	.1	6.3	2.0	4.3
	MEAN	25	38	74	.2	.1	.1	6.0	1.9	4.1
	±S.D.	20	5	24	0	0	0	0.4	0.2	0.5

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 38

CLINICAL CHEMISTRY VALUES OF FEMALE RATS AT TERMINATION OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	mg/100 ml BUN	mU/ml SGPT	mU/ml AP	mg/100 ml Bilirubin Total	mg/100 ml Bilirubin Direct	mg/100 ml Bilirubin Indirect	gm/100 ml Total Protein	gm/100 ml Albumin	gm/100 ml Globulin
0	75-912	17	37	40	.2	.1	.1	7.4	2.3	4.6
	75-913	14	49	78	.2	.1	.1	7.3	3.5	3.8
	75-914	13	37	42	.3	.1	.2	6.8	2.9	3.9
	75-915	13	30	36	.2	0	.2	7.0	2.8	4.2
	75-918	16	50	91	.2	.1	.1	6.5	2.8	3.7
	75-920	24	60	36	.3	.1	.2	5.9	2.1	3.8
	75-926	12	50	93	.1	.1	0	6.1	2.8	3.3
	75-927	17	39	38	.3	.1	.2	6.4	2.1	4.3
	75-929	16	32	47	.3	.1	.2	6.3	2.4	3.9
	75-932	30	38	184	.2	0	.2	5.6	2.3	3.3
	MEAN	17	42	68	.2	.1	.2	6.5	2.6	3.9
	±S.D.	6	10	47	.1	0	.1	0.6	0.4	0.4
30	75-1097	16	45	48	.2	.1	.1	6.0	2.6	3.4
	75-1098	30	36	76	.2	.1	.1	6.1	1.8	4.3
	75-1100	27	36	68	.3	.1	.2	6.2	2.9	3.3
	75-1102	Dead	—	—	—	—	—	—	—	—
	75-1104	22	34	38	.2	.1	.1	6.4	2.9	3.5
	75-1108	15	45	93	.1	.1	0	6.2	2.2	4.0
	75-1112	18	43	66	.2	.1	.1	6.4	2.7	3.7
	75-1115	22	30	57	.1	.1	0	6.4	2.9	3.5
	75-1116	18	27	29	.1	0	.1	6.7	3.0	3.7
	75-1117	17	38	44	.3	0	.3	6.7	1.8	4.9
	75-1119	8	37	69	.2	0	.2	6.3	3.2	3.1
	MEAN	19	37	59	.2	.1	.1	6.3	2.6	3.7
	±S.D.	6	6	19	.1	0	.1	0.2	0.5	0.5
10	75-1046	11	46	52	.2	.1	.1	6.6	3.0	3.6
	75-1047	17	30	75	.3	.1	.2	6.3	2.6	3.7
	75-1049	Dead	—	—	—	—	—	—	—	—
	75-1056	18	41	57	.4	.4	0	6.7	1.3	5.4
	75-1059	15	37	81	.1	0	.1	6.7	3.0	3.7
	75-1063	18	31	55	.3	.1	.2	5.9	2.3	3.6
	75-1070	16	40	34	.2	.1	.1	5.8	2.8	3.0
	75-1072	12	36	72	.2	.1	.1	6.5	2.9	3.6
	75-1086	19	34	31	.2	.1	.1	6.3	2.9	3.4
	75-1091	14	30	44	.2	.1	.1	6.4	2.9	3.5
	MEAN	16	40	56	.2	.1	.1	6.4	2.6	3.7
	±S.D.	3	9	18	.1	.1	.1	0.3	0.5	0.7
3	75-997	17	34	48	.3	.1	.2	6.5	2.1	4.4
	75-999	11	36	58	.2	.1	.1	6.6	2.5	4.1
	75-1007	19	26	31	.2	.1	.1	5.9	2.9	3.0
	75-1008	12	27	49	.4	.1	.3	5.6	1.8	3.8
	75-1009	15	48	54	.2	.1	.1	6.5	2.7	3.8
	75-1010	62	45	76	.3	.1	.2	6.5	1.4	5.1
	75-1011	42	27	110	.2	0	.2	6.4	1.9	4.5
	75-1012	340	94	114	.2	.1	.1	5.2	0.9	4.3
	75-1015	30	37	43	.2	0	.2	5.7	2.7	3.0
	75-1017	15	51	43	.2	0	.2	6.9	3.3	3.6
	MEAN	56	42	63	.2	.1	.2	6.2	2.2	4.0
	±S.D.	101	20	28	.1	0	.1	0.5	0.7	0.7

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

APPENDIX 39

TERMINAL ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS OF MALE RATS AT TERMINATION OF A 118 DAY DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Fasted Body Weight g	Organ Weights (g and g/100 g Body Weight)													
			Brain		Heart		Liver		Kidneys		Testes		Spleen		Thymus	
			g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g
0	75-594	415	1.84	0.44	1.15	0.22	10.51	2.53	2.52	0.61	4.02	0.97	0.62	0.15	0.36	0.09
	75-595	449	1.60	0.36	1.33	0.30	12.44	2.77	3.50	0.74	3.53	0.79	0.86	0.19	0.29	0.07
	75-596	484	1.86	0.39	1.38	0.29	11.30	2.34	3.25	0.67	4.42	0.91	0.80	0.17	0.39	0.08
	75-597	452	1.91	0.42	1.42	0.31	11.62	2.57	3.74	0.83	3.88	0.86	0.92	0.20	0.28	0.06
	75-598	468	1.86	0.40	1.44	0.31	11.77	2.51	2.93	0.63	3.75	0.80	0.93	0.20	0.30	0.06
	75-599	484	1.86	0.38	1.48	0.31	12.29	2.54	2.96	0.61	3.96	0.82	0.83	0.17	0.39	0.08
	75-600	517	1.95	0.38	1.50	0.29	14.26	2.76	3.47	0.67	4.55	0.88	0.88	0.17	0.27	0.05
	75-601	478	1.88	0.39	1.33	0.28	12.52	2.62	3.19	0.67	4.36	0.91	0.81	0.17	0.53	0.11
	75-602	458	1.82	0.40	1.51	0.33	17.10	3.73	4.09	0.89	3.97	0.87	1.10	0.24	0.30	0.07
	75-603	460	1.87	0.41	1.41	0.31	11.74	2.55	3.38	0.73	4.04	0.88	0.76	0.16	0.38	0.08
	MEAN	466	1.85	0.40	1.40	0.30	12.55	2.69	3.28	0.70	4.05	0.87	0.85	0.18	0.35	0.08
	±S.D.	27	0.09	0.02	0.11	0.02	1.87	0.39	0.44	0.09	0.31	0.06	0.12	0.03	0.08	0.02
30	75-624	464	1.84	0.40	1.35	0.29	12.78	2.76	3.85	0.83	4.19	0.90	0.75	0.16	0.33	0.07
	75-625	442	1.82	0.41	1.38	0.31	11.79	2.67	3.68	0.83	3.59	0.81	0.91	0.21	0.31	0.07
	75-626	510	1.85	0.36	1.48	0.29	14.44	2.83	3.97	0.73	3.64	0.71	1.14	0.22	0.42	0.08
	75-627	470	1.82	0.39	1.23	0.26	12.50	2.66	3.30	0.70	4.22	0.90	0.71	0.15	0.29	0.06
	75-628	524	1.93	0.37	1.79	0.34	17.90	3.41	4.31	0.82	4.21	0.80	1.04	0.20	0.42	0.08
	75-629	447	1.91	0.43	1.49	0.33	12.71	2.85	3.39	0.76	4.63	1.04	0.86	0.19	0.31	0.07
	75-630	536	1.98	0.37	1.34	0.25	15.03	2.80	4.31	0.80	4.21	0.78	0.85	0.16	0.41	0.08
	75-631	444	1.70	0.38	1.33	0.30	11.29	2.54	3.03	0.68	3.82	0.86	0.72	0.16	0.38	0.09
	75-632	440	1.79	0.41	1.35	0.31	13.71	3.12	3.94	0.90	3.74	0.85	0.99	0.23	0.48	0.11
	75-633	461	1.92	0.42	1.41	0.31	12.35	2.68	3.66	0.79	3.87	0.84	0.93	0.20	0.31	0.07
	MEAN	474	1.86	0.39	1.41	0.30	13.45	2.83	3.74	0.79 ^a	4.01	0.85	0.89	0.19	0.37	0.08
	±S.D.	36	0.08	0.02	0.15	0.03	1.94	0.26	0.42	0.06	0.33	0.09	0.14	0.03	0.06	0.01
10	75-614	462	1.71	0.37	1.37	0.30	13.01	2.81	3.28	0.71	4.42	0.90	0.97	0.21	0.40	0.09
	75-615	393	1.97	0.50	1.28	0.33	9.77	2.48	3.10	0.79	3.85	0.98	0.75	0.19	0.30	0.08
	75-616	419	1.82	0.43	1.22	0.29	10.38	2.48	2.90	0.69	3.52	0.84	0.92	0.22	0.40	0.10
	75-617	534	1.86	0.35	1.31	0.25	14.87	2.79	4.32	0.81	3.70	0.69	0.97	0.18	0.33	0.06
	75-618	573	1.98	0.34	1.52	0.26	15.64	2.73	3.89	0.68	4.06	0.71	0.94	0.16	0.53	0.09
	75-619	542	1.83	0.34	1.55	0.29	15.15	2.80	4.03	0.74	4.35	0.80	0.95	0.17	0.57	0.11
	75-620	468	1.77	0.38	1.51	0.32	11.93	2.55	3.14	0.67	4.48	0.96	0.96	0.20	0.27	0.06
	75-621	520	1.89	0.36	1.43	0.27	14.82	2.85	3.69	0.71	3.82	0.73	0.93	0.18	0.53	0.10
	75-622	543	1.91	0.35	1.46	0.27	15.89	2.93	3.86	0.71	3.96	0.73	0.97	0.18	0.29	0.05
	75-623	438	1.81	0.41	1.29	0.29	11.02	2.52	3.45	0.79	3.80	0.87	0.89 ^a	0.20	0.36	0.08
	MEAN	489	1.85	0.38	1.39	0.29	13.25	2.69	3.57	0.73	4.00	0.83	0.92	0.19	0.40	0.08
	±S.D.	61	0.08	0.05	0.12	0.02	2.32	0.17	0.46	0.05	0.32	0.11	0.07	0.02	0.11 ^a	0.02
3	75-604	503	1.92	0.38	1.41	0.28	14.16	2.82	3.54	0.70	3.90	0.78	0.99	0.20	0.42	0.08
	75-605	453	1.85	0.41	1.29	0.29	12.74	2.81	3.37	0.74	3.92	0.86	0.94	0.21	0.33	0.07
	75-606	545	1.80	0.33	1.45	0.27	18.03	3.31	4.21	0.77	4.46	0.82	0.93	0.17	0.47	0.09
	75-607	454	1.90	0.42	1.27	0.28	11.87	2.61	3.10	0.68	4.34	0.96	0.74	0.16	0.44	0.10
	75-608	404	1.79	0.44	1.33	0.33	11.53	2.86	3.15	0.78	4.12	1.02	0.77	0.19	0.39	0.10
	75-609	544	1.72	0.32	1.45	0.27	14.56	2.67	3.79	0.70	4.06	0.75	0.86	0.16	0.44	0.08
	75-610	468	1.93	0.41	1.37	0.29	12.82	2.74	3.60	0.77	4.08	0.87	0.77	0.16	0.22	0.05
	75-611	484	1.83	0.38	1.42	0.29	15.03	3.11	3.70	0.77	3.71	0.77	0.80	0.17	0.53	0.11
	75-612	492	1.93	0.39	1.49	0.30	12.95	2.63	3.36	0.68	4.04	0.82	1.01	0.20	0.25	0.05
	75-613	466	1.79	0.39	1.37	0.29	12.07	2.59	3.01	0.65	4.77	1.02	0.92	0.20	0.33	0.07
	MEAN	481	1.85	0.39	1.38	0.29	13.57	2.81	3.42	0.72	4.14	0.87	0.87	0.18	0.38	0.08
	±S.D.	43	0.07	0.04	0.07	0.02	1.95	0.23	0.37	0.05	0.31	0.10	0.10	0.02	0.10	0.02

^aSignificantly different from control means using analysis of variance and Dunnett's test, p<0.05.

TERMINAL ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS OF FEMALE RATS AT TERMINATION OF A 119 DAY DIETARY STUDY OF 2,4,5-F

Dose mg/kg/day	Animal Number	Sexed Body Weight	Organ Weights (g and g/100 g body weight)											
			Brain			Heart			Liver			Kidneys		
			g	g/100g	g/100g	g	g/100g	g/100g	g	g/100g	g/100g	g	g/100g	g/100g
3	73-634	175	1.70	0.32	0.39	0.32	0.32	7.34	2.35	1.71	0.32	0.34	0.14	0.73
	73-635	192	1.30	0.31	0.39	0.32	0.32	7.33	2.35	1.91	0.35	0.36	0.12	0.33
	73-636	109	1.96	0.53	0.39	0.32	0.32	8.07	2.81	2.10	0.68	0.74	0.24	0.38
	73-637	294	1.35	0.63	0.31	0.31	0.31	7.56	2.37	2.04	0.99	1.05	0.36	0.71
	73-638	287	1.30	0.63	0.31	0.35	0.35	8.30	2.89	1.03	0.36	1.23	0.45	0.35
	73-639	102	1.78	0.39	1.01	0.35	0.35	7.31	2.38	2.13	0.70	0.61	0.20	0.76
	73-640	279	1.35	0.62	0.72	0.34	0.34	7.31	2.35	1.36	0.52	0.52	0.21	0.66
	73-641	288	1.73	0.52	0.34	0.29	0.29	7.34	2.35	1.98	0.69	0.34	0.19	0.34
	73-642	114	1.72	0.55	0.34	0.27	0.27	8.33	2.76	2.13	0.68	0.46	0.15	0.90
	73-643	322	1.68	0.58	1.13	0.35	0.35	8.61	2.68	2.33	0.79	0.66	0.21	0.70
	MEAN	288	1.81	0.61	0.93	0.31	0.31	7.86	2.63	1.94	0.65	0.78	0.27	0.75
	±S.D.	14	0.08	0.03	0.12	0.04	0.04	0.35	0.11	0.39	0.11	0.25	0.09	0.10
30	73-664	290	1.66	0.37	0.91	0.31	0.31	7.64	2.63	1.95	0.67	0.76	0.26	0.75
	73-665	286	1.71	0.64	0.52	0.19	0.19	6.91	2.60	1.79	0.67	0.58	0.22	0.63
	73-666	275	1.76	0.64	0.90	0.33	0.33	7.42	2.70	1.81	0.66	0.66	0.24	0.75
	73-667	286	1.94	0.68	0.79	0.28	0.28	7.57	2.65	1.91	0.67	1.14	0.40	0.67
	73-668	278	1.70	0.61	1.08	0.39	0.39	6.55	2.36	1.79	0.64	0.81	0.29	0.44
	73-669	306	1.85	0.61	1.00	0.33	0.33	7.43	2.43	2.06	0.67	0.57	0.19	0.73
	73-670	287	1.77	0.62	0.98	0.34	0.34	8.12	2.81	2.05	0.72	1.14	0.40	0.73
	73-671	293	1.39	0.64	0.63	0.22	0.22	8.48	2.89	1.94	0.66	0.57	0.20	0.54
	73-672	325	1.86	0.57	0.86	0.29	0.29	8.79	2.71	2.35	0.72	0.83	0.26	0.84
	73-673	291	1.86	0.64	0.73	0.25	0.25	8.30	2.83	2.10	0.72	0.68	0.23	0.36
	MEAN	290	1.80	0.62	0.85	0.29	0.29	7.72	2.66	1.98	0.68	0.78	0.27	0.64
	±S.D.	17	0.09	0.03	0.13	0.06	0.06	0.70	0.17	0.17	0.03	0.22	0.08	0.15
10	73-654	175	1.79	0.65	0.99	0.36	0.36	6.33	2.37	1.80	0.65	0.61	0.22	0.64
	73-655	103	1.80	0.59	0.98	0.32	0.32	8.58	2.33	1.73	0.67	0.46	0.15	0.35
	73-656	325	1.30	0.35	0.98	0.30	0.30	9.43	2.80	2.33	0.72	0.67	0.21	0.72
	73-657	257	1.74	0.53	0.90	0.34	0.34	7.86	2.36	1.94	0.72	1.07	0.40	0.96
	73-658	279	1.85	0.66	0.84	0.30	0.30	7.33	2.63	1.81	0.65	0.92	0.33	0.78
	73-659	300	1.80	0.50	0.92	0.30	0.30	7.40	2.67	1.77	0.59	0.62	0.21	0.70
	73-660	330	1.64	0.50	1.04	0.32	0.32	8.80	2.61	2.04	0.62	0.73	0.22	0.79
	73-661	288	1.73	0.65	0.87	0.32	0.32	6.30	2.34	1.66	0.62	0.61	0.23	0.58
	73-662	302	1.78	0.59	0.92	0.31	0.31	7.37	2.80	1.94	0.64	0.47	0.15	0.75
	73-663	287	1.82	0.68	0.96	0.36	0.36	7.05	2.64	1.87	0.70	0.67	0.25	0.43
	MEAN	292	1.78	0.61	0.94	0.32	0.32	7.72	2.54	1.89	0.65	0.66	0.24	0.67
	±S.D.	24	0.06	0.06	0.06	0.02	0.02	0.91	0.17	0.19	0.05	0.19	0.08	0.18
3	73-644	285	1.39	0.56	1.06	0.37	0.37	7.41	2.60	2.05	0.72	1.46	0.51	0.61
	73-645	310	1.82	0.59	1.05	0.34	0.34	8.56	2.76	2.15	0.69	0.85	0.27	0.32
	73-646	280	1.79	0.54	0.91	0.33	0.33	7.27	2.60	1.77	0.63	0.75	0.20	0.66
	73-647	282	1.77	0.53	0.96	0.34	0.34	8.24	2.92	2.04	0.72	0.85	0.30	0.76
	73-648	301	1.33	0.61	0.97	0.32	0.32	8.28	2.75	2.11	0.70	1.03	0.34	0.69
	73-649	270	1.83	0.61	1.03	0.34	0.34	8.13	2.70	1.84	0.61	0.67	0.24	0.67
	73-650	270	1.80	0.67	0.92	0.34	0.34	7.13	2.67	1.84	0.68	0.86	0.24	0.71
	73-651	294	1.79	0.61	1.07	0.36	0.36	7.80	2.66	2.11	0.72	0.70	0.24	0.74
	73-652	287	1.70	0.37	1.12	0.38	0.38	9.13	3.14	2.45	0.83	0.71	0.24	0.77
	73-653	300	1.75	0.58	1.08	0.36	0.36	9.12	3.04	2.20	0.73	0.99	0.33	0.78
	MEAN	292	1.80	0.62	1.02	0.35	0.35	8.13	2.78	2.06	0.70	0.87	0.30	0.72
	±S.D.	12	0.05	0.03	0.07	0.02	0.02	0.76	0.13	0.20	0.06	0.25	0.09	0.06

No statistically significant differences from control means using analysis of variance and Dunnett's test, $p < 0.05$.

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APPENDIX -1

TERMINAL ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS OF MALE RATS AT TERMINATION OF A TWO-YEAR DIETARY STUDY OF 2,4,5-T

Dose mg/kg/day	Animal Number	Fasted Body Weight g	Organ Weights (g and g/100 g Body Weight)													
			Brain		Heart		Liver		Kidneys		Testes		Spleen		Thymus	
			g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g
0	75-678	439	1.95	0.44	2.14	0.49	14.86	3.38	5.22	1.19	2.64	0.60	0.96	0.22	0.11	0.03
	75-679	535	1.94	0.36	2.52	0.47	17.77	3.32	6.04	1.13	4.38	0.82	1.43	0.27	0.09	0.02
	75-680	588	2.06	0.35	2.12	0.36	21.36	3.63	5.76	0.98	4.01	0.68	1.51	0.26	0.08	0.01
	75-681	499	2.08	0.42	1.93	0.39	16.13	3.23	6.55	1.31	4.23	0.85	1.34	0.27	0.15	0.03
	75-732	458	1.98	0.43	2.06	0.45	18.97	4.14	10.05 ^a	1.20 ^a	4.09	0.89	1.25	0.27	0.06	0.01
	75-748	529	1.98	0.37	1.66	0.31	17.26	3.26	5.73	1.08	4.22	0.80	1.28	0.24	0.09	0.02
	MEAN	508	2.00	0.40	2.07	0.41	17.72	3.49	5.86	1.14	3.93	0.77	1.29	0.25	0.10	0.02
	±S.D.	±43	±0.06	±0.04	±0.28	±0.07	±2.27	±0.35	±0.49	±0.11	±0.65	±0.11	±0.19	±0.02	±0.03	±0.01
30	75-861	436	2.02	0.46	1.82	0.42	16.49	3.78	5.71	1.31	3.24	0.74	1.34	0.31	0.09	0.02
	75-862	586	2.13	0.36	1.69	0.29	14.58	2.49	4.95	0.84	3.98	0.68	1.54	0.26	0.15	0.02
	75-863	331	1.97	0.60	1.84	0.56	15.32	4.63	4.32	1.31	1.07	0.32	0.56	0.17	0.11	0.03
	75-864	650	2.10	0.32	1.83	0.28	18.90	2.91	5.34	0.82	4.27	0.66	1.28	0.20	0.09	0.01
	75-867	418	1.92	0.46	1.64	0.39	11.87	2.84	3.20	0.77	2.46	0.59	0.62	0.15	0.09	0.02
	75-871	652	2.08	0.32	1.79	0.27	14.58	2.24	4.20	0.64	4.90	0.75	1.13	0.17	0.15	0.02
	75-882	638	2.06	0.32	2.06	0.32	15.83	2.48	4.30	0.67	2.88	0.45	1.18	0.18	0.10	0.01
	75-884	551	2.06	0.37	1.76	0.32	12.63	2.29	4.04	0.73	4.76	0.86	1.26	0.23	0.15	0.03
	75-885	461	1.75	0.38	1.77	0.38	25.10 ^a	5.44 ^a	5.94	1.29	3.62	0.79	1.09	0.24	0.15	0.03
	75-889	472	1.89	0.40	1.71	0.36	15.48	3.28	5.46	1.16	3.85	0.82	1.40	0.30	0.09	0.02
	75-891	598	2.10	0.35	1.87	0.31	17.19	2.87	6.11	1.02	4.74	0.79	1.80	0.30	0.11	0.02
	75-894	521	2.03	0.39	1.67	0.32	14.47	2.78	4.94	0.95	3.76	0.72	1.27	0.24	0.13	0.03
	75-904	503	1.96	0.39	1.71	0.34	14.85	2.95	5.24	1.04	4.50	0.89	1.32	0.26	0.11	0.02
	75-906	581	2.13	0.37	2.21	0.38	16.95	2.92	4.37	0.75	4.13	0.71	1.20	0.21	0.12	0.02
	75-907	612	1.98	0.32	1.83	0.30	16.73	2.73	4.68	0.76	4.12	0.67	1.25	0.20	0.11	0.02
	75-909	522	1.97	0.37	1.98	0.37	17.02	3.20	7.71	1.45	4.24	0.80	0.96	0.13	0.08	0.01
	MEAN	534	2.01	0.39	1.82 ^b	0.35	15.52	2.96	5.03	0.97	3.78	0.70	1.20	0.23	0.11	0.02
	±S.D.	±92	±0.10	±0.07	±0.15	±0.07	±1.81	±0.61	±1.05	±0.26	±0.99	±0.15	±0.30	±0.05	±0.03	±0.01
10	75-814	599	2.00	0.33	2.14	0.36	18.50	3.09	5.39	0.90	4.17	0.70	1.38	0.23	0.12	0.02
	75-819	445	2.02	0.45	2.25	0.51	18.30	4.11	5.73	1.29	4.24	0.95	1.68	0.38	0.10	0.02
	75-844	580	2.01	0.35	1.82	0.31	17.83	3.08	5.11	0.88	4.12	0.71	1.23	0.21	0.16	0.03
	75-850	503	2.00	0.40	2.10	0.42	17.74	3.53	6.56	1.32	4.22	0.84	1.23	0.24	0.08	0.02
	MEAN	532	2.01	0.38	2.08	0.40	18.09	3.45	5.72	1.10	4.18	0.80	1.38	0.27	0.11	0.02
	±S.D.	±71	±0.01	±0.05	±0.19	±0.08	±0.36	±0.49	±0.68	±0.24	±0.05	±0.12	±0.21	±0.08	±0.04	±0.01
3	75-762	474	1.98	0.42	1.71	0.36	14.20	2.99	3.83	0.81	3.41	0.72	1.00	0.21	0.09	0.02
	75-771	556	2.19	0.39	1.81	0.33	18.62	3.35	5.64	1.02	4.31	0.78	1.41	0.25	0.11	0.02
	75-773	503	2.00	0.40	1.75	0.35	17.78	3.53	7.21	1.43	4.74	0.94	1.37	0.27	0.80	0.01
	75-774	453	1.90	0.42	1.79	0.40	13.71 ^a	3.03 ^a	7.25	1.60	1.33	0.29	1.25	0.28	0.09	0.02
	75-784	573	2.11	0.37	2.07	0.36	16.43	2.87	4.46	0.78	3.60	0.63	1.14	0.20	0.16	0.03
	75-794	512	2.00	0.39	1.94	0.38	18.23	3.56	6.07	1.19	3.97	0.78	1.10	0.21	0.14	0.03
	75-795	532	2.14	0.40	2.00	0.38	15.57	2.93	4.64	0.87	4.33	0.81	1.34	0.25	0.19	0.04
	75-800	791	2.04	0.26	1.90	0.24	23.33	2.95	4.38	0.55	4.09	0.52	1.13	0.19	0.21	0.03
	75-807	593	2.05	0.35	1.88	0.32	16.55	2.79	5.27	0.89	2.78	0.47	1.05	0.18	0.12	0.02
	MEAN	554	2.04	0.38	1.87	0.35	17.59	3.12	5.42	1.02	3.62	0.66	1.24	0.23	0.13	0.02
	±S.D.	±100	±0.09	±0.05	±0.12	±0.05	±2.74	±0.31	±1.23	±0.33	±1.03	±0.20	±0.18	±0.04	±0.05	±0.01

^aWeights excluded from calculations due to tumor or mass.^bStatistically significant difference from control mean using analysis of variance and Dunnett's test, p<0.05.

APPENDIX 12

TERMINAL ORGAN WEIGHTS AND ORGAN/BODY WEIGHT RATIOS OF FEMALE RATS AT TERMINATION OF A TWO-YEAR DIETARY STUDY OF 7,4,4-T

Dose mg/kg/day	Animal Number	Fasted Body Weight g	Organ Weights (g and g/100 g Body Weight)										Spleen		Thymus	
			Brain		Heart		Liver		Kidneys		Pancreas/Ovaries					
			g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g	g	g/100g
0	75-912	179	1.34	0.48	1.13	0.30	10.33	2.36	2.35	0.75	2.26	0.60	0.33	0.22	0.20	0.05
	75-913	168	1.79	0.49	1.19	0.32	9.13	1.46	2.34	0.69	1.35	0.23	1.32	0.17	0.10	0.03
	75-914	199	1.32	0.46	1.15	0.29	12.22	1.06	3.35	0.34	0.38	0.12	0.97	0.14	0.20	0.03
	75-915	166	1.91	0.32	1.10	0.30	9.32	2.33	2.49	0.68	0.71	0.19	0.50	0.14	0.17	0.05
	75-918	338	1.75	0.32	1.20	0.35	8.28	2.43	2.51	0.77	1.12	0.69	0.98	0.29	0.10	0.03
	75-920	402	1.81	0.45	1.35	0.34	13.68	1.40	1.49	0.37	1.73	0.43	0.98	0.24	0.05	0.01
	75-926	233	1.78	0.63	1.03	0.36	7.46	2.54	2.55	0.30	1.09	0.39	0.78	0.28	0.10	0.04
	75-927	169	1.38	0.31	1.38	0.41	11.30	2.38	3.04	0.32	1.48	0.57	1.36	0.23	0.07	0.02
	75-929	282	1.79	0.47	1.42	0.37	12.50	1.27	1.92	0.76	0.52	0.16	0.33	0.22	0.09	0.02
	75-932	523	1.33	0.35	1.31	0.35	14.21	2.72	1.31	0.63	0.57	0.13	1.49	0.43	0.02	0.00
	75-935	467	1.33	0.29	1.17	0.25	11.37	2.34	1.70	0.58	1.34	0.22	0.84	0.14	0.39	0.12
	75-936	400	1.74	0.44	1.31	0.33	13.90	1.48	1.31	0.38	1.23	0.31	0.66	0.17	0.12	0.03
	75-939	426	1.31	0.42	1.13	0.27	15.19	1.36	2.55	0.62	0.61	0.14	0.75	0.18	0.06	0.01
	75-942	369	1.36	0.30	1.63	0.44	12.17	1.32	1.34	0.90	1.72	0.47	0.97	0.26	0.06	0.02
	75-943	531	1.82	0.34	1.11	0.23	13.69	1.32	2.85	0.54	0.71	0.13	0.75	0.14	0.07	0.01
	75-946	447	1.79	0.60	1.31	0.33	13.19	1.40	2.97	0.66	0.99	0.22	0.98	0.22	0.08	0.02
	75-947	304	1.85	0.61	1.36	0.45	12.47	4.11	1.49	1.15	1.13	0.37	0.74	0.25	0.19	0.08
	75-948	341	1.83	0.34	1.78	0.33	20.08	1.71	1.46	0.64	0.85	0.16	1.67	0.11	0.12	0.02
	75-951	177	1.84	0.49	1.21	0.32	8.77	2.33	2.57	0.68	0.72	0.19	0.66	0.23	0.12	0.03
	75-952	434	1.30	0.41	1.31	0.30	16.07	1.71	4.16	0.96	1.00	0.23	0.33	0.19	0.04	0.01
	75-954	372	1.79	0.31	1.52	0.27	15.56	2.74	1.27	0.37	1.15	0.20	0.32	0.14	0.07	0.01
	75-961	446	1.31	0.28	1.19	0.18	15.15	2.38	1.33	0.32	0.56	0.09	0.60	0.09	0.09	0.01
	75-965	123	1.91	0.59	1.16	0.36	10.37	1.27	1.75	1.16	0.97	0.30	0.78	0.24	0.06	0.02
75-966	532	1.79	0.32	1.36	0.25	18.60	1.37	2.42	0.44	0.53	0.11	0.65	0.12	0.06	0.01	
75-971	407	1.85	0.45	1.50	0.34	13.48	1.31	3.01	0.74	0.75	0.19	0.96	0.23	0.07	0.02	
75-972	412	1.87	0.45	1.09	0.27	10.00	2.43	3.08	0.75	1.45 ^a	0.84 ^a	1.25	0.30	0.37	0.02	
75-973	274	1.30	0.46	1.41	0.51	11.47	4.19	1.63	1.33	5.32 ^a	1.02 ^a	0.98	0.36	0.12	0.04	
75-980	532	1.71	0.32	1.14	0.21	13.33	2.51	2.40	0.45	2.92 ^a	0.55 ^a	0.51	0.10	0.34	0.01	
75-981	324	1.70	0.32	0.76	0.30	10.33	1.19	2.97	0.32	0.74	0.23	0.53	0.16	0.06	0.02	
75-985	305	1.73	0.34	1.13	0.22	16.95	1.35	2.81	0.36	0.77	0.15	0.69	0.14	0.08	0.02	
75-987	420	2.10	0.50	1.31	0.31	11.22	2.67	2.96	0.70	1.15	0.27	0.71	0.17	0.15	0.04	
75-989	492	1.38	0.38	1.19	0.24	19.10	1.38	1.15	0.54	0.33	0.17	0.79	0.16	0.08	0.02	
75-991	463	1.32	0.39	1.28	0.28	24.22	5.23	1.51	0.76	0.66	0.14	0.87	0.19	0.08	0.02	
75-992	276	1.86	0.57	1.05	0.38	9.43	1.06	1.24	0.78	0.44	0.18	0.50	0.22	0.07	0.02	
75-993	175	1.97	0.71	1.11	0.40	5.89	2.14	2.13	0.79	0.32	0.12	0.58	0.21	0.08	0.03	
75-995	497	1.76	0.35	1.22	0.25	12.90	1.50	2.42	0.49	1.13	0.23	0.87	0.17	0.09	0.02	
MEAN	419	1.32	0.46	1.28	0.32	13.07	3.12	3.00	0.75	0.99	0.25	0.56	0.21	0.09	0.02	
S.D.	293	±0.07	±0.11	±0.20	±0.07	±3.92	±0.64	±0.67	±0.20	±0.43	±0.14	±0.36	±0.08	±0.04	±0.01	
30	75-1097	501	1.33	0.37	1.25	0.25	16.39	1.37	3.05	0.51	0.67	0.13	1.07	0.21	0.10	0.02
	75-1098	324	1.72	0.32	1.34	0.40	15.31	4.39	1.55	1.09	1.11	0.33	1.03	0.31	0.07	0.02
	75-1100	164	1.32	0.30	1.29	0.35	10.06	2.76	2.34	0.70	1.21	0.33	0.74	0.20	0.11	0.03
	75-1104	377	1.31	0.48	1.08	0.29	12.39	1.33	1.02	0.80	1.22	0.32	0.76	0.20	0.09	0.02
	75-1108	402	1.76	0.44	1.38	0.34	9.12	2.27	2.72	0.68	1.30	0.32	0.35	0.21	0.04	0.01
	75-1112	428	1.30	0.42	1.33	0.31	13.17	1.07	3.22	0.75	1.55	0.36	0.90	0.21	0.15	0.04
	75-1115	434	1.89	0.44	1.25	0.29	16.31	1.87	2.60	0.60	0.33	0.19	1.06	0.24	0.09	0.02
	75-1116	407	1.76	0.43	1.12	0.27	14.31	1.32	2.72	0.67	2.45	0.60	0.66	0.16	0.08	0.02
	75-1117	377	1.37	0.30	1.40	0.37	13.87	1.68	3.51	0.93	1.13	0.30	0.77	0.20	0.15	0.04
	75-1119	206	1.87	0.35	1.26	0.37	9.54	2.34	3.36	1.30	0.30	0.21	1.15	0.34	0.05	0.01
	75-1121	450	1.81	0.40	1.33	0.30	12.08	2.68	2.83	0.63	0.38	0.20	0.95	0.21	0.20	0.04
	75-1123	431	1.72	0.27	1.20	0.19	12.75	1.02	2.70	0.43	0.71	0.11	0.66	0.10	0.06	0.01
	75-1124	291	1.90	0.45	1.30	0.45	8.76	1.01	3.11	1.37	1.08	0.37	0.72	0.25	0.39	0.03
	75-1130	437	1.84	0.40	1.15	0.25	13.36	2.36	2.98	0.55	0.37	0.12	0.54	0.12	0.05	0.01
	75-1132	343	1.82	0.33	1.23	0.36	11.45	1.34	3.17	0.92	0.70	0.20	0.86	0.25	0.06	0.02
	75-1133	448	1.91	0.43	1.29	0.29	12.71	2.34	2.79	0.62	0.35	0.19	0.75	0.17	0.10	0.02
	75-1134	318	1.88	0.59	1.15	0.36	8.64	2.72	2.55	0.30	1.30	0.57	0.93	0.30	0.12	0.04
	75-1138	440	1.37	0.43	1.34	0.30	12.39	1.32	3.27	0.74	0.66	0.15	1.16	0.26	0.10	0.02
	75-1143	426	1.91	0.41	1.19	0.23	14.96	1.51	3.06	0.72	0.81	0.19	0.59	0.14	0.07	0.02
	75-1144	527	1.36	0.35	1.50	0.21	14.31	2.75	2.59	0.49	0.30	0.15	0.34	0.16	0.05	0.01
	75-1145	334	1.97	0.59	1.38	0.41	10.60	3.17	3.59	1.07	1.25	0.67	0.33	0.25	0.09	0.03
	MEAN	411	1.33	0.46	1.26	0.32	12.55	3.10	3.00	0.76	1.11	0.29	0.85	0.21	0.09	0.02
	S.D.	280	±0.06	±0.09	±0.10	±0.07	±2.47	±0.37	±0.35	±0.19	±0.31	±0.16	±0.18	±0.06	±0.04	±0.01
10	75-1046	445	1.78	0.40	1.36	0.31	10.42	2.34	3.18	0.72	1.23	0.23	0.97	0.22	0.10	0.02
	75-1047	391	1.82	0.47	1.46	0.37	11.58	2.36	2.71	0.69	0.30	0.20	1.14	0.29	0.08	0.02
	75-1054	313	1.77	0.57	1.20	0.38	11.73	5.75	1.73	1.19	1.12	0.36	0.79	0.25	0.30	0.03
	75-1059	119	1.82	0.57	1.10	0.35	8.31	2.76	1.76	0.37	1.62	0.51	0.71	0.22	0.10	0.03
	75-1061	395	1.89	0.48	1.27	0.32	12.41	1.34	2.84	0.72	1.16	0.29	0.96	0.24	0.08	0.02
	75-1070	427	1.88	0.44	1.19	0.28	11.78	2.76	2.70	0.53	0.83	0.19	0.80	0.19	0.11	0.03
	75-1072	323	1.87	0.58	1.01	0.31	8.57	2.66	2.52	0.78	1.38	0.43	0.73	0.23	0.16	0.05
	75-1086	414	1.85	0.45	1.33	0.32	10.28	2.48	3.02	0.73	0.71	0.17	0.82	0.20	0.17	0.04
	75-1091	351	1.87	0.53	1.28	0.36	9.48	2.70	2.81	0.30	1.16	0.33	0.82	0.23	0.12	0.03
	75-1092	493	1.32	0.37	1.86	0.34	14.20	2.88	3.40	0.73	0.62	0.13	0.91	0.18	0.23	0.03
	75-1095	410	1.45	0.45	1.15	0.23	12.52	3.05	1.74	0.67	1.35	0.33	0.72	0.18	0.07	0.02
	MEAN	389	1.34	0.48												

Ex.E-1

RESULTS OF A TWO-YEAR CHRONIC TOXICITY AND ONCOGENIC STUDY OF RATS INGESTING DIETS CONTAINING 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T)

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(Received 27 December 1978)

Abstract—Groups of Sprague-Dawley rats (50 males and 50 females) were maintained on diets supplying 1, 10 or 30 mg 2,4,5-T/kg body weight/day for up to 2 yr, with an interim autopsy (on an additional ten males and ten females per group) after 118–119 days. The highest dose level was associated with some degree of toxicity, including a decrease in body-weight gain and increases in relative kidney weight, in the volume of urine excreted and in the urinary excretion of coproporphyrin and uroporphyrin, plus slight morphological changes in the kidney, liver and lungs. The kidney changes involved, primarily, the presence of mineralized deposits in the renal pelvis. Parameters not adversely affected by this dose level included death rate, food consumption, the occurrence of palpable masses, haematological indices (red-cell count, haemoglobin, packed cell volume, total and differential white-cell counts, thrombocytes and reticulocytes), the results of routine urine analyses, urinary excretion of creatinine and δ -aminolaevulinic acid, serum-chemistry values (urea nitrogen, glutamic-pyruvic-transaminase and alkaline-phosphatase activities, bilirubin, total protein, albumin and globulin), weights of organs other than the kidneys, tumour incidence and gross and microscopic morphology of all the organ systems examined, with the exception of those mentioned above. At the intermediate dose level (10 mg/kg/day) only minimal effects were noted, primarily an increased incidence of mineralized deposits in the renal pelvis and, in the males and only during the early phase of the study, an increase in urinary excretion of coproporphyrin. At the lower dose level (3 mg/kg/day) there were no changes that were considered to be related to treatment throughout the 2-yr period. Thus, this study revealed no oncogenic response in rats, even when the duration of 2,4,5-T administration extended over most of their lifespan at a dosage high enough to induce toxicity.

INTRODUCTION

2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) has been used about 25 yr as a selective herbicide in the management of certain crops, pasture, rangeland and forests. In the production of 2,4,5-T, the starting material for the aromatic portion of the compound is tetrachlorobenzene, which is subsequently hydrolysed to 2,4,5-trichlorophenol. In the production of the trichlorophenol, several impurities may be formed under unfavourable conditions. One of these impurities is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which may be found at low levels in the trichlorophenol. Over the past decade, a concerted effort has been made to minimize the level of TCDD in 2,4,5-T, and commercial 2,4,5-T currently contains no more than 0.1 ppm TCDD.

Various toxicological studies have been conducted with 2,4,5-T over the past 25 yr. The single oral dose LD_{50} of 2,4,5-T has been reported to be 389 mg/kg in mice, 381 mg/kg in guinea-pigs, 500 mg/kg in rats and 310 mg/kg in chicks (Rowe & Hymas, 1954). The LD_{50} in dogs of 160 mg/kg, reported by Ditt & Hiratzka (1953), is lower than in other species, most probably because of their slower rate of urinary elimination of organic acids such as 2,4,5-T (Piper, Rose, Long & Gehring, 1973).

The compound 2,4,5-T is readily absorbed and rapidly excreted by mammals, including man. The half-life values for the clearance of ^{14}C activity from the plasma of rats given single oral doses of labelled 2,4,5-T at doses of 5, 50, 100 and 200 mg/kg were 4.7, 4.2, 19.4 and 25.2 hr, respectively (Piper *et al.* 1973). The half-lives for elimination from the body of these rats were 13.6, 13.1, 19.3 and 28.9 hr, respectively. In dogs given ^{14}C -labelled 2,4,5-T at 5 mg/kg, half-life values for clearance from the plasma and clearance from the body were 77.0 and 86.6 hr, respectively (Piper *et al.* 1973). This lower rate of clearance probably explains the greater toxicity of 2,4,5-T in dogs than in rats. When five human volunteers ingested 2,4,5-T, containing less than 0.05 ppm TCDD, in a dose of 5 mg/kg (Gehring, Kramer, Schwetz, Rose & Rowe, 1973), essentially all the 2,4,5-T was absorbed and excreted unchanged in the urine, with a half-life of 23.1 hr. This rate of excretion resembles more closely that observed in the rat than that in the dog.

In an unpublished subacute toxicity study conducted previously by this laboratory, no adverse effects were observed in rats fed 2,4,5-T at dose levels of 3 or 10 mg/kg/day for 90 days. At higher dose levels (30 or 100 mg/kg/day), there were increases in liver and kidney weights, elevations in serum enzymes

and other indications of slight toxicity. Drill & Hiratzka (1953) found no adverse effects in dogs given 2.5 or 10 mg 2,4,5-T/kg for 5 days/wk for 3 months; four dogs could not tolerate a higher dose level of 20 mg/kg and died during the experiment.

Highman, Gaines & Schumacher (1976) reported results of short-term toxicity studies in mice using samples of 2,4,5-T with varying contents of TCDD. These studies, which were conducted in conjunction with teratology studies, demonstrated multiple toxic effects from treatment with doses of 120 or 60 mg 2,4,5-T/kg; lower dose levels were not studied.

Smith, Schwetz, Murray, Crawford, John, Kociba & Humiston (1973) recently reported a three-generation reproduction study in rats ingesting 2,4,5-T. No adverse effects were seen at a dose level of 3 mg/kg/day. Higher dose levels of 2,4,5-T which were sufficient to cause signs of toxicity had no effect on the reproductive capacity of the rats, except for a tendency toward a reduction in neonatal survival at dose levels of 10 and 30 mg/kg/day.

Several studies have been conducted in mice over the past 10 yr to assess the potential oncogenicity of 2,4,5-T. Innes, Ulland, Valerio, Petrucelli, Fishbein, Hart, Palotta, Bates, Falk, Gart, Klein, Mitchell & Peters (1969) reported no increase in the incidence of tumours in two strains of mice given the maximum tolerated dose (21.5 mg/kg/day) of 2,4,5-T (containing approximately 30 ppm TCDD) by stomach tube from day 7 to day 28 of age. After the mice were weaned at 28 days of age, 2,4,5-T was mixed directly in the diet at a concentration of 60 ppm for approximately 18 months. In a study conducted for the National Cancer Institute by the Bionetics Research Laboratories (1968) but never published, weanling mice (two strains) were given a single dose of 215 mg 2,4,5-T/kg by sc injection and were observed for the following 18 months. The authors concluded that there was no increase due to 2,4,5-T in the incidence of tumours.

In an initial publication, Muranyi-Kovacs, Rudali & Imbert (1976) reported results of their study of two inbred strains of mice (C3Hf and XVIIG) given 2,4,5-T (100 mg/litre) in the drinking-water for 2 months from 6 wk of age. The mice were subsequently fed diets containing 80 ppm 2,4,5-T (approximately 12 mg/kg/day) for the rest of the lifetime study. On the basis of their method of combining tumour types, these authors reported a statistically significant increase in tumour incidence in C3Hf mice following 2,4,5-T treatment, but no significant differences in tumour incidence in the mice of the XVIIG strain. In a subsequent communication, Muranyi-Kovacs, Rudali & Imbert (1977) reported that no increase in tumours was noted in other studies in which they gave the same two strains of mice a series of neonatal sc injections of 2,4,5-T. Muranyi-Kovacs *et al.* (1976) stated, however, that the mouse may not be the best experimental model for testing the carcinogenicity of 2,4,5-T and recommended further testing in greater numbers of animals and in other species, such as the rat.

In order to evaluate the long-term toxicity and oncogenic potential of 2,4,5-T in rats, the study reported here was conducted. It involved the dietary administration of 3, 10 or 30 mg 2,4,5-T/kg/day to rats for up to 2 yr.

EXPERIMENTAL

Groups of 100 rats (50 males and 50 females) were maintained for up to 2 yr on diets supplying 3, 10 or 30 mg 2,4,5-T/kg body weight/day. The control group for this part of the study consisted of 86 males and 86 females. An additional ten rats of each sex were included for each treatment and control group for the interim kill on days 118 and 119.

Test material. The purified sample of 2,4,5-T was supplied by The Dow Chemical Company, Midland, MI. The identity of the 2,4,5-T was verified, prior to the initiation of the study, by elemental analysis, gas chromatography, solid-probe mass spectrometry, gas chromatography-mass spectrometry, nuclear magnetic resonance spectrometry and infra-red spectrometry. Within the limits of precision of each of these techniques, the material was essentially 2,4,5-trichlorophenoxyacetic acid. Assay by standard procedures indicated approximately 99% 2,4,5-trichlorophenoxyacetic acid. Other phenoxy acid impurities amounted to 1.3% (w/w) by gas chromatography. Using gas chromatography-mass spectrometry, TCDD, hexachlorodibenzo-*p*-dioxin, heptachlorodibenzo-*p*-dioxin and octachlorodibenzo-*p*-dioxin were not detected, the limits of detection being 0.33, 0.12, 0.40 and 0.40 ppb ($\mu\text{g/kg}$), respectively. The recrystallization solvent tetrachloroethylene, was present at a level of 0.22%.

Diet preparation. The test diets were prepared by dissolving an appropriate amount of 2,4,5-T in reagent-grade acetone and mixing the solution thoroughly with ground laboratory chow to give a 1% premix. This premix, after drying, was mixed with rat chow to prepare diets supplying 3, 10 and 30 mg 2,4,5-T/kg body weight/day. The body weights and food consumption of 20 rats of each sex per group were used as the basis for preparing these diets. Control rats were supplied with ground laboratory chow treated with an equivalent amount of acetone. Portions of each prepared diet from each dose level were collected during months 5, 7, 12, 19 and 23 and analysed for 2,4,5-T.

Experimental design. Male and female Sprague-Dawley rats, 7-8 wk old (from Spartan Research Animals, Haslett, MI) were randomly placed (using a table of random numbers) in suspended wire-bottomed cages (two rats/cage) for a 118/119-day and a 2-yr chronic toxicity study. Individual rats were identified by metal ear tags and dose levels were also identified by toe-clipping. Food (Purina Laboratory Chow, Ralston-Purina Company, St. Louis, MO) and water were available *ad lib*.

Clinical observations. Rats were observed for their general state of health and for possible toxicological responses during the study, observations being recorded when the rats were weighed. From month 6, observations were also recorded at each monthly examination for the detection of palpable masses. Body weights were recorded at approximately weekly intervals for all the rats scheduled for the interim kill at 118/119 days and also for 20 rats of each sex in the control and test groups during the first 3 months of the 2-yr part of the study. Thereafter, body weights of the latter rats were recorded at approximately monthly intervals. Food consumption was recorded

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approximately twice weekly for all the rats scheduled for the interim kill and during the first 4 months of the 2-yr study for 20 rats of each sex from each group. For the rest of the 2-yr study food consumption was recorded for up to 20 rats of each sex per group for 1 wk/month.

Blood samples for haematological determinations were collected from the tail veins of eight rats of each sex and group, on days 82/83 for the rats scheduled for the interim kill and on days 89/90, 364/365 and 726/727 for those in the 2-yr study (when group sizes permitted). The total erythrocyte count, total and differential leucocyte counts, thrombocyte and reticulocyte counts, packed cell volumes and haemoglobin concentrations were determined using automated techniques (Coulter Counter Model ZBI, from Coulter Electronics, Hialeah, FL) or manual procedures.

Urine samples were collected from the same numbers of animals and at the same times as the blood samples. The specific gravity and pH of the urine and the presence or absence of glucose, protein, ketones, bilirubin and occult blood were determined (Ames Bililabstix or Multistix, from Ames Company, Elkhart, IN; TS Meter, from AO Optical, Buffalo, NY) at each of these times, and urinary urobilinogen was determined on days 364/365 and 726/727 of treatment.

Urinary levels of creatinine, coproporphyrin and uroporphyrin with or without δ -aminolaevulinic acid (δ -ALA) were determined by Bio-Science Laboratories, Van Nuys, CA. Rats were housed individually in metabolism cages for collection of 24- or 48-hr urine samples, which were transported in glass jars wrapped in aluminium foil and packed in dry ice. Urine samples were collected from five rats of each sex and group on three occasions before day 106 of treatment and once just prior to the interim kill in the subsidiary study, and from four or five rats of each sex and group after 95-97, 188-190, 361-363, 564-566 and 698-700 days of the 2-yr treatment. Urine volumes were recorded for each individual rat.

At both the interim and terminal autopsies, serum samples were collected from a maximum of ten rats of each sex and group for the determination of urea nitrogen (BUN), activities of glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (SAP), total, direct and indirect bilirubin, and total protein, albumin and globulin. Automated procedures were used for these determinations (Technicon Auto-Analyzer, Technicon Corporation, Rye, NY).

Post mortem examination. Rats scheduled for the interim autopsy were deprived of food overnight after treatment for 118 or 119 days and then killed by decapitation. The eyes of all rats were examined, by means of a glass slide pressed gently against the cornea under bright fluorescent illumination. The eyes from a maximum of five rats of each sex and group were preserved in Zenker's fixative and those from the remaining rats were fixed in 10% buffered formalin. A complete gross pathological examination was performed on all the rats by a veterinary pathologist. Representative sections of all major organs and tissues, including liver, kidneys, heart, pancreas, spleen, brain (cerebrum, cerebellum and brain stem), spinal cord, peripheral (sciatic) nerve, pituitary gland,

stomach, small and large intestines, mesenteric lymph node(s), skeletal (thigh) muscle, salivary gland, testes, epididymis, accessory male sex glands, urinary bladder, uterus, ovary, trachea, oesophagus, aorta, thoracic lymph node(s), thymus, lungs, bronchi, integument, thyroid gland, parathyroid glands, adipose tissue, eyes, adrenal gland(s), and any grossly observed lesion, were preserved in formalin fixative. The weights of the liver, kidneys, brain, heart, thymus, spleen and testes or ovaries/uterus (as a unit) were recorded for all the rats.

All rats dying or killed during the course of the 2-yr study were also subjected to a gross pathological examination. Representative parts of the skull (including nasal turbinates and ear canal) along with any gross lesions suggestive of a significant pathological process of tumour formation were collected from each rat and preserved in formalin fixative.

A terminal autopsy was conducted on all rats surviving the 2-yr period of treatment. The organs and tissues weighed and preserved and the procedures used for these animals and for those autopsied during the 2-yr period were as described for the interim autopsies. At the terminal autopsy, smears of peripheral blood and femoral bone marrow were prepared for most rats, and filed for future reference if needed. Portions of fat, liver and kidney for possible 2,4,5-T analysis were saved from a maximum of five rats of each sex and group at both the interim and terminal autopsies.

Histological examination of tissues. Paraffin-embedded sections of tissues were stained with haematoxylin and eosin. Sections of the preserved tissues from the control and high-dose groups killed on days 118/119 and from all treated and control rats dying or killed during the 2-yr study or killed at termination were subjected to histological examination, but those taken from the lower-dose groups at the interim kill were not examined, because of a lack of any definite treatment-related effects in the high-dose group. Additional sections of liver and kidney from most control and top-dose rats killed after 118/119 days were stained for lipid content using Oil Red O. Additional sections of kidneys taken from five to seven female rats from the control and high-dose groups at the terminal kill were stained with Oil Red O, by Mallory's reaction for iron, with Ziehl Neelsen stain for acid fastness, by Dahl's method for calcium and by the periodic-acid-Schiff reaction (Luna, 1968).

Statistical evaluation of data. Haematological, urinary and clinical chemistry parameters, body weights, organ weights and organ/body weight ratios were analysed statistically by a one-way analysis of variance followed by Dunnett's Test, with $P < 0.05$ (Steel & Torrie, 1960). Data on mortality, palpable masses, gross pathology, histopathology and tumour incidence in the rats of the 2-yr study were analysed using Fisher's Exact Probability, $P < 0.05$, one-sided test (Siegel, 1956). For gross pathological observations, statistical evaluation of the cumulative findings for the entire 2-yr study involved comparison of the data of each of the treatment groups against those of the control group of that sex. The data were inspected and those suggestive of a difference from the control were analysed statistically. For histopathological observations and tumour incidences, the cumulative

Table 1. Summary of body-weights, clinical observations and organ weights in rats fed diets containing 2,4,5-T for up to 2 yr†

Dose of 2,4,5-T (mg/kg/day)	No. of rats/ group†	Body weight (g) on day				Urinary excretion					Kidney weights: 118/119- day study (g/100 g body weight)
		26	53	95	314	Termination of 118/119-day study		Termination of 2-yr study			
						Total 48-hr urine vol. (ml)	Copro- porphyrin (µg/48 hr)	Uro- porphyrin (µg/48 hr)	Copro- porphyrin (µg/48 hr)	Uro- porphyrin (µg/48 hr)	
0	86 ± 10	385 ± 21	444 ± 30	498 ± 36	627 ± 53	Males					0.70 ± 0.09
3	50 ± 10	385 ± 25	447 ± 34	503 ± 41	631 ± 59	38 ± 8	17.5 ± 4.9	4.1 ± 1.8	14.6 ± 6.0	4.7 ± 1.8	0.72 ± 0.05
10	50 ± 10	386 ± 25	449 ± 36	508 ± 43	635 ± 59	40 ± 10	23.1 ± 4.2	4.5 ± 2.0	15.2 ± 3.6	3.0 ± 0.9	0.73 ± 0.05
30	50 ± 10	375 ± 21	437 ± 30	492 ± 38	616 ± 50	36 ± 14	24.6 ± 9.6*	5.0 ± 3.1	9.7 ± 4.4	3.0 ± 1.3	0.73 ± 0.05
						49 ± 11*	43.6 ± 9.3*	7.7 ± 4.7*	22.8 ± 13.0	4.0 ± 1.5	0.79 ± 0.06*
0	86 ± 10	253 ± 14	278 ± 16	301 ± 17	361 ± 28	Females					
3	50 ± 10	254 ± 12	279 ± 15	306 ± 17	363 ± 26		9.6 ± 1.9		5.8 ± 1.9		
10	50 ± 10	253 ± 15	273 ± 15	299 ± 17	365 ± 28	NE	10.3 ± 3.5	NE	6.5 ± 2.3	NE	NE
30	50 ± 10	244 ± 12*	266 ± 15*	288 ± 17*	347 ± 27*	NE	9.2 ± 0.8	NE	9.8 ± 2.3	NE	NE
						NE	16.4 ± 7.0*	NE	8.0 ± 9.8	NE	NE

NE = No treatment-related effect

†Selected to depict the effects considered to be related to treatment.

‡In 2-yr + 118/119-day studies.

Values are means ± SD and those marked with an asterisk differ significantly (by analysis of variance and Dunnett's Test) from control values: *P < 0.05.

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data of each dose group were compared with those of the control group of that sex. The exact number of tissues examined was used as the total group size in each analysis performed. The data were inspected and those suggestive of a statistical difference from the control were analysed.

RESULTS

Dietary content of 2,4,5-T

The results of analyses of feed samples on five occasions indicated that the dosage levels of 3, 10 and 30 mg 2,4,5-T/kg/day equated with approximately 85, 230 and 677 ppm of 2,4,5-T in the diet. Repeated analyses of the feed samples indicated generally good agreement between the intended and the analytically measured concentrations of 2,4,5-T. On one occasion, analysis of the diet for the group of rats given 3 mg/kg/day indicated a somewhat higher than expected 2,4,5-T content, but this was considered to have a negligible impact on the outcome of the study.

Body weights

There were no statistically significant differences from control means in the mean body weights of any of the treated male and female rats in the 113/119 day study. Mean body weights of females given 30 mg 2,4,5-T/kg/day showed a statistically significant decrease four times during the course of the 2-yr study (Table 1), and those of the males on this dose showed a trend towards slightly lower values during most of the 2-yr period. Males and females given 3 or 10 mg 2,4,5-T/kg/day did not differ statistically from the control body weights, except at one period when the mean body weights of males given 10 mg 2,4,5-T/kg/day were statistically increased. This isolated observation was considered of no toxicological significance.

Food consumption data

There were no consistent deviations in the rate of the food consumption of males or females given any of the three dose levels during the course of either the 113/119-day or the 2-yr study. The few sporadic cases in which there was a statistical increase or decrease in food consumption between the control and various treatment groups followed no consistent trend, and were considered of no toxicological significance.

Mortality

The incidence of deaths in the group of male rats given 30 mg 2,4,5-T/kg/day showed a statistically significant decrease during the latter part of the 2-yr study, a finding considered to be secondary to the less severe chronic renal disease in these rats. The mortality in the females given this high dose level showed no difference from the control pattern. Four or five times during the study there were statistically significant increases in deaths in males and females given 10 mg 2,4,5-T/kg/day, while males given 3 mg/kg/day showed statistically significant decreases at four intervals. Females on this low dose level showed no differences from control values. These differences in mortality patterns in rats given 3 or 10 mg 2,4,5-T/kg/day showed no dose-related effects and were considered to be of no toxicological significance.

Palpable masses and animal observations

The first palpable mass was noted in a male rat of the control group. There were no statistically significant differences between control and treated groups of male rats except during months 13 and 14, when the group given 10 mg 2,4,5-T/kg/day had an increased incidence of palpable masses. This was considered of no toxicological significance, because of its isolated occurrence and lack of a dose- or temporal-response relationship. There were no differences in the incidence rates of palpable masses in groups of treated females except for statistically significant increases during months 9 and 10 in the group receiving 30 mg/kg/day and during month 14 in that group receiving 10 mg/kg/day. These observations were considered of no toxicological significance, since they were isolated occurrences and did not occur during the latter phases of the study. Examination of the rats during the course of the 2-yr study revealed no overt clinical evidence of toxicity attributable to these three levels of treatment with 2,4,5-T.

Haematology and urine analyses

Repetitive evaluation of all haematological parameters revealed no statistical differences from control values at any dose level at any time during the course of the subchronic or chronic parts of the study. Routine urine analyses also revealed no changes considered to be related to ingestion of 2,4,5-T. On one occasion there was a statistical decrease in the specific gravity of the urine of male rats given 10 mg 2,4,5-T/kg/day, but because of its isolated occurrence and lack of a dose response this was considered to be of no toxicological significance.

Repetitive evaluation of urinary porphyrins did reveal observations that were considered to be related to the higher dose levels of treatment (Table 1). In males receiving 30 mg 2,4,5-T/kg/day, there were statistically significant increases in total urine volume and in the excretion of coproporphyrin and uroporphyrin. In males receiving 10 mg/kg/day, there was a significant increase in coproporphyrin excretion, but only in the early phase of the study. In females receiving 30 mg 2,4,5-T/kg/day, excretion of coproporphyrin was increased at most of the sampling times; excretion of uroporphyrin showed a statistically significant decrease after 119 days but an increase after 366 days. No significant differences in total urinary volume or in the excretion of creatinine, coproporphyrin, uroporphyrin or δ -ALA were recorded for male rats on 3 mg/kg/day or for females on 3 or 10 mg/kg/day at any time during the study.

Clinical chemistry

Terminal analyses of serum samples for BUN, SGPT, SAP, bilirubin (total, direct and indirect), total protein, albumin and globulin after 113/119 days or 2 yr showed no alterations considered to be related to 2,4,5-T treatment at any dose level. SGPT showed a statistically significant increase in the group of females given 10 mg/kg/day for 119 days, but this was considered to be of no toxicological significance, in view of its isolated nature, the lack of dose response and the absence of any similar finding at the end of the 2-yr study.

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Table 2. Summary of major gross and histopathological observations in rats maintained on diets containing 2,4,5-T for 2 yr†

Lesion	Dose (mg/kg. day)...	No. of rats affected/no. examined							
		Males				Females			
		0	3	10	30	0	3	10	30
Kidney									
Mineralized deposits in pelvis			NE	NE	NE	1/86	1/50	5/50*	8/50*
Localized reaction adjacent to mineralized deposits			NE	NE	NE	25/86	19/50	22/50	37/50*
Moderate to severe chronic renal disease	56/86	28/50	30/50	22/50*	6/86	6/50	2/50	1/50	
Increased pigment in renal tubular cytoplasm			NE	NE	NE	0/86	0/50	0/50	11/50*
Mineralization secondary to renal disease seen in:									
pulmonary alveoli	13/86	8/50	4/50	0/50*		NE	NE	NE	
myocardium	5/86	3/50	2/50	0/50		NE	NE	NE	
myocardial blood vessels	12/86	6/50	5/50	0/50*		NE	NE	NE	
gastric mucosa and muscularis	25/85	13/49	9/49	1/50*		NE	NE	NE	
Liver									
Enlargement	11/86	3/50	7/50	1/50*		NE	NE	NE	
Focal biliary hyperplasia	9/86	6/50	8/50	14/50*		NE	NE	NE	
Periportal inflammation	29/86	7/50*	9/50	23/50*		NE	NE	NE	
Focal aggregates of reticulo-endothelial cells adjacent to degenerate or necrotic hepatocytes			NE	NE	NE	36/86	17/50	21/50	34/50*
Multiple foci of hepatocellular alteration (swollen hepatocytes)			NE	NE	NE	15/86	4/50	6/50	3/50*
Focal hepatocellular cytoplasmic vacuolization	34/86	20/50	12/50*	8/50*		NE	NE	NE	
Lungs									
Focal interstitial inflammation	21/86	5/50*	5/50*	27/50*		NE	NE	NE	
Focal accumulations of alveolar macrophages in alveoli	23/86	16/50	13/50	29/50*		NE	NE	NE	
Focal cholesterol clefts	6/86	4/50	5/50	9/50*		NE	NE	NE	
Focal accumulations of secretory material in alveoli			NE	NE	NE	0/86	2/50	2/50	4/50*
Cardiovascular system									
Dilated/flaccid ventricle	28/86	15/50	7/50	9/50	0/86	1/50	2/50	3/50*	
Periarteritis	20/86	12/50	8/50	8/50		NE	NE	NE	
Left atrial thrombosis	12/86	6/50	4/50	3/50		NE	NE	NE	

NE = No treatment-related effect

†Selected from the results of examination of all organ systems to depict the effects considered to be related to treatment.

The tumour incidence in all the organ systems was considered to be unaffected by any level of treatment.

Values marked with an asterisk differ significantly (by Fisher's Exact Probability Test) from the corresponding control incidence: * $P < 0.05$.

Organ weights

A statistically significant increase in the relative weight of the kidneys of male rats killed after being given 30 mg 2,4,5-T/kg/day for 118 days was considered to be related to treatment (Table 1). No other organ-weight changes were noted at the interim autopsies. At the terminal autopsy after the 2-yr treatment, the only statistically significant difference was a decrease in the absolute heart weight of males given 30 mg/kg/day; this was considered to be of highly questionable toxicological significance because of an absence of any discernible gross or histopathological changes in these hearts. No alterations considered to be related to treatment were found in the weights of brain, liver, testes, spleen, thymus or uterus/ovaries at any time or at any dose level.

Gross and histopathological observations

The major pathological observations considered to

be related to 2,4,5-T treatment are summarized in Table 2. Full details of the gross and histopathological findings for both the 2-yr and the shorter study are on file with the authors.

The kidneys of female rats receiving 30 mg 2,4,5-T/kg/day showed gross and microscopic evidence of an increased incidence of mineralized deposits in the renal papillae or pelvis (Fig. 1); these mineralized deposits were sometimes accompanied by a localized reaction of the adjacent renal-pelvis epithelium. Other renal effects attributed to this dose of 2,4,5-T included an increased content of pigment (Fig. 2) within the cytoplasm of the proximal convoluted tubular epithelial cells (in females), and a decrease in the severity of the chronic nephropathy (Fig. 3) that normally occurs spontaneously, especially in the males, in this strain of rat. The special stains applied to sections of kidneys of the control and high-dose groups indicated similar staining reactions for both groups.

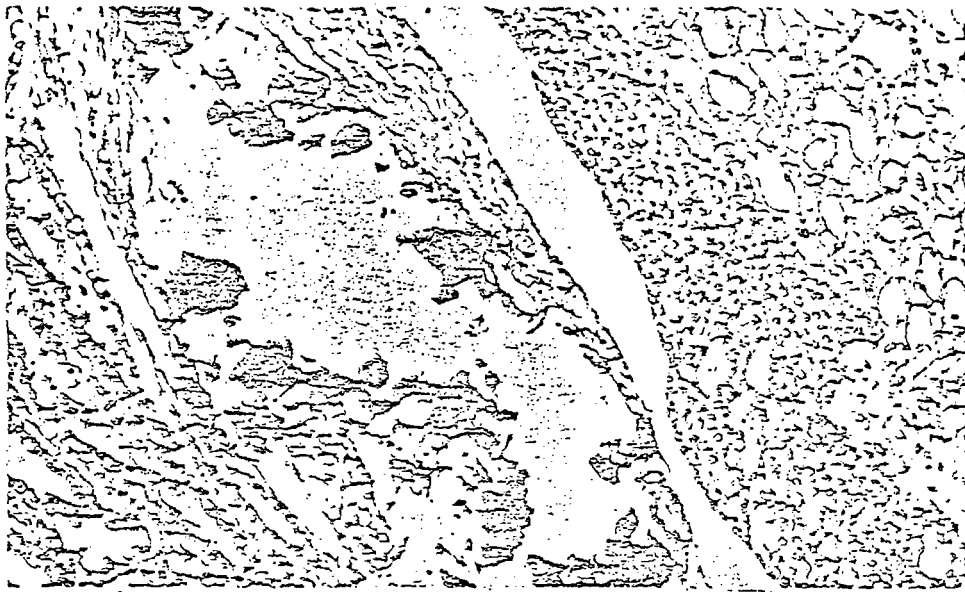


Fig. 1. Mineralized deposit in the renal papilla adjacent to the renal pelvis of the kidney of a rat given 30 mg 2,4,5-T/kg/day for 2 yr. Haematoxylin and eosin $\times 100$.

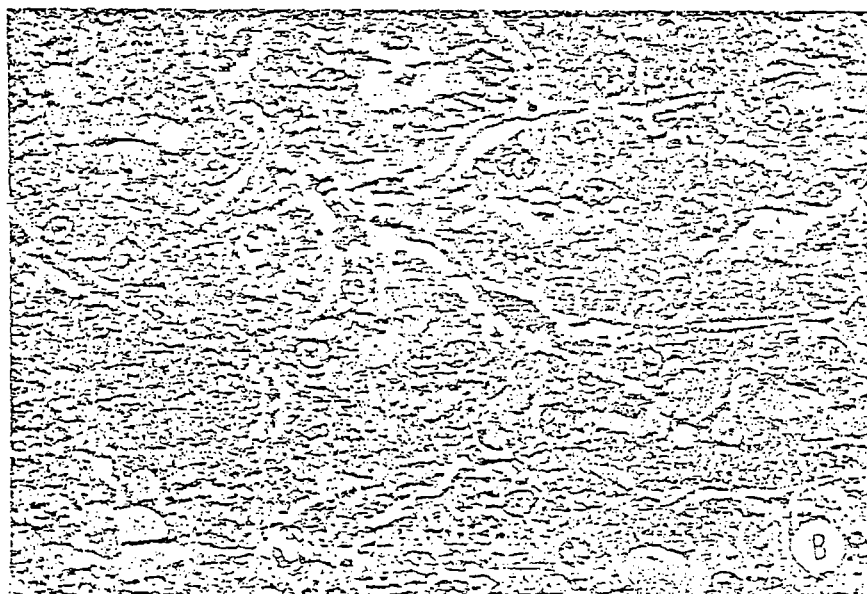


Fig. 2. (a) Increased content of pigment, staining positive for iron, in the renal tubular epithelial cells of a female rat given 30 mg 2,4,5-T/kg/day for 2 yr, compared with that from a control rat (b). Mallory's stain $\times 400$.

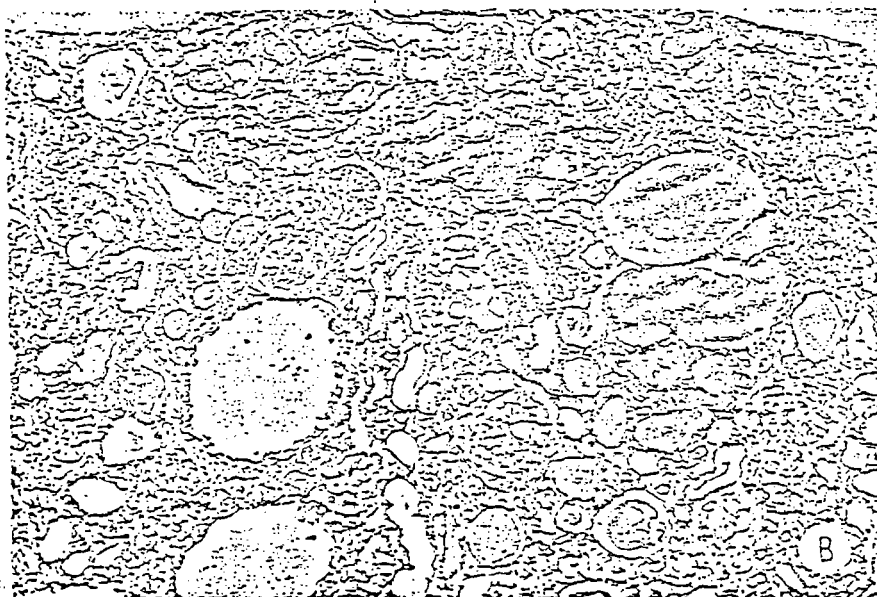


Fig. 3. Chronic nephropathic lesion in the kidney of a rat given 30 mg 2,4,5-T/kg/day (a) compared with the more severe lesion in a control rat (b). Haematoxylin and eosin $\times 40$.

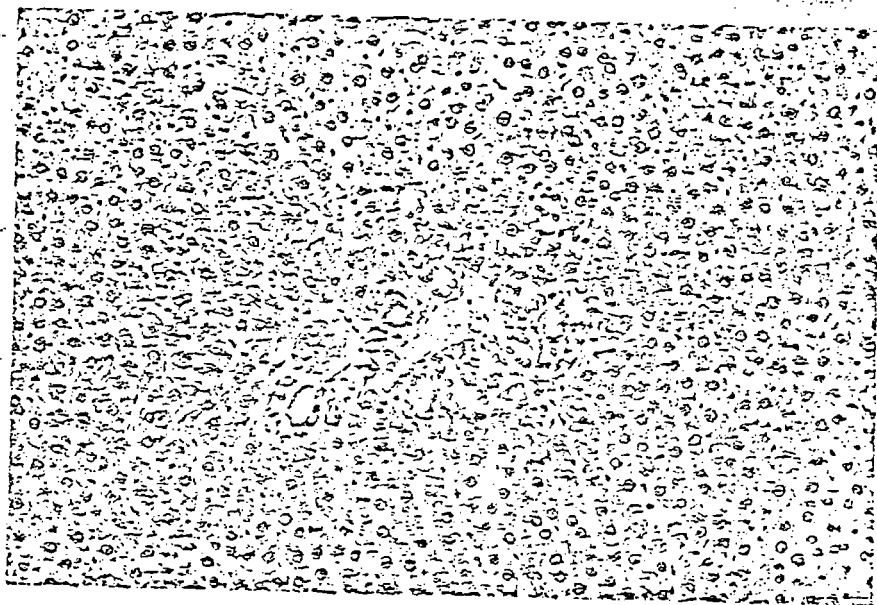


Fig. 4. Maximal response in the liver of a rat given 30 mg 2,4,5-T/kg/day, comprising a slight degree of focal biliary hyperplasia and periportal inflammation. Haematoxylin and eosin $\times 100$.

except that the increased pigment noted in the renal tubular cells of the high-dose group gave an increased positive reaction with Mallory's stain for iron (see Fig. 2). At the intermediate dose level (10 mg/kg/day), the females showed an increased incidence of mineralized deposits in the renal pelvis of female rats and statistically significant increases in the incidence of minimal degenerative and inflammatory changes, including focal renal tubular atrophy and renal aggregations of lymphoid cells. The latter increases, however, were considered to be a reflection of an overall decrease in the severity of the geriatric chronic renal disease noted in this group. A statistically significant decrease in the incidence of mineralized deposits in the renal pelvis of male rats given 10 mg 2,4,5-T/kg/day may or may not have been the result of treatment. In the males and females on 3 mg/kg/day there were no renal lesions of any type that were considered to be related to treatment.

The decrease in the severity of the spontaneous chronic nephropathy noted in male rats receiving 30 mg 2,4,5-T/kg/day (Fig. 3) was accompanied by a secondary decrease in the incidence of mineralization of the pulmonary alveoli, myocardium, myocardial blood vessels, and gastric mucosa and muscularis.

Although the kidney was the primary target organ, the liver was also affected slightly by lifetime treatment with 30 mg 2,4,5-T/kg/day, the males showing a decreased incidence of liver enlargement and an increased incidence of focal biliary hyperplasia and periportal inflammation (Fig. 4), and the females an increased incidence of focal aggregation of reticuloendothelial cells adjacent to degenerate or necrotic hepatocytes and a decreased incidence of multiple foci of hepatocellular change (swollen hepatocytes). The incidence of focal hepatocellular cytoplasmic vacuolization was decreased in the livers of males given 10 or 30 mg/kg/day. Although the incidence of grossly visible liver nodules was higher in the control group of females than in the treatment groups, this control incidence was also somewhat higher than that generally encountered in our controls. Livers of rats given 3 mg 2,4,5-T/kg/day showed no changes attributable to treatment.

The lungs showed some changes that, directly or indirectly, may have been the result of treatment with 30 mg 2,4,5-T/kg/day; the males had an increased incidence of focal pulmonary interstitial inflammation, focal accumulations of alveolar macrophages and cholesterol clefts, while a few females showed focal accumulations of secreted material in the alveoli. At the intermediate and lower dose levels there were no pulmonary changes attributable to treatment, with the possible exception of an increased incidence of focal interstitial fibrosis in the lungs of females on the intermediate dose level. As this change was not seen at the higher dose level, it was of highly questionable toxicological significance.

Examination of the tissues of the cardiovascular system revealed no lesions that could definitely be attributed to the ingestion of 2,4,5-T. In the group given 30 mg/kg/day, the females showed a statistically significant increase in the incidence of hearts with a dilated, flaccid ventricle, but the opposite trend occurred in the males. Males given either 10 or 30 mg/kg/day showed an apparent trend toward a decrease

in the incidence of both periarteritis and left atrial thrombosis, but these observations may not have been the result of 2,4,5-T treatment.

No other gross or histopathological changes considered to be attributable to 2,4,5-T ingestion were detected in any organs.

Tumour incidence

The incidence of tumours in male and female rats is detailed in Tables 3 and 4 and summarized in Table 5. The same spectrum of tumours observed historically in rats of this strain were noted in the liver, nasal turbinates/hard palate, lungs, pancreas, kidney, urinary bladder, testes, ovary, uterus, musculo-skeletal tissue, oral cavity, tongue, salivary glands, stomach, small intestine, large intestine, subcutaneous tissues, integument, mammary gland, ear canal, brain, peripheral nerves, pituitary gland, cranial cavity, adrenal glands, eye, lymph nodes, thymus, spleen, mesentery, thyroid and parathyroid glands. Statistical analyses of these data showed that the incidence of each type of tumour in any of the 2,4,5-T-treated groups was comparable to that in the control group, with the exception of the incidence of interfollicular C-cell adenoma of the thyroid, which was increased significantly in females given 3 mg/kg/day. This observation was not considered to be related to treatment, since there was no dose response and the female control group had an unusually low incidence of thyroid adenomas compared to the historical control data (up to 17%). Thus, none of the tumours listed in Tables 3 and 4 were considered to be related to treatment with any of the dose levels of 2,4,5-T. Neither were the total numbers of tumours per group, the average numbers per rat or the times of observation of tumours affected by any of the levels of treatment, these data in each case being comparable to those of the control groups (Table 5).

DISCUSSION

During the course of this 2-yr study, the 2,4,5-T treatment had no adverse effects on the onset of mortality, food consumption, the development of palpable masses, haematology, routine urine analyses, urinary excretion of creatinine or δ -ALA, serum clinical chemistry, organ weights (except the relative weight of the kidney at the interim kill in rats given 30 mg/kg/day), tumour incidence and gross and microscopic morphology of all organ systems of the body except the kidney, liver and possibly lung. Nevertheless, the highest dose level (30 mg 2,4,5-T/kg/day) predictably caused some degree of toxicity. This included a decrease in body-weight gain, increases in total urine volume, urinary coproporphyrin and uroporphyrin, an increase in relative kidney weights and morphological alterations in the kidney, liver and possibly lung.

In the kidney, these changes consisted mainly of mineralized deposits in the renal papillae or pelvis, sometimes accompanied by a resultant localized reaction of the adjacent epithelium of the renal pelvis. The chronic nephropathy normally encountered in aged rats was decreased in rats given 30 mg 2,4,5-T/kg/day. Morphological alterations in the liver attributed to treatment with 30 mg/kg/day included an

Table 3. Tumour incidence in male rats maintained on diets containing 2,4,5-T for up to 2 yr

Tumours or tumour-like lesions	Dose (mg/kg/day) ... No. of rats examined ...	No. of rats found to be affected* in:											
		Months 13-18				Month 19-terminal kill				Whole period of study (total)†			
		0 14	3 12	10 22	30 9	0 69	3 37	10 27	30 41	0 86	3 50	10 50	30 50
Hepatocellular carcinoma		0	0	0	0	3	1	0	1	3	1	0	1
Keratoacanthoma of nasal turbinates/hard palate		1	0	0	0	0	0	0	0	1	0	0	0
Squamous cell carcinoma of hard palate		0	0	0	0	1	1	1	0	1	1	1	0
Mixed adenoma of hard palate		0	0	0	0	1	0	0	0	1	0	0	0
Pulmonary adenoma		0	1	0	0	0	0	0	0	0	1	0	0
Pancreatic acinar adenoma		2	2	2	3	23	15	7	17	26	17	9	20
Pancreatic acinar adenocarcinoma		0	0	0	0	2	0	0	0	2	0	0	0
Pancreatic islet-cell adenoma		0	2	1	0	12	8	6	7	13	10	7	7
Pancreatic islet-cell adenocarcinoma		1	0	0	0	0	0	1	0	1	0	1	0
Renal pelvis transitional-cell adenoma		0	0	0	0	0	0	1	0	0	0	1	0
Renal tubular adenoma		0	0	0	0	0	0	0	1	0	0	0	1
Papillary adenoma of renal pelvis		0	0	0	0	1	0	0	0	1	0	0	0
Renal carcinoma		0	0	0	0	1	0	0	0	1	0	0	0
Transitional-cell carcinoma of urinary bladder with metastasis		0	0	0	0	1	0	0	0	1	0	0	0
Interstitial-cell adenoma of testis		0	0	0	1	4	1	2	2	4	1	2	3
Mesothelioma of testis		0	0	0	0	1	0	0	0	1	0	0	0
Sertoli cell tumour of testis		1	0	0	0	0	0	0	0	1	0	0	0
Chondrosarcoma of rib		0	0	0	0	1	0	0	0	1	0	0	0
Oral papilloma		0	0	0	0	1	0	0	0	1	0	0	0
Squamous-cell carcinoma of tongue		0	0	0	1	1	1	0	3	1	1	0	4
Salivary-gland adenoma		0	0	0	0	1	0	0	0	1	0	0	0
Squamous papilloma of stomach mucosa		0	0	0	0	3	1	0	0	3	1	0	0
Mucocystadenocarcinoma or adenocarcinoma of small intestine		0	0	0	0	2	2	0	0	2	2	0	0
Fibrosarcoma of small intestine		0	0	0	0	0	0	0	1	0	0	0	1
Lymphosarcoma of small intestine		0	0	1	0	1	0	0	0	1	0	1	0
Undifferentiated sarcoma of small intestine		0	0	0	1	0	0	0	0	0	0	0	1
Lymphosarcoma of small or large intestine with metastasis		0	0	0	0	1	0	0	1	1	0	0	1
Papillary adenoma or mucocystadenoma of small intestine		0	0	0	0	1	1	0	0	1	1	0	0
Subcutaneous fibroma		1	1	0	0	3	7/5	1	3	4	8/6	2	3
Subcutaneous adenocarcinoma		0	0	0	1	0	0	0	0	0	0	0	1
Malignant fibrous histiocytoma with metastasis		0	0	0	0	0	1	0	0	0	1	0	0
Malignant fibrous histiocytoma without metastasis		0	0	0	0	1	0	0	0	1	0	0	0
Subcutaneous liposarcoma		0	0	0	0	0	0	0	1	0	0	0	1
Subcutaneous myxoma		0	0	0	0	0	1	0	0	0	1	0	0

Subcutaneous carcinoma	0	0	0	0	0	0	0	1	0	0	0	1
Mammary-gland fibroadenoma/adenofibroma	0	0	1	0	1	2	0	1	1	2	1	1
Mammary-gland adenocarcinoma without metastasis	0	0	0	0	1	0	0	0	1	0	0	0
Mammary-gland adenocarcinoma with metastasis	0	0	0	0	1	0	0	0	1	0	0	0
Mammary-gland carcinosarcoma	0	0	0	0	1	0	0	0	1	0	0	0
Squamous papilloma of integument	0	0	0	0	1	0	1	1	1	0	1	1
Cutaneous adnexal carcinoma with metastasis	0	0	1	0	0	0	0	0	0	0	1	0
Fibrosarcoma adjacent to ear tag	0	0	1	0	0	0	0	0	0	0	1	0
Cutaneous adnexal cystadenoma	0	0	0	0	1	0	0	0	1	0	0	0
Zymbal-gland carcinoma of ear canal	1	0	0	0	3	0	1	1	4	0	1	1
Subcuticular undifferentiated sarcoma with metastasis	0	0	0	0	1	0	0	0	1	0	0	0
Astrocytoma or mixed glial neoplasm of brain	0	1	1	0	1	0	0	1	1	1	1	1
Granular cell myoblastoma of brain	0	0	0	0	0	1	1	1	0	1	1	1
Mediastinal malignant schwannoma or malignant schwannoma of cranial nerve	0	0	0	0	1	1	0	0	1	1	0	0
Schwannoma of cranial nerve	0	0	0	0	0	0	1	0	0	0	1	0
Pituitary adenoma formation(s)	2	1	3	0	20	9	10	10	22	10	13	10
Pituitary adenocarcinoma without invasion of brain	0	0	1	0	4	2	1	3	4	2	2	3
Pituitary adenocarcinoma with invasion of brain	0	0	0	1	1	0	0	0	1	0	0	1
Neurofibroma in cranium	0	0	0	0	1	0	0	0	1	0	0	0
Adenoma of adrenal cortex	0	0	1	0	1	3	1	3	1	3	2	3
Adenocarcinoma of adrenal cortex	0	0	0	0	1	1	0	0	1	1	0	0
Adrenal pheochromocytoma—unilateral or bilateral	3	2	4	1	33	10	14	19	36	12	18	20
Adrenal pheochromocytoma—malignant	0	0	0	0	7	4	1	1	8	4	1	1
Adrenal ganglioneuroma	0	0	0	0	0	0	0	1	0	0	0	1
Retro-ocular squamous-cell carcinoma	0	0	0	0	0	0	0	1	0	0	0	1
Intraorbital undifferentiated sarcoma	0	0	0	0	0	0	1	0	0	0	1	0
Mesenteric lipoma	0	0	0	0	1	0	0	0	1	0	0	0
Generalized lymphosarcoma	0	1	0	0	1	0	0	0	2	1	0	0
Thymic lymphosarcoma	0	0	0	0	0	1	0	0	0	1	0	0
Splenic lymphosarcoma	0	0	0	0	1	0	0	0	1	0	0	0
Thyroid interfollicular C-cell adenoma	0	0	0	1	6	3	2	6	6	3	2	7
Thyroid interfollicular C-cell adenocarcinoma without metastasis	0	0	0	0	1	1	0	0	1	1	0	0

*There were no statistical differences (by Fisher's Exact Probability Test, $P < 0.05$) between control values and those for any treated groups. Where multiple tumours of the same classification occurred in individual rats, the data are listed as the total no. of tumours of that type/no. of rats bearing them.

†During months 1-6 there was only one tumour noted (a generalized lymphosarcoma) in one control rat. Tumours occurring during months 7-12 included one pancreatic acinar adenoma (control), one pancreatic islet-cell adenoma (control), one subcutaneous fibroma (10 mg/kg/day) and one adrenal pheochromocytoma, malignant (control). All of these tumours are included in the total tabulation.

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Table 4. Tumour incidence in female rats maintained on diets containing 2,4,5-T for up to 2 yr

Tumour or tumour-like lesions	Dose (mg/kg/day)...	No. of rats examined...	No. of rats found to be affected† in:											
			Months 13-18				Month 19-terminal kill				Whole period of study (total)†			
			0	3	10	30	0	3	10	30	0	3	10	30
			17	11	11	9	67	37	36	39	86	50	50	50
Hepatocellular hyperplastic nodule(s)			1	0	1	0	3	2	2	1	4	2	3	1
Hepatocellular carcinoma(s)			1	0	0	0	1	0	0	0	2	0	0	0
Pancreatic islet-cell adenoma			1	0	0	0	7	1	3	2	8	1	3	2
Pancreatic islet-cell adenocarcinoma			0	0	0	0	0	0	0	1	0	0	0	1
Pancreatic acinar adenoma			0	0	0	0	0	0	1	0	0	0	1	0
Renal haemangioma			0	0	0	0	0	0	0	1	0	0	0	1
Granulosa-cell neoplasm of ovary			0	0	0	0	4	1	1	0	4	1	1	0
Stromal polyp of uterus			1	0	2/1	1	13	4	8/7	11/10	14	4	11/9	12/11
Malignant schwannoma—uterus (metastasis), or vagina			0	0	0	0	1	0	0	1	1	0	0	1
Adenocarcinoma of uterus			0	0	0	0	4	0	1	0	4	0	1	0
Myxosarcoma of uterus			0	0	1	0	0	0	0	0	0	0	1	0
Schwannoma of uterus			0	1	0	0	0	0	1	0	0	1	1	0
Leiomyosarcoma of uterus with metastasis			0	0	0	0	0	0	1	0	0	0	1	0
Fibroma of uterus/vagina			0	0	0	0	1	1	0	0	1	1	0	0
Paravertebral rhabdomyosarcoma with metastasis			0	0	0	1	0	0	0	0	0	0	0	1
Fibroma of gingiva			0	0	0	0	0	0	0	1	0	0	0	1
Squamous-cell carcinoma of tongue			0	0	0	0	0	0	0	1	0	0	0	1
Adenoma of tongue			0	0	0	0	1	0	0	0	1	0	0	0
Squamous polyp or papilloma of stomach			0	0	0	0	3	1	0	0	3	1	0	0
Mucocystadenocarcinoma or adenocarcinoma of small intestine			0	0	0	0	0	1	0	1	1	1	0	0
Lymphosarcoma of small intestine			0	0	0	0	0	0	0	1	0	0	0	1
Leiomyoma or leiomyosarcoma of small intestine			0	0	0	0	1	0	1	0	1	0	1	0
Subcutaneous lipoma			0	0	0	0	0	1	0	0	0	1	0	0
Subcutaneous fibroma			0	0	0	1	0	0	0	0	0	0	0	1
Subcutaneous fibrosarcoma			0	0	0	0	0	0	0	1	0	0	0	1
Subcutaneous carcinosarcoma			0	0	0	0	0	1	0	0	0	1	0	0
Subcutaneous myxosarcoma			0	0	0	0	0	1	0	0	0	1	0	0
Mammary-gland fibroadenoma/adenofibroma			25/13	9/7	12/9	11/7	113/52	38/24	49/28	43/25	138/65	47/31	62/38	55/33
Mammary-gland adenoma			3/2	1	0	1	12/8	6/5	4/3	8/7	15/10	7/6	4/3	9/8
Mammary-gland adenocarcinoma without metastasis			3/2	0	0	1	4	4	1	5	7/6	4	1	6
Mammary-gland adenocarcinoma with metastasis			0	1	0	1	1	1	0	2	1	2	0	4
Mammary-gland cystadenoma			0	1	0	0	2	1	6/4	3	2	2	6/4	3
Mammary-gland fibroma			1	0	0	0	3	1	3	3/2	4	1	3	3/2
Mammary-gland cystifibroadenoma/cystadenofibroma			0	0	0	0	13/11	10/7	9/7	5	13/11	10/7	9/7	5
Mammary-gland unclassified malignant neoplasm (carcinoma)			0	0	0	0	0	0	0	0	1	0	0	0

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Mammary-gland cystadenocarcinoma	0	0	0	0	0	0	0	1	0	0	0	1
Squamous papilloma or keratoacanthoma of integument	0	0	0	0	2	0	0	0	2	0	0	0
Zymbal-gland carcinoma of ear canal	0	0	0	0	1	0	0	0	1	0	0	0
Adenoma of Zymbal gland	0	0	0	0	0	1	0	0	0	1	0	0
Pituitary adenoma formation(s)	8	3	1	3	34	20	19	16	42	23	20	19
Pituitary adenocarcinoma without extension or invasion of brain	2	0	0	0	4	6	3	5	6	6	3	5
Pituitary adenocarcinoma with extension of brain	1	0	0	0	4	2	2	2	5	2	2	2
Pituitary adenoma arising from pars intermedia	0	0	0	0	1	0	0	0	1	0	0	0
Neurofibrosarcoma in cranium	0	0	0	0	0	0	1	0	0	0	1	0
Adenoma of adrenal cortex	2	0	0	2	4	2	4	1	6	2	4	3
Adrenal pheochromocytoma, unilateral	1	0	0	0	5	3	2	2	6	3	2	2
Adrenal pheochromocytoma, malignant	0	0	0	0	1	0	0	0	1	0	0	0
Mesenteric lipoma	0	0	0	0	1	0	0	0	1	0	0	0
Abdominal haemangiosarcoma	0	0	1	0	0	0	0	0	0	0	1	0
Granulocytic leukaemia	0	0	0	0	0	0	1	0	0	0	1	0
Generalized monocytic leukaemia	0	0	0	0	0	1	0	0	0	1	0	0
Generalized lymphosarcoma	1	0	1	0	1	0	0	1	2	1	1	1
Intra-abdominal lymphosarcoma or carcinosarcoma with metastasis	0	0	0	0	1	0	0	0	1	0	0	0
Lymphangioma of mesenteric lymph node	0	0	0	0	1	0	0	0	1	0	0	0
Thymic lymphosarcoma	0	0	0	0	0	0	0	1	0	0	0	1
Splenic myeloid leukaemia	0	0	0	0	0	1	0	0	0	1	0	0
Thyroid interfollicular C-cell adenoma	0	1	0	1	3	9	2	5	3	10*	2	6
Thyroid interfollicular C-cell adenocarcinoma without metastasis	0	0	0	0	3	2	0	1	3	2	0	1
Thyroid interfollicular C-cell adenocarcinoma with metastasis	0	0	0	0	1	0	1	0	1	0	1	0
Parathyroid adenoma	0	0	0	0	1	0	0	1	1	0	0	1

†Where multiple tumours of the same classification occurred in individual rats, the data are listed as the total no. of tumours of that type/no. of rats bearing them.

‡During months 1-6, there was only one tumour noted (a generalized lymphosarcoma) in one rat given 3 mg/kg/day. Tumours occurring during months 7-12 included one stromal polyp of the uterus (10 mg/kg/day), one mammary-gland fibroadenoma/adenofibroma in both the 10- and 30-mg/kg/day groups, one mammary-gland adenocarcinoma with metastasis (30 mg/kg/day) and one mammary-gland unclassified malignant neoplasm (carcinoma) (control). All of these tumours are included in the total tabulation. The value marked with an asterisk differs significantly (by Fisher's Exact Probability Test, * $P < 0.05$) from the control value.

Table 5. Summary of data on renal tumours in rats maintained on diets containing 2,4,5-T for up to 2 yr

Dose of 2,4,5-T (mg/kg/day)	No. of rats/group	Total no. of tumours/ group	Total no. of tumours/ rat	No. of tumour-bearing rats/rats autopsied					Total*
				During months				At terminal kill	
				7-12	13-18	19-24			
Males									
0	86	172	2.0	1/1	1/2	8/14	60/63	6/6	76/86 (88%)
3	50	89	1.8	0/0	0/1	9/12	26/23	9/9	44/50 (88%)
10	50	71	1.4	0/0	1/1	15/22	20/23	4/4	40/50 (80%)
30	50	97	1.9	0/0	0/0	6/9	24/25	14/16	44/50 (88%)
Females									
0	86	308	3.6	0/0	1/2	16/17	30/31	36/36	83/86 (97%)
3	50	140	2.8	1/1	0/1	10/11	18/18	19/19	48/50 (96%)
10	50	147	2.9	0/1	1/2	11/11	25/25	11/11	48/50 (96%)
30	50	151	3.0	0/0	2/2	9/9	18/18	21/21	50/50 (100%)

*Values for treated groups showed no statistically significant differences from the control values when analysed by Fisher's Exact Probability Test ($P < 0.05$).

increased incidence of periportal inflammation, focal biliary hyperplasia and inflammatory cell aggregations adjacent to degenerate hepatocytes; these findings were accompanied by a decreased incidence of multiple foci of swollen hepatocytes and focal cytoplasmic vacuolization. Lungs of rats given 30 mg 2,4,5-T/kg/day showed indications of focal pulmonary inflammation, including focal accumulations of alveolar macrophages and cholesterol clefts.

Only minimal toxicity was associated with the intermediate dose level (10 mg/kg/day), primarily an increased incidence of mineralized deposits in the renal pelvis. An increase in the urinary excretion of coproporphyrin was noted in males on this dose level only during the early phase of the study. At the lower dose level (3 mg/kg/day) there were no effects attributable to 2,4,5-T treatment throughout the 2-yr study.

The absence of any tumours considered to be the result of treatment with 2,4,5-T at any of the three dose levels concurs with the results of most of the oncogenic studies on 2,4,5-T previously reported. Innes *et al.* (1969) found no oncogenic response in either of two strains of mice given the maximum tolerated dose of 215 mg 2,4,5-T/kg/day, and the Bionetics Research Laboratory (1968) noted no increase in tumours in mice of two strains given a single sc injection of 215 mg 2,4,5-T/kg. The only published oncogenic study reporting an increase in tumours allegedly due to treatment with 2,4,5-T is one of the four studies conducted by Muranyi-Kovacs *et al.* (1976 & 1977). In these studies, mice of the C3H strain given a single dose level of 80 ppm (approximately 12 mg/kg/day) of 2,4,5-T had a statistically significant increase of the so-called 'non-incident' types of tumours, but mice of the XVIIG strain given identical 2,4,5-T treatment showed no increase in tumour incidence. When Muranyi-Kovacs *et al.* (1977) gave neonatal mice of these same two strains a series of sc injections of 2,4,5-T there was no resultant increase in tumour incidence in either strain. It is interesting to note that in the one study in which Muranyi-Kovacs *et al.* reported an oncogenic response to 2,4,5-T, no specific target tissue showed the alleged response. This is contrary to the vast majority of cases, in which a positive oncogenic response typically

affects specific target tissue(s), from which the resulting increase in tumours originates.

Overall, therefore, the results of this study concur with those of all but one of the previously reported oncogenic studies on 2,4,5-T. Rats given 10 or 30 mg 2,4,5-T/kg/day for 2 yr showed some dose-related toxicity but no oncogenic response, while in those given 3 mg/kg/day there were no adverse effects related to treatment.

REFERENCES

- Bionetics Research Laboratories (1968). Evaluation of Carcinogenic, Teratogenic and Mutagenic Activities of Selected Pesticides and Industrial Chemicals. Report No. NCI-DCCP-CG-1973-1-1, issued August 1968; Bethesda, MD.
- Drill, V. A. & Hiratzka, T. (1953). Toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. A report on their acute and chronic toxicity in dogs. *Archs ind. Hyg. occup. Med.* 7, 61.
- Gehring, P. J., Kramer, C. G., Schweiz, B. A., Rose, J. Q. & Rowe, V. K. (1973). The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to man. *Toxic. appl. Pharmac.* 26, 352.
- Highman, B., Gaines, T. B. & Schumacher, H. G. (1976). Sequential histopathologic, hematologic, and blood chemistry changes induced in mice by a technical and a purified preparation of 2,4,5-trichlorophenoxyacetic acid. *J. Toxicol. envir. Hlth* 1, 469.
- Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucci, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I. & Peters, J. (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. *J. natn. Cancer Inst.* 42, 1101.
- Luna, L. G. (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd Ed. McGraw-Hill Book Company, Inc., New York.
- Muranyi-Kovacs, I., Rudali, G. & Imbert, J. (1976). Bioassay of 2,4,5-trichlorophenoxyacetic acid for carcinogenicity in mice. *Br. J. Cancer* 33, 626.
- Muranyi-Kovacs, I., Rudali, G. & Imbert, J. (1977). Study on the carcinogenicity of 2,4,5-T in mice. Abstract, Fourth Meeting of the European Association for Cancer Research, University of Lyon, September 1977.
- Piper, W. N., Rose, J. Q., Leng, M. L. & Gehring, P. J. (1973). The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to rats and dogs. *Toxic. appl. Pharmac.* 26, 339.

Rowe, V. K. & Hymas, T. A. (1954). Summary of toxicological information on 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. *Am. J. vet. Res.* 15, 622.

Siegel, S. (1956). *Non-parametric Statistics for the Behavioral Sciences*. McGraw-Hill Book Company, Inc., New York.

Smith, F. A., Schwetz, B. A., Murray, F. J., Crawford, A. A.,

John, J. A., Kociba, R. J. & Humiston, C. G. (1978). Three-generation reproductive study in rats ingesting 2,4,5-trichlorophenoxyacetic acid in the diet. Abstract, 17th Annual Meeting of Society of Toxicology, March 1978. *Toxic. appl. Pharmac.* 45, 293.

Steel, R. G. & Torrie, H. H. (1960). *Principles and Procedures of Statistics*. McGraw-Hill Book Company, Inc., New York.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

BEFORE THE ADMINISTRATOR

In re:

The Dow Chemical Company, et al.

FIFRA Docket Nos. 415, et al.

DIRECT TESTIMONY OF DR. ROY E. ALBERT ^{*/}

Scheduled Date of Testimony: September 23, 1980

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*/ EPA Exhibit No. 32A. Dr. Albert's direct testimony is a revision of his statement for his appearance in this proceeding on March 18, 1980. Dr. Albert's original statement was assigned Exhibit 32, and this revised statement is designated as Exhibit 32A.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
BEFORE THE ADMINISTRATOR

In re:

The Dow Chemical Co., et al.)

) FIFRA Docket No. 415, et al.
)
)
)

DIRECT TESTIMONY OF DR. ROY E. ALBERT^{*/}

(UPDATE)

INTRODUCTION

My name is Roy Albert. I am Deputy Director of the Institute of Environmental Medicine, New York University Medical Center, and Chairman of EPA's Carcinogen Assessment Group (CAG). CAG is responsible for reviewing data relating to the possible carcinogenicity of compounds which EPA has authority to regulate and for assessing the risk of cancer these compounds pose to the human population. During the past three years, as Chairman of CAG, I have participated in the evaluation of approximately 100 compounds, including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-trichlorophenoxypropionic acid (silvex), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), an unavoidable contaminant of commercial 2,4,5-T and silvex, for possible carcinogenic potential.

*/ EPA Exhibit No. 32A.

CAG has evaluated several laboratory animal studies of the carcinogenicity of TCDD, 2,4,5-T and silvex ^{*/} and several epidemiological studies of the association between the risk of cancer among humans and exposure to 2,4,5-T, other chemically related chemicals, and TCDD. CAG has concluded that this body of information provides substantial evidence that TCDD, and therefore commercial 2,4,5-T and commercial silvex, pose a carcinogenic risk to exposed humans. CAG has also concluded that this body of information provides highly suggestive evidence that the pure 2,4,5-T component of commercial 2,4,5-T may pose a carcinogenic risk to exposed humans. In addition, based on the laboratory animal studies, CAG has concluded that TCDD appears to be one of the most potent known carcinogens.

In this testimony, I first describe some of the policies and principles which EPA (and CAG) follow in determining whether chemical substances pose human cancer risks. I then provide an analysis of the carcinogenicity of TCDD, 2,4,5-T, and silvex based on CAG's review of the relevant laboratory animal studies and epidemiological studies. Finally, I summarize CAG's quantitative assessment of part of the cancer risk posed by some routes of exposure to 2,4,5-T, silvex, and TCDD.

I. EPA's Cancer Policy

The Agency's assessment of the carcinogenic risk associated with the uses of 2,4,5-T and silvex is founded on principles

*/ CAG's updated "Risk Assessment on 2,4,5-T, Silvex, and TCDD," (the CAG Report) is appended as Exhibit 33a.

developed and followed by EPA as well as other federal regulatory agencies and research institutions charged with measuring the risks associated with toxic chemicals in the environment. The Agency analyzes carcinogenic risks in accordance with its "Interim Guideline for Carcinogen Risk Assessment" (Interim Guideline). [Exhibits 34, and 35]. The policies described in the Interim Guideline are consistent with a recent report of the Interagency Regulatory Liaison Group (IRLG) entitled "Scientific Basis for Identification of Potential Carcinogens and Estimation of Risks" (IRLG Report). [Exhibit 36]. The IRLG report reflects the consensus of scientists and policymakers of four major federal regulatory agencies, including EPA, which regulate carcinogenic substances as well as senior scientists from two major federal agencies involved in cancer research.*/_

According to the Agency's cancer principles, the best evidence that a chemical substance is a human carcinogen comes from a combination of epidemiological studies and experimental animal data. Substantial evidence is provided

*/_ The four agencies comprising the IRLG at the time the IRLG Report was written were the Consumer Product Safety Commission (CPSC), Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA). The IRLG Report was written by scientists and policymakers from all these Agencies with the assistance of senior scientists from the National Cancer Institute (NCI) and the National Institute of Environmental Health Sciences (NIEHS). Scientists from the Food Safety and Quality Service of the Department of Agriculture, which subsequently joined the IRLG, reviewed and concurred in the report after it was completed.

by animal tests which demonstrate that the substance induces, in one or more animal species, malignant tumors or benign tumors, which are generally recognized as precursors of malignant tumors. Suggestive evidence includes positive short term in vitro tests such as mutagenicity and cell transformation tests.

While epidemiological cancer studies (i.e., studies of cancer incidences in human populations) can provide the most direct evidence that a chemical poses a cancer risk to humans, such evidence is frequently not available because studies have not been done. Furthermore, human populations do not live in controlled environments and, therefore, are subject to many factors which may affect cancer incidence. Thus, the force of epidemiological evidence increases by the repetition of similar findings under different circumstances, all of which involve exposure to the agent being investigated. Another limitation of epidemiological cancer studies lies in the fact that development of cancer requires a long period, often 5-40 years after initial exposure to carcinogens. This latency period makes it nearly impossible to promptly detect by epidemiologic studies the carcinogenicity of substances newly introduced into the environment .

Because it is often difficult to determine the carcinogenicity of a substance in humans by epidemiological means alone, cancer tests on laboratory animals are heavily relied

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upon by regulatory agencies and the scientific community to make this determination. A close qualitative similarity has been demonstrated in the nature of the response of laboratory animals and humans to carcinogenic substances. Currently available evidence indicates that almost all agents known to cause cancer in humans also induce oncogenic effects in laboratory animals [Exhibit 36]. Therefore, the induction of tumors in laboratory animals by a substance is regarded as substantial evidence that the substance is a human carcinogen.

The Agency has adopted the no-threshold concept for cancer induction by complete carcinogens (i.e., substances which themselves alone cause cancer). According to this concept, any exposure to a complete carcinogen, however small, will confer some risk of cancer on the exposed population. Thus, if epidemiologic studies or laboratory animal studies indicate that a chemical substance is a likely human carcinogen, the Agency considers that substance to pose a carcinogenic risk to exposed humans at any level of exposure, no matter how small. The no-threshold concept is recommended in the IRLG Report as an appropriate regulatory policy.

II. Carcinogenicity of TCDD, 2,4,5-T, and silvex in Laboratory Animals

Several cancer bioassay studies have been conducted on the effects of TCDD using rats and mice. These studies provide substantial evidence that TCDD administered alone causes cancer in rats and mice at exceedingly low dose levels.

Therefore, in view of the no-threshold concept for cancer induction, these studies provide substantial evidence that TCDD is a complete carcinogen and is likely to pose a risk of cancer to humans at any level of human exposure.

There is evidence that TCDD is both a promoting agent and a cocarcinogen. A cancer promoter is an agent which, when applied after a complete carcinogen or a cancer initiator,^{*/} increases the incidence of tumors and shortens the latency period for tumor development. This enhancement can occur even if the exposure to the cancer promoter occurs long after the exposure to the complete carcinogen or initiator. A chemical is a cocarcinogen if the incidence of tumors induced by that compound and another carcinogen to which an animal is simultaneously exposed is greater than what would be expected if the effects of the two compounds were only additive. TCDD has been shown to be a powerful promoting agent in the rat liver and a cocarcinogen in the mouse skin. It is possible that the promoting and cocarcinogenic effects may extend to other organs in other species including man.

^{*/} A cancer initiator is an agent which causes irreversible transformation of a cell into a latent tumor cell (i.e., a cell capable of developing into a tumor).

Since commercial 2,4,5-T and silvex are unavoidably contaminated with low levels of TCDD, the laboratory animal studies on TCDD provide substantial evidence that commercial 2,4,5-T and silvex are likely to pose a risk of cancer to humans at any level of exposure.

One study in which rats were administered specially purified 2,4,5-T containing no detectable TCDD provides highly suggestive evidence that pure 2,4,5-T, independent of any TCDD contamination, is carcinogenic in rats. Thus, commercial 2,4,5-T may pose a risk of cancer to exposed humans, which is in addition to the risk of cancer due to its TCDD contaminant.

Several other chronic laboratory animal studies have been conducted on 2,4,5-T and a few have been conducted on silvex. Because of deficiencies in design or conduct as well as other factors, none of these studies provides significant evidence of either the oncogenicity or non-oncogenicity of commercial 2,4,5-T and silvex.

In summary, data from the animal cancer studies on TCDD, 2,4,5-T, and silvex, considered as a whole, provide substantial evidence that TCDD, commercial 2,4,5-T, and commercial silvex pose a carcinogenic risk to exposed humans at any level of exposure.

A. Laboratory Animal Studies on TCDD

1. Rat Studies

CAG reviewed two chronic toxicity studies involving

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the administration of TCDD to rats. In these studies, exceedingly low dose levels of TCDD induced a carcinogenic effect.

Kociba et al.^{*/} (scientists at Dow Chemical Company) administered TCDD mixed with food to groups of male and female Sprague-Dawley rats (Spartan substrain) at TCDD doses of 0.1 ug, 0.01 ug, and 0.001 ug/kg body weight per day)^{**/} per kilogram of rat body weight per day for up to two years. At the highest dose level (0.1 ug/kg/day), TCDD induced statistically significant increases of several types of cancers in both male and female rats. In high dose female rats, statistically significant increases in liver (hepatocellular) carcinomas, carcinomas of the lung, and carcinomas of the hard palate and/or nasal turbinates were observed. The increases in the liver tumors and lung carcinomas were very highly statistically significant ($p < .001$ for both types of tumors). In high dose male rats, statistically significant increases in carcinomas of the hard palate and/or nasal turbinates and in carcinomas of the tongue were observed. In addition, TCDD induced a very highly significant increase ($p < .001$) in the combined incidence of

^{*/} The published report of this study, a letter with an attachment providing supplemental data, and some additional unpublished data are appended as Exhibits 13, 14, and 15, respectively.

^{**/} Doses of 0.1 ug, 0.01 ug, and 0.001 ug/kg body weight per day were administered by feeding the rats diets containing 2.2 ppb, 0.22 ppb, and 0.022 ppb TCDD (1 ppb = 1 part per billion or 10^{-9} gram TCDD/gram of food).

liver carcinomas and neoplastic nodules of the liver^{*/} in the middle dose group (0.01 ug/kg/day). Thus, the results of this study provide very strong evidence that TCDD is a carcinogen in rats at exceedingly low doses.^{**/}

The National Cancer Institute (NCI) recently released a camera ready report of its cancer bioassay of TCDD (Exhibit 577). In this bioassay, male and female Osborne-Mendel rats were administered TCDD by oral intubation at doses of 0, 0.01, 0.05, and 0.5 ug/kg/week (equivalent to 0, 0.001, 0.007, and 0.07 ug/kg/day, respectively). The results from this study indicate that TCDD induced statistically significant increases in tumors of the thyroid gland (follicular cell adenomas or carcinomas) at the high ($p < .001$), middle ($P < .01$), and low ($p = .042$) doses in the male rats. The results also indicate that TCDD induced in female rats a statistically significant increase of each of the following tumors in the high dose group: carcinomas and neoplastic nodules of the liver ($p = 0.001$), subcutaneous fibrosarcomas (a

^{*/} It is appropriate to combine the incidences of all neoplasms arising from the same type of cell for purposes of evaluating the carcinogenicity of a test compound in a laboratory animal study. It is especially appropriate to combine hepatocellular carcinomas and neoplastic nodules of the liver because neoplastic nodules have clearly been shown to be precursors of hepatocellular carcinomas. Squire and Levitt (1975) [Exhibit 16]; Hirota and Williams (1979) [Exhibit 18]; Farber (1976) [Exhibit 37].

^{**/} At CAG's request, Dr. Robert A. Squire, a pathologist at Johns Hopkins School of Medicine, evaluated all liver tissue sections from all dose groups and all other tissue sections from the control and high dose groups. His diagnoses are summarized in Exhibit 760 and in Appendix B to Exhibit 33A. The discussion in the text regarding the results of the Kociba TCDD study are correct whether we take into account Dr. Kociba's or Dr. Squire's diagnoses.

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cancer of the fibrous tissue beneath the skin) ($p=0.023$), and adrenal cortical adenomas ($p=0.039$). These results confirm the findings of the Kociba study that TCDD induces observable carcinogenic effects in rats at exceedingly low doses.

2. Mouse Studies on TCDD

CAG has reviewed three studies involving the administration of TCDD to mice.

In two companion studies to the NCI cancer bioassay on rats described above, mice were administered low doses of TCDD by oral intubation and by skin application. In the oral bioassay (Exhibit 577) male B6C3F1 mice were administered TCDD by oral intubation at doses of 0.001, 0.05, and 0.5 ug/kg/week (equivalent to 0, 0.001, 0.007, and 0.07 ug/kg/day, respectively) and to female mice at doses of 0, 0.04, 0.2, and 2.0 ug/kg/week (equivalent to 0, 0.006, 0.03, and 0.3 ug/kg/day) for up to two years. The results indicate that among female mice TCDD induced statistically significant increases in the incidence of hepatocellular carcinomas ($p=0.014$), thyroid tumors (follicular cell adenomas), fibrosarcomas, and histiocytic lymphomas. The results indicate that among the male mice, TCDD induced a statistically significant increase in the incidence of hepatocellular carcinomas ($p = 0.002$) in the high dose group.

In the skin application bioassay (Exhibit 578) male and female Swiss Webster mice were administered TCDD in acetone by application to the skin three times a week at doses of 0.001 and 0.005 ug per male and female mouse, respectively (equivalent to approximately 0.01 and 0.07 ug/kg/day, respectively) for up

to two years. A statistically significant increase in fibrosarcomas of the skin was found among treated female mice compared to controls.

In summary, both the oral and skinpainting NCI studies on mice provide substantial evidence that TCDD is carcinogenic in mice.

In the third study, conducted by Toth et al., [Exhibit 20] TCDD was administered to outbred Swiss male mice by gavage (feeding through a tube which extends from the mouth to the stomach) in a sunflower oil vehicle at doses of 7.0 ug, 0.7 ug, and 0.007 ug TCDD/kg body weight per week over a period of a year. A matched control group of mice, which were identical to the treated animals except for the administration of TCDD were administered the sunflower oil vehicle. In conjunction with a companion study on another compound, three other control groups were also maintained.

A statistically significant increase in the incidence of liver tumors was observed in the middle dose group (0.7 ug TCDD/kg/week) when compared to the matched sunflower oil control group. The incidence of liver tumors in the middle dose group was also significantly greater than the incidence of these tumors in the pooled controls (all four control groups combined). The high dose group (7.0 ug TCDD/kg/week) had an increased incidence of liver tumors compared to the matched control group, but this increase was not statistically significant.

Discrepancies in survival between the treated groups and the control groups may have tended to increase the tumor

incidence in the middle dose group in comparison to the controls and decreased the tumor incidence in the high dose group in comparison to the controls. Therefore, CAG has concluded that the results of this study provide suggestive evidence of an oncogenic effect.

B. Promotion and Cocarcinogenicity Studies On TCDD In Laboratory Animals

A promotion study by Pitot et al. (Exhibit 574) indicates that TCDD is a very potent liver cancer promoter. In this study, some groups of rats received a single dose of diethylnitrosamine (DEN), a known complete carcinogen, for purposes of cancer initiation. All groups of rats had partial hepatectomies (removal of part of the liver) to cause cell proliferation. To test the promoting effects of TCDD, beginning seven days after treatment with DEN, two groups of DEN-treated rats received biweekly subcutaneous injection of TCDD at doses of 0.14 and 1.4 ug/kg body weight, respectively (equivalent to 0.01 and 0.1 ug/kg/day, respectively) for 28 weeks. Different control groups received no compound, DEN alone, or TCDD alone at the above doses. Compared to the control groups, which developed no liver tumors,^{*/} a highly statistically significant increase in liver cancers (hepatocellular

*/ While TCDD administered alone induced observable liver tumors at exceedingly low doses in the Kociba and NCI studies, the fact that no liver tumors were found among the control groups receiving TCDD alone in the Pitot study is not unexpected. This follows from the fact that TCDD was administered in the Pitot study for 28 weeks (rather than for approximately two years as in the Kociba and NCI studies) and from the fact that only 5 animals were used in each TCDD control group.

carcinomas) was found in the group receiving DEN and the higher TCDD dose, providing strong evidence of a promoting effect by TCDD. In addition, the presence of liver tumors (hepatocellular neoplastic nodules) among three of five animals in the group receiving DEN and the lower TCDD dose in comparison to none in the control groups is indicative of a promoting effect. Thus, TCDD has liver tumor promoting effects down to the lowest dose tested (0.01 ug/kg/day).

A study conducted by Kouri et al. (Exhibit 575) provides substantial evidence that TCDD acts as a cocarcinogen in mice when administered to mice simultaneously with 3-methylcholanthrene (MCA) (a carcinogen). In this study, a single dose of MCA was administered subcutaneously to DBA/2 Cum mice. A single dose of TCDD was administered either subcutaneously or intraperitoneally (into the abdomen) either simultaneously or 2 days before the administration of the MCA. Two dose levels of TCDD were administered (1 ug/kg and 100 ug/kg). The incidence of fibrosarcomas at the site of MCA inoculation when TCDD was administered simultaneously with MCA at the higher dose (100 ug/kg) was significantly greater than the incidence of fibrosarcomas which occurred when MCA was administered alone. Since TCDD administered alone as a single dose in controls did not result in an increase of fibrosarcomas, the study provides evidence that TCDD acts as a cocarcinogen. That is, it acts synergistically with another carcinogen to produce a carcinogenic effect greater than the carcinogenic effects of the two carcinogens added together.

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D. Potency of TCDD

The carcinogenic potency of a chemical refers to the strength of the carcinogenic effect induced by that chemical. TCDD appears to be a more potent carcinogen than aflatoxin B₁ which was previously considered to be the most potent carcinogen known. This conclusion follows from a comparison of the carcinogenic potency of TCDD in the Kociba et al. study (Exhibits 13, 14, and 15) to the carcinogenic potency of aflatoxin B₁ in a chronic study by Wogan et al. (Exhibit 40) in which aflatoxin B₁ induced hepatocellular carcinomas in male Fisher rats at an oral dose of 50 ppb in the diet. CAG estimated the respective potencies of TCDD and aflatoxin B₁ in these two studies by calculating the slope (B) of the linear one-hit model for the two compounds.^{*/} Potency (B) was calculated for each compound according to the following formula:

$$B = \frac{1}{d} \ln \frac{1 - P_c}{1 - P_t}$$

d = dose inducing a significant increase in carcinomas in the respective studies on TCDD and aflatoxin;

P_c = tumor incidence in control animals in the respective studies;

P_t = tumor incidence in treated animals in the respective studies at dose d.

^{*/} The linear one-hit model is a mathematical model which was previously used by CAG for purposes of estimating the probability that an observable case of cancer will be induced by doses of a carcinogen which are lower than doses which have empirically been demonstrated to cause carcinogenic responses. While CAG now uses a different mathematical model for estimating cancer risks (see discussion below), it is appropriate to use the linear one-hit model for estimating potency.

This calculation was made on the basis of the lowest dose level at which TCDD or aflatoxin B₁ caused a significant increase in hepatocellular carcinomas, the incidence of hepatocellular carcinomas at the respective dose levels, and the spontaneous incidence of this type of cancer in the control animals of each study. The following table (copied from Exhibit 33) shows that TCDD is approximately three times more potent a carcinogen than aflatoxin B₁ (0.110/0.032 = 3.45).^{*/}

TABLE 23. COMPARISON OF CARCINOGENIC POTENCY OF TCDD WITH AFLATOXIN

	<u>TCDD</u>	<u>Aflatoxin</u>
Author	Kociba et al.	Wogan et al.
Species	Sprague-Dawley rats	Fischer rats
Sex	Female	Male
Tumor incidence in controls (Pc)	1/86	0/18
Dose(d), Tumor incidence in treated animals (Pt)	2.2 ppb, 11/49	50 ppb, 20/25
Carcinogenic Potency (B)	0.110 (ppb) ⁻¹	0.032 (ppb) ⁻¹

^{*/} The calculation of relative potency was based only on the incidence of hepatocellular carcinomas in both studies because the classification of other liver lesions in the Wogan study was ambiguous.

D. Laboratory Animal Studies on the Carcinogenicity of 2,4,5-T

1. Rat Studies

CAG has reviewed two chronic toxicity studies involving the administration of 2,4,5-T to rats. The results of one of these studies provides highly suggestive evidence that 2,4,5-T itself (i.e., aside from any TCDD contamination) is carcinogenic in rats. The results of the other rat study do not appear to provide evidence of a carcinogenic response. Aspects of both studies limited their sensitivity for purposes of detecting an oncogenic response.

The study providing suggestive evidence that 2,4,5-T is carcinogenic in rats was conducted by scientists at Dow (Kociba et al.)^{*/} [Exhibits 21 and 22] These scientists administered specially purified 2,4,5-T containing no detectable TCDD (level of detection = 0.33 parts per billion TCDD) in the diet to groups of male and female Sprague-Dawley rats (Spartan substrain) at doses of 30, 10, and 3 mg of 2,4,5-T/kg body weight per day for up to two years. The authors concluded that no carcinogenic response was observed. However, the incidence of squamous cell carcinomas on the tongue which they observed in the high dose group males was marginally statistically significant compared to the incidence of this tumor in the controls ($p = 0.063$). In addition, the dose-related trend for the incidence of tongue

^{*/} The published report of this study and certain unpublished data from this study are appended as Exhibits 21 and 22, respectively.

carcinomas in males diagnosed by the authors is statistically significant by the Cochran Armitage test ($p < 0.03$). ^{*}/ Dr. Robert A. Squire, evaluated tongue tissues from male rats in the Kociba 2,4,5-T study at CAG's request. Dr. Squire diagnosed one more high dose male rat animal with a squamous cell carcinoma of the tongue than the author reported. (Exhibit 760 and Appendix B, Exhibit 33A). When Dr. Squire's diagnoses are considered, the incidence of squamous cell carcinomas in the high dose male rats is statistically significant compared to the incidence in the controls ($p = .025$). In addition, when the incidence of carcinomas of the tongue in the high dose males (using either Squire's or Kociba's diagnoses) is compared to the incidence of these tumors in historical control Sprague-Dawley male rats from the Dow laboratories, the incidence is highly statistically significant ($p < 0.001$). (Exhibit 576). These results provide highly suggestive evidence that specially purified 2,4,5-T induced a carcinogenic effect in rats.

The highly suggestive evidence provided by this study is particularly noteworthy in light of the fact that this study was relatively insensitive for purposes of detecting a carcinogenic effect. This insensitivity resulted from the very early mortality

^{*}/ It is assumed that the incidence of tumors induced by a carcinogen increases as exposure to the carcinogen increases. Such an effect in a cancer study is known as a dose-related trend. Observation of a dose-related trend in a cancer study, by itself, may provide suggestive evidence of a carcinogenic effect.

among all groups of males and females which reduced the number of animals at risk for late developing tumors. The insensitivity of the study was further increased by the relatively high incidence of spontaneous tumors in some organs and tissues in the control animals (e.g., hepatocellular carcinomas and hepatocellular neoplastic nodules). A high incidence of spontaneous tumors in controls makes it difficult to detect small increases in tumor incidence in the treated groups.

In the other chronic rat study on 2,4,5-T [Exhibit 23], Leuschner et al. fed commercial grade 2,4,5-T (containing 0.05 ppm TCDD) dissolved in acetone and mixed with the diet to Sprague-Dawley (SIV 50 substrain) rats at doses of 30, 10, and 3 mg 2,4,5-T/kg body weight/day for up to 130 weeks. The authors concluded that no carcinogenic effect was observed. However, a statistically significant increase in interstitial cell tumors of the testes in the high dose group as well as a significant dose-related trend for these tumors were observed when comparison is made to the matched controls, which were bred at the same laboratory as the treated animals and which were also fed the acetone vehicle.

The results are not statistically significant when the incidence of testicular tumors in the high dose group is compared to the incidence of this tumor in a non-matched control group of animals discussed in the study as well as in historical controls (provided to EPA by Leuschner, attachment to Exhibit 33A). In each of these control groups, the incidence of testicular tumors is comparable to that in the high dose group. However, the reported non-matching

controls as well as the historical controls were untreated controls, rather than vehicle (acetone) treated controls, and therefore may not be appropriate for comparison. Thus, the results of this study are equivocal.

It should be noted that the highest dose of 2,4,5-T administered in this study was apparently less than the maximum tolerated dose (MTD). Therefore, the sensitivity of this study for detecting an oncogenic effect was less than prescribed by standard protocols. Although the adequacy of the histopathological examination of the tongues in the Leuschner study is not clear at the present time,^{*/} the unusual site of tumor formation in the tongue in the Kociba study and the apparent lack of reproducibility of this tongue tumor response in the Leuschner study, reduced our judgment of the strength of evidence of the carcinogenicity of pure 2,4,5-T provided by the Kociba study from substantial to highly suggestive.

2. Mouse Studies

Several chronic mouse studies have been conducted on 2,4,5-T contaminated with low levels of TCDD. No significant increases of any specific tumor type were reported in any of these studies. However, because of deficiencies in their design, the reporting of their results, and other factors, these studies fail to demon-

*/ EPA recently requested Dr. Leuschner to cut cross-sections from the tongues of control and high dose male rats in his study which had been preserved. No sections had been made previously. The request was made by overseas telephone conversations. Due to some misunderstanding, longitudinal sections rather than cross-sections were cut.

strate that commercial 2,4,5-T is not likely to be oncogenic in mice.

Bionetics Research Laboratories conducted, as part of a large-scale cancer screening test on 140 chemicals, two sets of studies in which 2,4,5-T contaminated with TCDD was administered in daily oral doses to two strains of mice and in a single subcutaneous dose to two strains of mice. [Exhibits 24 and 25] The use of very small groups of animals in both sets of studies made the studies very insensitive for purposes of detecting an oncogenic effect. This insensitivity was increased still further by the termination of the studies after only 18 months, which is short of the two years or greater life-expectancy of mice. Moreover, the subcutaneous study was extremely insensitive since the test compound (i.e., the 2,4,5-T) was administered only once. In a properly designed oncogenicity study, the test compound is ordinarily administered every day for nearly the entire lifetime of the animals tested.

In other chronic mouse studies on commercial 2,4,5-T, Muranyi-Kovacs et al. administered 2,4,5-T orally to mice from two different strains [Exhibit 26] and subcutaneously to mice from the same two strains [Exhibit 27]. No significant increases in tumors at any specific sites were observed. However, these studies fail to provide significant evidence of the noncarcinogenicity of 2,4,5-T in mice. This conclusion for the oral study is based on the following reasons: 1) the study was very insensitive for the purpose of detecting an oncogenic effect because insufficient numbers of animals were used in the treatment groups and because the maximum tolerated dose was apparently not

used; 2) since only "macroscopically altered" tissues were preserved for histopathologic evaluation, many microscopic tumors may not have been detected; 3) some mice were arbitrarily excluded from the calculation of tumor incidence; and 4) the histology data on all animals were not available. The subcutaneous study cannot be considered as evidence of the lack of carcinogenicity of 2,4,5-T because the study was so incompletely reported that the details of its methodology are unclear. In addition, this study was very insensitive because insufficient numbers of animals were used in the treatment groups, and only a few subcutaneous doses were administered.

C. Laboratory Animal Studies on The Carcinogenicity of Silvex

The very few chronic animal toxicity studies on silvex which have been conducted have not demonstrated a carcinogenic response. However, because of their deficiencies, these studies do not constitute significant evidence of the non-carcinogenicity of silvex.

Oral and subcutaneous mouse studies on silvex were conducted by Bionetics Research Laboratories as part of the same large-scale screening test of which the Bionetics 2,4,5-T studies described above were a part. [Exhibits 24 and 25]. While no significant increase in the incidence of any type of tumor was observed in either the oral or subcutaneous study, these studies do not provide significant evidence of the non-oncogenicity of silvex because they suffered from the deficiencies described above for the Bionetics study on 2,4,5-T.

Dow conducted a study (Exhibits 28, 29 and 30) in which groups of 30 male and 30 female Dow-Wistar rats were fed diets containing Kurosal SL (potassium salt of silvex) for up to 24 months. Some animals were intentionally killed before the conclusion of the experiment. Therefore, at most, only about 21 or 22 animals of each sex in each group were maintained until the end of the experiment.*/

No significant increases in tumors were reported. However, because very small groups of animals were used and the maximum tolerated dose apparently was not used in females, this study was a very insensitive screen for carcinogenicity. Therefore, this study cannot be considered as decisive evidence of the non-carcinogenicity of silvex in rats.

Dow also conducted a study (Exhibits 29, 30, and 31) in which groups of three beagle dogs of each sex (initially four dogs) were fed silvex for two years. No increases in tumors were observed. However, this study does not constitute a valid cancer study because its duration was far less than the life expectancy of a beagle dog and because the sizes of the animal groups were exceedingly small.

*/ Because of the inadequate reporting of the results, the extent to which additional animals died before the end of the experiment cannot be determined. It is possible that considerably fewer than 21 or 22 animals survived.

III. Epidemiologic^{*/} Studies of the Carcinogenicity of
2,4,5-T and TCDD

The evidence of carcinogenicity of commercial 2,4,5-T and silvex and their TCDD contaminant provided by studies on laboratory animals is supported by epidemiologic studies of people exposed to 2,4,5-T, other chemically related herbicides (known collectively, as phenoxyacetic acids), and TCDD. Two Swedish epidemiologic studies reporting highly significant associations between a certain type of cancer and exposure to phenoxyacetic acids provide strongly suggestive evidence for the carcinogenicity of 2,4,5-T and/or TCDD. Suggestive, but weaker, evidence is provided by other epidemiologic studies.

The two Swedish epidemiologic studies providing strongly suggestive evidence of the carcinogenicity of 2,4,5-T and TCDD are both case-control studies. A case-control study is one in which a subject group (or group of cases) who share a common disease is selected at the beginning of the study. The purpose of a case-control study is to compare the cases to a control group (i.e., a group without the disease but similar to the cases with respect to factors other than the factors being studied) to determine if the cases share exposure patterns not seen in the controls. This type of study is well suited for rare diseases with long periods of induction.

^{*/} An epidemiological study is a study of the patterns and causes of disease in human populations.

In one of the Swedish case-control studies, Hardell and Sandstrom (Ref. 1) investigated the possible association in northern Sweden between sarcoma of soft connective tissue (i.e., cancer of such tissues as smooth muscle and fat) and exposure to phenoxyacetic acids and chlorophenols, whether considered separately or together. In northern Sweden, occupational exposure to phenoxyacetic acids took place in both forestry and agricultural work. Occupational exposure to chlorophenols took place mostly in sawmill work and paper pulp production.*/

In the second Swedish case-control study, the same group of epidemiologists (Eriksson et al. 1979, Ref. 2) investigated in southern Sweden the possible association between soft-tissue sarcomas and exposure to phenoxyacetic acids and chlorophenols. Occupational exposure to phenoxyacetic acids in southern Sweden was primarily through agricultural use.

In both studies, controls (people without soft tissue sarcoma) were selected to match the cases with respect to age, sex, vital status, and place of residence in order to eliminate the possibility that these factors would affect the results of the studies. In both studies, information regarding occupational exposure to phenoxyacetic acids and chlorophenols was obtained primarily through questionnaires and interviews. The investigators

*/ The principal phenoxyacetic acids used as herbicides were 2,4,5-T and 2,4-D, only the former of which is known to be contaminated with TCDD. Chlorophenols are also known to contain chlorinated dibenzodioxin.

took great care to minimize the possibility that people with sarcoma would overreport exposure to these chemicals and therefore bias the results.

The two studies indicated approximately fivefold to sevenfold increases in the risk of developing soft-tissue sarcoma among people occupationally exposed to phenoxyacetic acids and chlorophenols, whether considered singly or together, in comparison to people not exposed to these chemicals. The estimated increases in risk were highly unlikely to have resulted solely by chance.

A third case-control study by Hardell et al. (1980)(Ref. 3) provides suggestive evidence of an association between another type of cancer and exposure to 2,4,5-T and/or TCDD. In this study, the investigators found a fivefold to sixfold increase in the risk of developing malignant lymphoma (cancer of lymphatic tissue) among people exposed to phenoxyacetic acids and chlorophenols, whether considered separately or together. The reliance which can be placed on this study at this time is limited somewhat by the fact that the methods and results were incompletely documented in the published report of the study. However, the researchers have indicated that an additional report of this study is in preparation.

Two cohort studies of people occupationally exposed to phenoxyacetic acids and/or to TCDD provide suggestive evidence that 2,4,5-T and/or TCDD may cause stomach cancer in humans. A

cohort study is one in which a group of individuals with a known history of exposure to the factor being investigated (phenoxyacetic acids in one study and TCDD in the other) is followed over time to see if any adverse health effects develop in comparison to a control group of unexposed or less highly exposed persons.

In one of these cohort studies (Axelson, et al. 1980, Ref.4), 348 Swedish railroad workers with at least 46 days of herbicide exposure (greater than one spray season) between 1955 and 1972 were followed through October 1978. The workers were grouped on the basis of their primary herbicide exposure; those previously exposed to phenoxyacetic acids (2,4,5-T and 2,4-D) only, to amitrole (aminotriazole) only, and to both types of herbicides. Workers with primary exposure to phenoxyacetic acids only showed a mortality rate from stomach cancer that was six times higher than expected from Swedish national mortality rates specific for age, sex, and calendar year. Even though this was not likely to be a chance finding ($p = 0.044$), firm conclusions about the carcinogenicity of 2,4,5-T and TCDD cannot be drawn from this study because the number of stomach cancer deaths observed was small.

In the second cohort study, Thiess and Frentzel-Beyme (1977) (Ref. 5) followed 75 workers exposed to TCDD during and after a 1953 runaway reaction at a trichlorophenol manufacturing facility in Ludwigshafen, West Germany. These workers had a

sevenfold to eightfold increase in the rate of stomach cancer mortality in comparison to the expected rate ($p < 0.05$). Again, however, firm conclusions cannot be drawn from this study because the number of stomach cancer deaths observed was relatively small.

In another cohort study (the "Nitro study"), workers exposed to TCDD in a trichlorophenol processing accident in Nitro, W.Va. (Zack and Suskind, 1980, Ref. 6) were followed. A threefold increase in deaths from cancers of the lymphatic and hematopoietic system was found in comparison to the expected rate ($p = 0.06$), but this result also was based on a small number of observed deaths.

In addition, single deaths from soft-tissue sarcoma were found in the Nitro study and in a Midland, Michigan study on workers exposed to TCDD in the manufacture of trichlorophenol (Cook, et al. 1980, Ref. 7). The authors of the Nitro study noted this type of cancer as rare. These findings are consistent with a carcinogenic effect from the TCDD exposures in both studies.

These, as well as two additional cohort studies, reported results that did not show increased stomach cancer mortality rates in groups of workers exposed to phenoxyacetic acids and/or TCDD. The additional two studies were of 2,4,5-T herbicide production workers in Midland, Michigan (Ott, et al. 1980, Ref. 8) and Finnish phenoxyacetic acid herbicide applicators (Riihimaki, et al. 1978, Ref. 9). Because the Nitro study and

the two Midland studies had insufficient statistical power for purposes of detecting the increased stomach cancer risks suggested by the Swedish and West German cohort studies discussed above, they should not be considered to be inconsistent with those studies.

The Finnish study reported a larger number of expected cancer deaths than the Nitro or the two Midland studies and therefore was more powerful than those studies for purposes of detecting small increases in risk. However, the Finnish study was designed to include subjects who were exposed to herbicides for a considerably shorter period of time than the Swedish cohort study discussed above (Ref. 4). In addition, certain inconsistencies in the data from the Finnish study will require clarification in order for the results to be adequately interpreted.

IV. Conclusions and Qualitative Risk Assessment of the Carcinogenicity of TCDD, 2,4,5-T and Silvex

The laboratory animal data provide substantial evidence that TCDD is likely to pose a carcinogenic risk to exposed humans as a very potent carcinogen and as a very potent cancer promoter, and also provide highly suggestive evidence that essentially pure 2,4,5-T is a carcinogen. Evidence that TCDD is a potent carcinogen is provided by the Kociba study on rats, and the NCI studies on both rats and mice. These studies indicate that TCDD administered alone at exceedingly low doses induces a carcinogenic response in several organs. On the basis of the Kociba study, it appears that TCDD is one of the most potent carcinogens known.

The highly suggestive evidence that essentially pure 2,4,5-T is carcinogenic is provided by the Kociba 2,4,5-T study.

Strongly suggestive human evidence that 2,4,5-T and/or TCDD pose a carcinogenic risk is provided by the two Swedish epidemiological studies demonstrating a strong association between increased risk of developing soft tissue sarcoma and exposure to 2,4,5-T and other phenoxyacetic acids. Suggestive but weaker evidence of increased cancer risk is provided by other epidemiological studies.

The laboratory animal data and the epidemiological data considered as a whole provide very substantial evidence that TCDD is carcinogenic in human and strongly suggestive evidence that essentially pure 2,4,5-T is carcinogenic in humans.

In accordance with the no-threshold concept, TCDD, as a carcinogen, poses a human cancer risk at any level of exposure no matter how small; the degree of cancer risk posed by TCDD is directly proportionate to the level of exposure to TCDD.

The evidence that TCDD is a potent cancer promoter and is a cocarcinogen is provided by the Pitot and Kouri studies, respectively. These studies demonstrated that very low doses of TCDD significantly enhanced the induction of liver and skin tumors by other carcinogens in rats.

The human population is exposed to a large number of carcinogens in the environment. Therefore, it is possible that exposure to a potent promoter such as TCDD could increase the number of cancers induced by environmental carcinogens and shorten the latency period for the development of cancer. This enhanced

induction of tumors by TCDD acting as a promoter would be in addition to the tumor induction by TCDD as a carcinogen. There is no theoretical basis for making even ballpark estimates of the risk posed by promoters and cocarcinogens to exposed persons because the mechanism for promotion is not well understood and because the degree of total exposure of the human population to the numerous carcinogens in the environment cannot be well quantified. However, it is possible that TCDD could significantly increase human cancer as a promoter or cocarcinogen at exceedingly low levels of TCDD exposure.

The question arises as to whether the carcinogenic action of TCDD by itself such as exhibited in the studies of Kociba and the NCI studies on rats and mice could be due to the action of TCDD as a carcinogen and/or a promoting agent. There is evidence that TCDD can be metabolized to an electrophilic metabolite (Ref. 10) which could react with DNA and thereby produce genetic damage of the sort that is associated with the induction of cancer. However, the reactivity of this metabolite with cellular protein is extremely high and, to date, the degree of interaction with DNA that has been demonstrated is low. This may be peculiar to the tissues that have been examined for this reaction so far but may not be generally applicable to the reaction of TCDD with DNA in the body. Furthermore, TCDD has a chemical structure which makes it likely that it could intercalate into DNA and also act as a genotoxic carcinogen (Exhibit 33A). Promoting agents, when administered alone, characteristically produce a relatively small increase in the occurrence of tumors

and those tumors that are increased seem to be of the type that would be expected to occur spontaneously as the result. This is not characteristic of TCDD, particularly in relation to its ability to induce squamous carcinomas of the lung and of the hard palate and turbinates. Squamous carcinomas of the lung are exceedingly uncommon in the rat in contrast to adenomas of the lung. For these reasons the CAG believes that it is prudent, given the present state of knowledge, to regard TCDD as a complete carcinogen as well as a promoting and cocarcinogenic agent.

Commercial 2,4,5-T and silvex pose a carcinogenic risk to exposed humans because they contain low levels of TCDD as a contaminant. In addition, based on the highly suggestive evidence of the Kociba study on specially purified 2,4,5-T, CAG concludes that the pure 2,4,5-T component of commercial 2,4,5-T may pose a cancer risk to humans at any level of exposure.

V. Quantitative Risk Assessment of TCDD and 2,4,5-T

A. General

In this part of my testimony, I summarize CAG's quantitative estimates of the carcinogenic risks posed to humans by certain sources of exposure to TCDD from the use of commercial 2,4,5-T or silvex in the U.S. CAG assessed the risks posed by TCDD only as a complete carcinogen, not as a promoter or cocarcinogen, because as discussed above there is no basis for quantitatively estimating the risks posed by cancer promoters or cocarcinogens.

However, it should be noted that the risks posed by TCDD as a promoter or cocarcinogen may be higher than those risks which have been calculated by CAG.

I also summarize CAG's quantitative estimates of the carcinogenic risks which may be posed by certain sources of exposure to the pure 2,4,5-T component of commercial 2,4,5-T. It should be kept in mind that the evidence for the carcinogenicity of essentially pure 2,4,5-T is only highly suggestive, not substantial.

CAG's quantitative estimate of human cancer risks posed by TCDD and 2,4,5-T were derived from dose-response data from the laboratory animal cancer studies on these compounds and from estimates of human exposure to these compounds. CAG used Dr. Squire's diagnoses of tumor incidences in the rats from Dr. Kociba's TCDD and 2,4,5-T studies in making its risk estimates. Exposure estimates used by CAG in its risk assessment were those provided by the Agency's Hazard Evaluation Division (HED) (See, Appendix F to Exhibit 33A).

In making quantitative estimates of cancer risks from human exposure information and from dose-response data provided by animal cancer studies, CAG uses a mathematical risk extrapolation model. The mathematical model used by CAG in its risk assessment of 2,4,5-T and TCDD was the linearized multistage extrapolation model, which was recently adopted by CAG in response to public comments expressing dissatisfaction with the "one-hit" extrapo-

lation model previously used by CAG.^{*/} The linearized multistage model has also been adopted by the National Academy of Sciences Drinking Water Panel.

The linearized multistage model is based in the assumption of a linear non-threshold dose response relationship at low doses. Further details describing this model are provided in the CAG Report (Exhibit 33A) and in the document, "Procedure for Calculating Air Quality Criteria" which was prepared by CAG and is attached as Appendix G to the CAG Report (Exhibit 33A).

It should be noted that CAG made estimates of risk for only some routes of exposure to TCDD and 2,4,5-T. CAG's analysis does not attempt to assess cancer risks resulting from all routes of exposure to these compounds because, as explained in HED's exposure analysis quantitative exposure estimates could be made for only some potential exposure routes.

It should also be noted that HED expressed in its exposure analysis document certain qualifications and reservations concerning the quantified exposure analyses contained in that document. CAG's quantitative risk estimates should be viewed in light of these qualifications and reservations.

^{*/} The risk estimates calculated by CAG for TCDD, 2,4,5-T, and silvex by the linearized multistage model are approximately the same as those which would be calculated by the use of the one-hit model. However, the linearized multistage model is methodologically more sound than the one-hit model because it gives a more systematic way of utilizing all the available data.

B. Quantitative Risk Assessment in General

Before I summarize CAG's quantitative risk assessment with respect to 2,4,5-T and silvex, I would like to discuss the functions and limitations of quantitative risk assessments in general.

The purpose of a quantitative risk assessment is to provide Agency decisionmakers with a rough approximation of the degree of risk posed by exposure to a carcinogenic compound. It will be clear from the discussion below that one cannot hope to generate an estimate of the degree of risk posed by a compound that is more accurate than a crude, "ball-park" figure. A quantitative risk assessment can give a decisionmaker a feel for the degree of risk posed by one compound in comparison to the risks posed by other compounds. Agency decisionmakers are aware that, although CAG expresses its risk assessments in terms of specific numbers, those numbers probably do not describe with precision what will happen in the real world.

The principal reasons why quantitative risk assessments can be only rough approximations of risk are the following:

1. Mathematical models for such assessments involve extrapolating from results observed in laboratory experiments in which animals are exposed to a compound to situations in which people will be exposed to the compound. This procedure introduces a number of variables whose impact can be substantial. First, there are uncertainties about the relative sensitivities of

different species (e.g., rats and people) to carcinogenic compounds. Moreover, there are variations in sensitivity to carcinogens within each species.

2. Extrapolation from high doses administered to experimental animals to relatively low exposures likely to be experienced by people may be inaccurate because of uncertainties about the shape of the dose-response relationship. The mathematical model adopted by CAG for risk extrapolation is intended to provide a reasonably conservative assessment of risk.

3. Human exposure to the compound, an essential part of a quantitative risk assessment, often can be estimated only roughly.

4. Quantitative risk assessments based solely on animal data relate to individual compounds and do not take into account the fact that these compounds may act as promoters or have synergistic effects. This is an especially serious limitation when strong evidence exists, as in the case of TCDD, that the chemical under consideration is a potent cancer promoter and is a cocarcinogen.

B. Summary of CAG's Quantitative Risk Estimates

The quantitative risk assessment for lifetime cancer risks from exposure to the TCDD contaminant of commercial 2,4,5-T and silvex and to 2,4,5-T itself have been estimated for worker exposure and to a limited extent, for food contamination. In estimating lifetime cancer risks from the linearized multistage

model, CAG assumed that the general population and local populations would be exposed to dietary sources of TCDD over a 70 year lifetime and that applicators of 2,4,5-T or silvex would be occupationally exposed over a 40 year period. Although the risk estimates are expressed as numbers, they should be considered to be only very rough estimates of risk and subject to the qualifications and limitations discussed above.

It should be noted that CAG's risk estimates took into account the percentage of total acres for each use which were in fact treated according to recent useage patterns. For almost all uses, only a very small percentage of the total acreage which could potentially be treated consistently with the pesticide labeling have in fact been treated. If the percentage of acreage treated were to increase in the future, the estimated cancer risks to the general population posed by food contaminated with TCDD or 2,4,5-T would increase proportionately. Similarly, the number of workers applying 2,4,5-T and silvex and therefore exposed to these compounds as well as TCDD would increase proportionately.

The individual lifetime risks of developing cancer which are projected for workers involved in the application of 2,4,5-T or silvex in forestry, range, rice, and rights-of-way uses are provided in Tables QA-19 and QA-20 in the CAG Report (Exhibit 33A). These estimates are based on the assumption that the workers do not generally wear special protective clothing.

Workers involved in different uses may have different risks because the average application rates and therefore average exposures may differ. In addition, different categories of workers within each use may have different risks.

Workers involved in forestry application of 2,4,5-T are projected to have individual lifetime risks of developing cancer ranging from 10^{-4} up to 3×10^{-3} , depending on the category of worker.

Workers involved in the application of 2,4,5-T to rights-of-way are projected to have individual lifetime risks of developing cancer ranging from 2×10^{-4} to 5×10^{-3} . Most categories of workers for this use have risks greater than 10^{-3} .

Workers involved in the application of 2,4,5-T to rangelands have projected risks ranging from 7×10^{-8} for flaggers to 2×10^{-4} for mixer-loaders. Projected risks for workers involved in 2,4,5-T application on rice fields range from 7×10^{-8} to 8×10^{-5} .

Each of the projected risks described above take into account the risk posed by TCDD as well as the possible risk posed by the pure 2,4,5-T component of commercial 2,4,5-T. The contribution of each of the two components to the total risk are approximately equal. Since pure silvex has not been demonstrated to be carcinogenic, the risks to workers who apply silvex are estimated only on the basis of the TCDD component of silvex. Thus, the projected individual lifetime risks of developing cancer for silvex applicators are about half of those for 2,4,5-T applicators.

The number of applicators in each category for each use are provided in Tables QA-19 and QA-20 of Exhibit 33A.

TCDD contamination of beef resulting from the use of 2,4,5-T and silvex on rangeland and directly treated pasture is projected to result in individual lifetime risks of developing cancer of approximately 2×10^{-4} in local populations which exclusively consume beef produced locally on grazing land treated every five years with silvex or 2,4,5-T. It is estimated that combined size of such local populations could be as high as 12,000 persons. Individual lifetime cancer risks in the general population of the U.S. resulting from consumption of beef contaminated with TCDD beef is projected to be approximately 2×10^{-6} . This route of exposure is projected to result in approximately 8 cases of cancer per year among the general population.

It is estimated that TCDD contamination of dairy products resulting from the use of 2,4,5-T or silvex on pastures may result in individual lifetime risks of developing cancer as high as 5×10^{-4} . The percentage of dairy cattle grazing on 2,4,5-T or silvex treated pasture could not be estimated. Therefore, risks to the general population resulting from consumption of dairy products contaminated with TCDD could not be estimated.

It is estimated that TCDD contamination of deer or elk meat as a result of the use of 2,4,5-T or silvex on forests may result in individual lifetime risks of developing cancer as high as 10^{-4} and 3×10^{-5} if twelve meals of deer meat and elk meat, respectively, are consumed each year for a lifetime. More or less consumption would lead to proportionate increases or decreases.

Roy E. Albert by RPS
Roy E. Albert, M.D.

EXHIBITS

EXHIBIT NO.

- *13 - Kociba, R.J., Keyes, D.G., Carreon, R.M., Wade, C.E., Dittenber, D., Kalnins, R., Frauson, L., Park, C.N., Hummel, R., and Humiston, C.G. 1978a. Results of a Two-year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Rats. Toxicol. Appl. Pharmacol. 46:279-303
- *14 - Kociba, R.J. 1979a. Letter and three tables sent to H. Warnick, Special Pesticide Review Division, U.S. Environmental Protection Agency, from R.J. Kociba, Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical U.S.A., Midland, Mich. February 20, 1979
- *15 - Kociba, R.J., Keyes, D.G., Carreon, R.M., Wade, C.E., Dittenber, D., Kalnins, R., Frauson, L., Park, C.N., Hummel, R., and Humiston, C.G. 1977. Results of a Two-year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Rats. Unpublished study. Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical, U.S.A., Midland, Mich. September 28, 1977
- *16 - Squire, R.A., and Levitt, M.H. 1975. Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Res. 35:3214-3215
- *18 - Hirota, N., and Williams, G.M. 1979. Persistence and growth of rat liver neoplastic nodules following cessation of carcinogen exposure. J. Natl. Cancer Inst. 63:1257-1265
- *20 - Toth, K., Somfai-Relle, S., Sugar, J., and Bence, J. 1979. Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxy-ethanol containing dioxin and of pure dioxin in Swiss mice. Nature 278:548-549
- *21 - Kociba, R.J., Keyes, D.G., Lisowe, R.W., Kalnins, R.P., Dittenber, D.D., Wade, C.E., Gorzinski, S.J., Mahle, N.H., and Schwetz, B.A. 1979b. Results of a two-year chronic toxicity and oncogenic study of rats ingesting diets containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Food Cosmet. Toxicol. 17:205-221
- *22 - Kociba, R.J., et al. 1978b. Results of a Two-Year Chronic Toxicity and Oncogenesis Study of Rats Ingesting Diets Containing 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T). Unpublished study. September 27, 1978

Asterisks (*) indicate Exhibits which have been previously transmitted with the testimony of Dr. Dawn G. Goodman.

EXHIBIT NO.

- *23 - Leuschner, F., Leuschner, A., Hubscher, F., Dontenwill, W., and Rogulja, P.V. 1979. Chronic Oral Toxicity of 2,4,5-T, Batch No. 503, Control No. 153574 b--Called for Short '2,4,5-T' -- in Sprague-Dawley (SIV 50) rats. Unpublished study. Laboratorium fur Pharmakologie and Toxikologie, Hamburg. April 9, 1979. Pp 1-29, 39-41
- *24 - Bionetics Research Labs, Inc. 1968. Evaluation of Carcinogenic, Teratogenic, and Mutagenic Activities of Selected Pesticides and Industrial Chemicals. Vol. 1. Pp 1-53, 57, 67, 75, 97-110, 225-228
- *25 - Innes, J.R.M., Ulland, B.M., Valerio, M.G., Petrucelli, L., Fisbein, L., Hart, E.R., Pallotta, A.J., Bates, R.R., Falk, H.L., Gart, J.J., Klein, M., Mitchell, I., and Peters, J. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. 42:1101-1114
- *26 - Muranyi-Kovacs, I., Rudali, G., and Imbert, J. 1976. Bioassay of 2,4,5-trichlorophenoxyacetic acid for carcinogenicity in mice. Br. J. Cancer 33:626-633
- *27 - Muranyi-Kovacs, I., et al. 1977. Study of the Carcinogenicity of 2,4,5-T in Mice. Paper presented to the Fourth Meeting of the European Association for Cancer Research. September 13-15, 1977
- *28 - Dow Chemical Company. 1965. Results of Two-Year Dietary Feeding Study in Rats of Kurosai SL (Potassium Salt of Silvex) Unpublished study. Pp 1-14, 38, 43-46, 73-95.
- *29 - Gehring, P.J., and Betso, J.E. 1978. Phenoxy acids: Effects and fate in mammals. In Ramel, C., ed. Chlorinated phenoxy acids and their dioxins. Ecol. Bull. (Stockholm) 27:122-133
- *30 - Mullison, W.R. 1966. Some toxicological aspects of silvex. Presentation at the Southern Weed Conference, Jacksonville, Fla., January 18, 1966
- *31 - Dow Chemical Company. 1965. Results of a Two Year Dietary Feeding Study in Dogs of Kurosai SL (Potassium Salt of Silvex). Unpublished study. Pp 1-5.

Asterisks (*) indicate Exhibits which have been previously transmitted with the testimony of Dr. Dawn G. Goodman.

EXHIBIT NO.

- 33A - Carcinogen Assessment Group. EPA. Risk Assessments on 2,4,5-T, silvex, and TCDD. June 16, 1980.
- 34 - U.S. Environmental Protection Agency. Interim procedures and guidelines for health risk and economic impact assessments of suspected carcinogens. 41 F.R. 21402-21405 (May 25, 1976).
- 35 - Albert, R.E., R.E. Train, and E. Anderson 1977. Rationale Developed by the Environmental Protection Agency for the Assessment of Carcinogenic Risk. J. Natl. Cancer Inst. 58(5) 1537-1541.
- 36 - Work Group on Risk Assessment of the Interagency Regulatory Liaison Group (IRLG). 1979. Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks. 44 F.R. 39858-39879 (July 6, 1979).
- 37 - Farber, E. 1976. Putative precursor lesions: summary and some analytical considerations. Cancer Research 36:2703-2705
- 40 - Wogan, G. et al. 1974. Carcinogenic effects of low dietary levels of aflatoxin B₁ in rats. Food Cosmet. Toxicol. 12: 681-685
- 574 - Pitot, H.C., T. Goldsworthy, and A. Poland. Promotion by 2,3,7,8-Tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Submitted for publication to Cancer Research.
- 575 - Kouri, R.E., et al., 1978. 2,3,7,8-tetrachlorodibenzo-p-dioxin as cocarcinogen causing 3-methylchloronothrene-initiated Subcutaneous Tumors in Mice Genetically "Nonresponsive" at Ah Locus. Cancer Research 38: 2777-2783.
- 576 - Historical Control Data on Incidence of Squamous Cell Carcinomas of the Tongue in Male Sprague-Dawley Rats from Dow Laboratories.
- 577 - National Cancer Institute. Bioassay of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin for Possible Carcinogenicity (Gavage Study). U.S. Dept. of Health and Human Services Publication No. (NIH) 80-1765. 1980.
- 578 - National Cancer Institute. Bioassay of 2,3,7-8-tetrachloro-dibenzo-p-dioxin for possible carcinogenicity (dermal study) U.S. Dept. of Health and Human Services Publication No. (NIH) 80-1757. 1980.

REFERENCES

1. Hardell, L. and Sandstrom, A. 1979. Case-control study: softtissue sarcomas and exposure to phenoxyacetic acids and chlorophenols. Br. J. Cancer 39: 711-717.
2. Eriksson, M. Hardell, L., Berg, N.O., Moller, T. and Axelson, O. 1979. Case-control study on malignant mesenchymal tumors of the soft tissue and exposure to chemical substances. Lakartidningen 76: 3872-3875 (Translation).
3. Hardell, L., Eriksson, M. and Lenner, P. 1980. Malignant lymphoma and exposure to chemical substances, especially organic solvents, chlorophenols and phenoxy acids. Lakartidningen 77: 208-210 (Translation).
4. Axelson, O., Sundell, L., Andersson, K. Edling, C., Hogstedt, C. and Kling, H. 1980. Herbicide exposure and tumor mortality: an updated epidemiological investigation on Swedish railroad workers. Sc. and J. Work Environ. Health (in press).
5. Thiess, A.M. and Frentzel-Beyme, R. Mortality study of persons exposed to dioxin following an accident which occurred in the BASF on 13 November 1955. Proceedings of MEDICHEM Congress V, San Francisco, September 5-9, 1977 (in press).
6. Zack, J.A. and Suskind, R.R. 1980. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J. Occup. Med. 22: 11-14.
7. Cook, R.R., Townsend, J.C., Ott, M.G., and Silverstein, "Mortality Experience of Employees Exposed to 2,3,7,8-Tetra chlorodibenzo-p-Dioxin (TCDD)," (Pre-publication copy) (1980).
8. Ott, M.G., Holder, B.B. and Olson, R.D. 1980. A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. J. Occup. Med. 22: 47-50.
9. Riihimaki, V., Asp, S., Seppalainen, A.M. and Hernberg, S. 1978. Symptomatology, morbidity and mortality experience of chlorinated phenoxy acid herbicide (2,4-D; 2,4,5-T) sprayers in Finland. A clinical and epidemiological study. Working paper for an IARC working group meeting on coordination of epidemiological studies on the long-term hazards of chlorinated dibenzodioxins and chlorinated dibenzofurans, Lyon, France, January 10-11, 1978.
10. Guenther, T., Fysh, J. and Nebert, D.W. 1979. 2,3,7,8-Tetra-chlorodibenzo-p-dioxin: covalent binding of reactive metabolic intermediates principally to protein in vitro. Pharmacology 19: 12-22.

- *11. Bionetics Research Labs, Inc. 1968. Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. Vol. I.
- *12. Leuschner, F., Leuschner, A., Hubscher, F., Dontenwill, W. and Rogulja, P.V. 1979. Chronic oral toxicity of 2,4,5-T, Batch No. 503, Control No. 153574 b - called for short '2,4,5-T' in Sprague-Dawley (SIV 50) rats. Unpublished study. Laboratorium fur Pharmakologie and Toxikologie, Hamburg. April 9, 1979.

*/These references were used as references 1 and 6 in the direct testimony of Dr. Dawn G. Goodman.

CERTIFICATE OF SERVICE

I hereby certify that copies of the foregoing DIRECT
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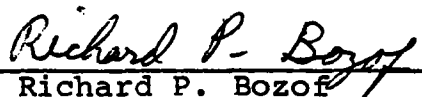
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21633

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
BEFORE THE ADMINISTRATOR

MAR 2 1980

In re:

The Dow Chemical Company, et al.

FIFRA Docket Nos. 415, et al.

DIRECT TESTIMONY OF MR. RONALD THOMAS */

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*/ This is the first of two documents containing Mr. Thomas' direct testimony. A portion of Mr. Thomas' testimony contains data which Dow claims to be confidential. This testimony is presented in a second document which has been filed UNDER SEAL for use in camera, and served only on the Administrative Law Judge, the Hearing Clerk, and the Dow Chemical Company.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
BEFORE THE ADMINISTRATOR

In re:

The Dow Chemical Company, et al.

)
) FIFRA Docket Nos. 415, et al.
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DIRECT TESTIMONY OF MR. RONALD THOMAS

INTRODUCTION

. My name is Ronald Thomas. I am currently employed as a supervisory chemist in the Residue & Special Projects Unit, Chemistry Section, Chemical & Biological Investigations Branch, Benefits & Field Studies Division, Office of Pesticide Programs, Environmental Protection Agency. Since 1956 I have been employed as an analytical chemist by federal regulatory agencies, namely the Food and Drug Administration, the U.S. Department of Agriculture, and the Environmental Protection Agency. Much of my career as a chemist has been in the field of pesticide residue analysis, and I currently specialize in that field. My curriculum vitae with a list of some of my publications is attached to the end of this statement.

My testimony is intended to describe the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in commercial samples of 2,4,5-T and silvex products. Based on the work

done in my own laboratory, EPA contract laboratories, and others, I have concluded that commercial 2,4,5-T and silvex products manufactured in the United States in 1977-78 routinely contain TCDD at levels in the 10-30 ppb range. Dow discovery documents demonstrate the presence of TCDD in some batches of 2,4,5-T at levels higher than 30 ppb during this time period.

HISTORICAL BACKGROUND

My laboratory is responsible for investigating and attempting to resolve various analytical problems for the Office of Pesticide Programs, including analysis of air, soil, water and commodity samples for pesticide residues, and evaluation or development of analytical methodologies for detection and quantification of pesticide residues. From 1970 to the present, we have been involved in the analysis of the dioxin content of pesticide products, including the analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in 2,4,5-T and silvex products.

The work in my laboratory has entailed the clean-up of samples by chromatographic means and the use of gas chromatography (GC) and low-resolution mass spectrometry (MS) for dioxin detection.^{1/} The limit of detection for dioxin achieved by the equipment in my laboratory is 0.1 ppm, with a margin of error of approximately 20% in the determinations. Between 1970 and 1978, my laboratory screened several hundred pesticide products for dioxin content. Dioxin levels greater than 0.1 ppm

^{1/} A short discussion of the theoretical basis of mass spectrometry is provided in Appendix A.

were detected in pre-1975 samples; however, for pesticide products manufactured after 1975, we did not find any samples with a dioxin content exceeding 0.1 ppm (100 ppb). The work performed in my laboratory from 1970-1972 is detailed in a paper published in 1972 (Exhibit A).

In 1977-78, the methodology for dioxin detection improved, and equipment became available which allowed a limit of detection of 0.01 ppm (10 ppb) for low-resolution GS/MS. In 1978, the Office of Pesticide Programs (OPP) undertook a project to determine the levels of dioxin in technical grade pesticide products likely to contain TCDD, including 2,4,5-T and silvex. Since my laboratory did not have the equipment to detect dioxin below the 0.1 ppm level, OPP entered into a contract with Gulf South Research Institute (GSRI), which had the equipment and the technical expertise to detect dioxin at a 0.01 ppm limit of detection level. My laboratory conducted an on-going quality control program for the GSRI contract throughout the duration of the contract. In this capacity, we had the responsibility of advising the contractor on the appropriate methodology, techniques and reporting methods; transmitting coded pesticide samples and reference samples for analysis; reviewing the results of the analytical determinations (for the reference samples and duplicates of the samples) while the study was in progress to assure that quality control was maintained; and providing any needed assistance and advice for the project.

On the basis of this monitoring program, we were able to conclude with great assurance that the contract and the analytical work had been carried out in a proper scientific manner commensurate with the state of the art of low resolution dioxin analysis. The results of the study, which will be discussed in greater detail in my ensuing testimony, establish that TCDD is present in commercial technical grade 2,4,5-T and silvex manufactured from 1977-78 at levels ranging from 0.01-0.03 ppm (10-30 ppb).

GULF SOUTH RESEARCH INSTITUTE (GSRI) STUDY
ON TCDD LEVELS IN TECHNICAL GRADE
2,4,5-T AND SILVEX AT THE PPB LEVEL

In 1978-1979, Gulf South Research Institute (GSRI), New Orleans, Louisiana, analyzed technical grade 2,4,5-T and silvex for TCDD at the 10 ppb level under an EPA contract.^{2/} The project was carried out under the direction of Joseph G. Montalvo, Jr., James F. Ryan, III, and Roy Flagg; the EPA Project Officer for the contract was Patricia Ott, Environmental Fate Branch of the Hazard Evaluation Division. The quality control for the contract was monitored by the EPA Residue & Special Projects Unit, under my supervision. The samples for the analysis were procured by the Enforcement Division of the Environmental Protection Agency from the manufacturing plants of the major manufacturers of the pesticide products in 1977-1978;

^{2/} In addition to various 2,4,5-T and silvex products, the study included the analysis of 2,4,5-trichlorophenol, an organophosphate, and a chlorinated phenolic antiseptic for TCDD content.

all the samples under study were taken from production tanks, bins or drums. The samples were coded by EPA in order to keep the sample contents anonymous; decoding information was provided to the Gulf South Research Institute only after completion of the assays and reporting of the TCDD data.

GSRI used low resolution gas chromatography/mass spectrometry (GC/MS) to determine the total TCDD content in technical grade samples of 2,4,5-T, the isooctyl ester of 2,4,5-T, the butoxyethyl ester of 2,4,5-T, the butoxypropyl ester of silvex and the isooctyl ester of silvex. A copy of the final contract report submitted by GSRI is attached (Exhibit B). The analytical method which was used to determine the levels of TCDD in the pesticide samples is described in detail in Exhibit B.

In order to assess dioxin recovery, an aliquot of a standardized radioactive solution of ^{37}Cl -2,3,7,8-TCDD was added to each sample prior to the sample clean-up procedure. The samples were prepared for analysis by caustic potassium hydroxide saponification of the pesticide followed by extraction of the neutral TCDD into hexane. The hexane solution was cleaned-up by extracting several times with sodium hydroxide solution and sulfuric acid, and was then dried, concentrated and chromatographed on alumina. The eluate was concentrated under nitrogen for GC/MS analysis.

The total TCDD in the technical grade samples was determined by low resolution gas chromatography/mass spectrometry

using a Hewlett-Packard 5985 GC/MC in the electron ionization source configuration. The standard addition injection technique was used and selective ion monitoring at 320/322 m/e^{3/} was performed for all samples. Complete mass spectra over the 150-350 range were obtained for all samples which gave a positive selective ion monitoring (SIM) scan. Six qualitative criteria for TCDD detection were developed to delineate the likelihood of TCDD detection in the analytical measurement system; these criteria are summarized in Table I of Exhibit B. For positive TCDD detection the criteria included (1) a recovery of ³⁷Cl-TCDD between 50 and 120 percent, inclusive; (2) both 320 and 322 m/e peaks at the TCDD retention time region; (3) well-defined 320 and 322 m/e peaks whose retention time exactly equals that of TCDD standards; (4) a signal-to-noise ratio of at least 2.5 times background at m/e 322; (5) a ratio of 320/322 m/e peak areas in the proper isotopic proportion and (6) TCDD confirmation by molecular ion spectra. The confidence in the TCDD detection was highest if all six criteria were met, and decreased as fewer criteria were met.

Samples were processed in sets which included internal standards and EPA supplied quality controls.^{4/} When the recovery

3/ TCDD gives a characteristic 320/322 m/e ratio; the presence of the expected 320/322 ratio provides a strong indication of the presence of TCDD, if other necessary criteria are met.

4/ TCDD standards in benzene were supplied by Aubry Dupuy, Toxicant Analysis Center, EPA.

fell within the specified contract range of 50-120% for a given sample, a duplicate assay was not performed; in cases where the recovery fell outside the specified range, a duplicate assay on an aliquot of the extract was performed. Contract requirements were satisfied if the average of the two recovery values fell within the specified range. Where the average recovery fell outside the specification range for the two aliquots, the original sample was reextracted and assayed again. The submission of at least two coded duplicates by my laboratory for each sample assured that the TCDD determinations were each based on a minimum of two duplicate analyses.

The contract did not require GSRI to report detectable values that fell below 10 ppb. Thus, although the mean detection limit achieved by GSRI was 4.7 ± 3.7 ppb, any result below 10 ppb was reported as non-detectable. In some samples, however, the presence of TCDD was indicated at levels below 10 ppb even though these samples were reported as non-detectable for TCDD at the 10 ppb level. The results of the study are summarized in Table I.

TABLE I
SUMMARY OF GSRI STUDY

PESTICIDE	NO. OF SAMPLES	NO. OF SAMPLES (PERCENT)		
		POSITIVE	DOUBTFUL [*]	NON-DETECTABLE
2,4,5-T	3	1(33.3%)	1(33.3%)	1(33.3%)
butoxyethyl ester of 2,4,5-T	10	9(90%)	1(10%)	0
isooctyl ester of 2,4,5-T	3	0	3(100%)	0
butoxyethyl ester of silvex	7	6(86%)	1(14%)	0
isooctyl ester of silvex	1	1(100%)	0	0

^{*}/ A sample was judged doubtful if it did not meet all the six criteria for positive TCDD detection.

TCDD was detected at levels ranging from 10 to 30 ppb in 9 of the 10 samples of the butoxyethyl ester of 2,4,5-T, in 1 of the 3 samples of 2,4,5-T, and was not detectable in three samples of the isooctyl ester of 2,4,5-T. For the silvex samples, TCDD was detected in 6 of 7 samples of the butoxypropyl ester and in the one sample of the isooctyl ester of silvex at levels of 10-30 ppb.

The results of the GSRI study are consistent with studies on 2,4,5-T and its derivatives performed, using methods similar to those employed by GSRI, by Buser and Bosshardt in 1974 and by Dow (Ramstad, Mahle, and Matalon) in 1977. The

Buser and Bosshardt study, which describes a gas chromatographic method for TCDD determination in technical 2,4,5-T (with confirmation by mass spectrometry of samples with a TCDD content greater than 100 ppb) and a gas chromatography/mass spectrometry method for TCDD determination in a 2,4,5-T alkyl ester and a 2,4,5-T amine salt, is attached as Exhibit C. Table II contains a summary of the results of the Buser and Bosshardt study. The TCDD content of these 2,4,5-T products ranged from 2 ppb to approximately 2000 ppb (or 2 ppm).

TABLE II
SUMMARY OF BUSER AND BOSSHARDT STUDY

<u>Pesticide</u>	<u>No. of Samples</u> ^{a/}	<u>TCDD Content (ppb)</u>
2,4,5-T (obtained from a German manufacturer prior to 1971)	3	300-600
2,4,5-T (obtained from a German manufacturer after 1971)	4	40-80
2,4,5-T (obtained from an American manufacturer prior to 1971)	1	~ 2000
2,4,5-T (obtained from an American manufacturer after 1971)	1	2
2,4,5-T alkyl ester (obtained from commercial sources in Switzerland in June, 1973)	2	20-50
2,4,5-T amine salt (obtained from commercial sources in Switzerland in June, 1973)	3	20-70

a/ The paper does not specify the number of duplicate determinations performed.

The Dow study, which describes an automated adsorption chromatographic system for the clean-up of commercial samples of 2,4,5-T ester for TCDD analysis by gas chromatography/mass spectrometry, is detailed in Exhibit D. The results of this study are summarized in Table III. The TCDD content of these 2,4,5-T samples ranged from 8 ppb to 26 ppb.

TABLE III
SUMMARY OF DOW STUDY

<u>Pesticide</u>	<u>No. of Samples</u>	<u>TCDD Content (ppb)</u> ^{a/}
1-isobutoxy-2-propyl ester of 2,4,5-T	1 ^{b/}	26 ^{c/}
butoxypropyl ester of 2,4,5-T	1 ^{d/}	8

a/ The detection limit for TCDD was 5 ppb.

b/ The isobutoxypropyl ester was analyzed 18 times.

c/ The standard deviation was + 2 ppb.

d/ The butoxypropyl ester sample was analyzed at least in duplicate.

Exhibit E details a Dow method for the determination of TCDD in 2,4,5-T and related materials which is typical of the current state of the art in dioxin analysis.

Appendix B contains a summary of confidential Dow documents obtained in discovery which report the presence of dioxin in commercial samples of 2,4,5-T and related products. Because Dow claims that this information is confidential, this testimony has been filed UNDER SEAL for use in camera.

APPENDIX A

Mass spectrometry is one of the most important tools available to the analytical chemist for the analysis, identification, and confirmation of organic compounds. Gas chromatography (GC) is often used in conjunction with mass spectrometry (MS) to achieve a separation of the components that contributes to the overall sensitivity of the analytical determination.^{1/}

There are many variations in the instrumentation of modern GC/MS equipment, but basically all GC/MS systems contain (1) an inlet system for the test substance; (2) an ion source which bombards the test substance to produce ion fragments; (3) a mass analyzer which accelerates the ions at rates which depend on the mass/charge (m/e) ratio for the individual ions; (4) a collector on which ions of different m/e values are individually focused; and (5) a data processing system which gathers the spectral information and prints this information out on request.

The two most widely used mass analyzers are (1) the magnetic analyzer, which usually achieves the acceleration of the ions by varying the magnetic field at a constant electric potential and (2) the quadrupole system, which depends on the oscillation of the ions at varying radio frequency levels in a constant electrostatic field to achieve different acceleration rates for the ions generated in the source.

^{1/} In gas chromatography, the components of a sample travel through a chromatographic column by means of a carrier gas with retention times that depend on the physical and chemical characteristics of the components, the type of column used, and the running conditions for the instrument.

The most widely used technique for producing fragments in mass spectrometry is electron impact ionization, which produces a mass spectrum that is distinctive for the compound undergoing ionization. In electron impact ionization mass spectrometry, a substance is bombarded with an electron beam (usually 70 electron volts) to produce positive ion fragments, sometimes including a "parent ion", which gives the exact molecular weight of the compound. The largest fragmentation peak in the spectrum is called the "base peak" and is assigned a value of 100%; the other peaks, including the parent peak, are usually given as percentages of the base peak. To identify and/or quantify the molecular species under investigation, the analytical determination focuses on the fragmentation pattern, i.e. the m/e peaks, the parent peak and its isotope contributions, and the intervals between the peaks, as well as the abundance of the m/e species in the spectrum. Of particular importance are the isotope contributions to the parent peak (P), which are represented by the $P + 1$ and $P + 2$ peaks; reference to tables allows the determination of the molecular formula for a test compound on the basis of the P, $P + 1$, and $P + 2$ peaks. Compounds containing halogens (chlorine, fluorine, bromine and iodine) display a very distinctive set of peaks representing the isotopic contribution of the heavy halogen isotopes; for each halogen containing species, these

peaks appear in characteristic ratios which can be found in standard reference tables.^{2/}

The other prevalent method for generating ions is the chemical ionization method.^{3/} In chemical ionization mass spectrometry, electrons are used to ionize a reagent gas which subsequently reacts with the sample molecules to produce ions. Since the ions produced by chemical ionization have a much lower internal energy than those produced by electron impact ionization, chemical ionization mass spectra usually exhibit intense ions in the molecular weight region, while electron impact ionization spectra contain a preponderance of highly fragmented ions. Chemical ionization mass spectrometry has a comparable sensitivity to electron ionization mass spectrometry, as well as the advantage of allowing a choice of reagent gases to best achieve the characterization of the sample. The mass spectra obtained by chemical ionization are often quite different from those obtained using electron impact ionization. Sample preparation, which usually entails such procedures as solvent extraction and chromatography, is similar for the two methods.

^{2/} The most abundant ions in the electron impact ionization mass spectrum of TCDD are those of the molecular ion chlorine isotope cluster at m/e 320, 322, 324, and 326. The ions at m/e 320 and 322 are the most intense.

^{3/} In recent years, other methods such as field ionization and field desorption have received some attention, but these methods are not widely used.

For quantitative purposes, a technique known as selective ion monitoring (SIM) can be used to increase the sensitivity of the analytical determination. In this method, the instrument is set to detect only selected key ions, and a lower limit of detection is achieved for the ions of interest.

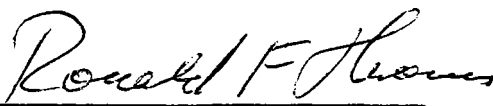

Ronald F. Thomas

EXHIBIT LIST

Exhibit A

Woolson, E.A., Thomas, R.F. and Ensor, P.D.J. 1972. Survey of Polychlorodibenzo-p-dioxin Content in Selected Pesticides. J. Ag. Ed. Chem. 20: 2.

Exhibit B

Monalvo, J.G., Ryan, J.F. and Flagg, R. Analysis of Technical Grade Pesticides for TCDD at the ppb Level. EPA Project No. 68-01-3981. Physical Engineering Sciences Division, Gulf South Research Institute, New Orleans, Louisiana.

Exhibit C

Buser, H. and Bosshardt, H. 1974. Determination of 2,3,7,8-TCDD at ppb Levels in Technical Grade 2,4,5-T in 2,4,5-T Alkylester and 2,4,5-T Amine Salt Herbicide Formulations by Quadrapole Massfragmentography. J. Chrom. 90: 71-77.

Exhibit D

Tore-Ramstad, Mahle, N.H. and Matalon, R. 1977. Automated Cleanup of Herbicides by Adsorption Chromatography for Determination of 2,3,7,8-TCDD. An. Chem. 49:

Exhibit E

Dow Chemical Company. Method ML-AM-75-34. Determination of TCDD in 2,4,5-T and Related Materials. Applicable to 2,4,5-T, Silvex and Chlorinated Phenols (unpublished).

Exhibit F

Documents which Dow claims contain confidential data on Dow's analysis of TCDD content in commercial 2,4,5-T produced between 1975 and 1979. (Filed Under Seal for use in in camera).

CERTIFICATE OF SERVICE

I hereby certify that copies of the foregoing
DIRECT TESTIMONY OF MR. RONALD THOMAS were hand delivered
or mailed first class postage prepaid on February 26,
1980 to the persons on the attached list.

Cara S. Jablon
Cara S. Jablon

February 26, 1980

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MAR 2 1980

EXHIBIT A

Survey of Polychlorodibenzo-*p*-dioxin Content in Selected Pesticides

MAR 2 1980

Edwin A. Woolson,* Ronald F. Thomas,¹ and Peter D. J. Ensor

One-hundred-twenty-nine samples of 17 different pesticides derived from chlorophenols were examined for polychlorinated dibenzo-*p*-dioxins by electron capture gas chromatography (ec-gc). The method of cleanup involved a concentrated sulfuric acid extraction of impurities from hexane and a mild nitration of the chlorophenol extracts. Seventy-six percent of the samples analyzed contained less than 0.1 $\mu\text{g/g}$ of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the technical material; whereas 7% contained between 0.1 to 1.0 $\mu\text{g/g}$, and 9% contained

greater than 10 $\mu\text{g/g}$ TCDD. No TCDD was detected in the 20 tri-, tetra-, or pentachlorophenol samples examined. However, high levels of other dioxins were found. All samples which contained more than 1.0 $\mu\text{g/g}$ of dioxin by ec-gc were confirmed by gas chromatography using a flame ionization detector (fid-gc), a microcoulometric detector (mc-gc), *p* values, uv irradiation, and/or gas chromatography/mass spectrometry (gc-ms). Samples of phenoxy herbicides from current production contained less than 0.5 $\mu\text{g/g}$ TCDD.

Polychlorodibenzo-*p*-dioxins may be contaminants in chlorophenols or in pesticides which use chlorophenols in the manufacturing process. Chlorinated dibenzo-*p*-dioxins are hazardous materials and may cause skin eruptions, teratogenesis, and are toxic to animals at low levels. The dioxins are formed usually when the reaction temperature for making *o*-chlorophenol by hydrolysis exceeds 160°C under pressure. The reaction may be an alkaline hydrolysis of polychlorobenzene or a chlorination of phenol to form a polychlorophenol.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, Figure 1) is the dioxin that may be formed when the reaction temperature for making 2,4,5-trichlorophenol from tetrachlorobenzene exceeds 160°C (Schultz, 1968). In chlorinating phenol to make tetra- or pentachlorophenol, heat must be supplied to the reaction mixture in order to maintain the mix as a melt. If too much heat is supplied, hexa-, hepta-, and/or octachlorodibenzo-*p*-dioxins may be formed. Since little or no heat is required to form di- and trichlorophenols by chlorination, there is little likelihood that di-, tri-, or tetrachlorodibenzo-*p*-dioxins will be formed in this process.

Interest in dioxins originated in 1957 from outbreaks of "chick edema (CE) disease." The disease was characterized by hydroparicardium in chickens. In 1958 a toxic substance, isolated from an unsaponifiable fraction of feed fats which caused CE, was identified (Cantrell *et al.*, 1969) by single crystal X-ray crystallography as 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HCDD). In 1970, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was implicated as a potential teratogen in pregnant rats (Courtney *et al.*, 1970). Later tests indicated that the teratogenesis may have been caused by 27 $\pm$ 8 ppm of TCDD present as contaminant in the 2,4,5-T.

In order to assess possible environmental contamination with TCDD or higher chlorinated dioxins through pesticidal materials, samples of 17 pesticides based on use of chlorophenols in their manufacturing process were collected from U.S. Department of Agriculture's former Pesticide Regulation Division (PRD) laboratories. Additionally, samples of 2,4,5-T from one manufacturer, covering a 3-year period, were collected. A method was developed for the analysis

of dioxins in the 17 different pesticides. The method developed and results of the survey are reported in this paper.

PROCEDURE

Materials. No attempt should be made to analyze for dioxins without proper safety procedures. The following solvents were distilled in glass or were of pesticide grade quality: hexane, methanol, petroleum ether, acetonitrile, benzene, and diethyl ether.

The following apparatuses were required: chromatography columns (450 $\times$ 19 mm i.d.) with Teflon stopcock, gas chromatograph (gc), preferably equipped with a Ni⁶³ detector (flame ionization or microcoulometric detectors also desirable for confirmation steps), glass gc columns (1.83-m $\times$ 4-mm), a sun lamp with maximum output at 310 nm, and thin-layer chromatography (tlc) apparatus.

The following compounds were examined for polychlorodibenzo-*p*-dioxins: (2,4-dichlorophenoxy)acetic acid, 2,4-D; 4-(2,4-dichlorophenoxy)butyric acid, 2,4-DB; 2-(2,4-dichlorophenoxy)propionic acid, 2,4-DP; (2,4,5-trichlorophenoxy)acetic acid, 2,4,5-T; 2-(2,4,5-trichlorophenoxy)propionic acid, silvex; 2-(2,4-dichlorophenoxy)ethyl sodium sulfate, sesone; 3,6-dichloro-*o*-anisic acid, dicamba; pentachlorophenol, PCP; tetrachlorophenol; trichlorophenol; 2,4-dichlorophenyl *p*-nitrophenyl ether, nitrofen; *O*-(2,4-dichlorophenyl) *O*-methyl isopropylphosphoramidothioate, DMPA; 2-(2,4,5-trichlorophenoxy)ethyl 2-dichloropropionate, erbon; tris[2-(2,4-dichlorophenoxy)ethyl]phosphite, 2,4-DEP; *O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothionate, ronnel; 2,4,5,4'-tetrachlorodiphenyl sulfone, tetradifon; and *O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate, VC-13, Nemacide.

The cleanup procedure consisted of three steps which were dependent on the nature of impurities found in and the complexity of the sample.

Step A. TECHNICAL SALTS AND ACIDS OF PHENOXY ACIDS. One-hundred milliliters of MeOH was added to 3.00 g of herbicide and 10 ml of 5 *N* KOH contained in a 1000-ml separatory funnel. Two-hundred milliliters of H₂O and 100 ml of hexane were added after 20 min and shaken. The aqueous phase was reextracted with 100 ml of hexane and the extracts were combined. The hexane extracts were washed twice with 100 ml of 1 *N* NaOH, twice with 1 *N* HCl, and twice with 100 ml of H₂O. The extracts were dried over anhydrous Na₂SO₄, transferred to a 250-ml beaker, and evaporated to ca. 10 ml. The sample was transferred to a pre-

Plant Science Research Division, Agriculture Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

¹ Present address: Pesticide Regulation Division, Environmental Protection Agency, Beltsville, Maryland 20705.

Table I. Relative Retention Times of Several Chlorinated Hydrocarbon Insecticides and Polychlorodibenzo-*p*-dioxins on Five Gas Chromatography Columns^a

	Relative retention times ^b on columns				
	5% OV-225	1.5% OV-17 + 2.0% QF-1 (1:1)	5% OV-17	5% UCW-98	15% QF-1 + 10% DC-200 (1:1)
Aldrin	1.00 (2.17) ^c	1.00 (1.80)	1.00 (0.91)	1.00 (1.26)	1.00 (4.2)
<i>p,p'</i> -DDE	2.63	2.50	1.98	1.67	1.90
<i>p,p'</i> -TDE	5.84	3.62	3.19	2.14	2.64
<i>p,p'</i> -DDT	5.62	4.17	<i>d</i>	2.70	3.14
Endrin	3.69	2.78	3.08	1.90	2.64
Endrin, Δ keto	15.76	6.89	8.57	3.25	5.48
2,7-CDD ^e	1.29	1.00	1.21	0.83	1.02
2,3,7-CDD	1.94	1.67	2.09	1.51	1.64
tri-CDD	2.30	1.83	7.10	1.57	1.83
tetra-CDD ^f	2.86	2.50	2.31	2.22	2.38
tetra-CDD ^f	3.27	2.52	2.33	2.24	2.52
2,3,7,8-CDD	4.33	3.44	3.41	2.86	3.19
penta-CDD	8.29	6.56		5.08	5.48
hexa-CDD (1)	11.61	9.28	7.18	7.06	7.26
hexa-CDD (2)	13.13	10.56	8.24	7.94	8.09
hexa-CDD (3)	16.45	12.44	11.10	9.97	9.45
hepta-CDD (1)	23.32	18.39	13.19	13.09	13.26
hepta-CDD (1)	26.96	21.17	15.82	14.68	14.88
octa-CDD	46.31	36.28	23.41	24.52	24.05

^a Column conditions: length, 1.8-m $\times$ 4-mm glass; solid support, Chromosorb W, 80-100 mesh; injector, 240°C; column, 220°C; detector, Ni⁶³, 310°C; flow rate, 80-100 ml/min. ^b $\pm$ 1%. ^c Time in minutes in parentheses. ^d Not resolved from TDE. ^e Chlorodibenzo-*p*-dioxin = CDD. ^f More than one isomer was present in the tri-, tetra-, hexa-, and heptachlorodibenzo-*p*-dioxins. Known isomers have the positions numbered.

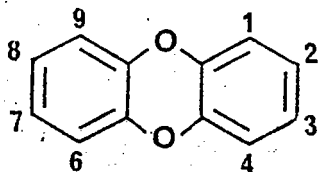


Figure 1. Structure of dibenzo-*p*-dioxin. Chlorines may be attached at the 1,2,3,4,6,7,8, and/or 9 positions to yield chlorodibenzo-*p*-dioxins

washed (petroleum ether) 15-g Al_2O_3 column (450 $\times$ 19 mm i.d.) and eluted with 100 ml of petroleum ether followed by 50 ml of 5% (v/v) diethyl ether in petroleum ether, which were discarded. One-hundred milliliters of 50% (v/v) diethyl ether in petroleum ether was collected, evaporated just to dryness, and adjusted to volume with hexane. The amount of each dioxin present was determined.

Step B. LOW ORGANIC MATTER SOILS, PHENOL-BASED INSECTICIDES, OIL, AND ESTER FORMULATIONS OF PHENOXY HERBICIDES. The following steps were performed prior to the Al_2O_3 column in Step A. The extract was shaken with concentrated H_2SO_4 until the acid was clear (not yellow or cloudy after standing ca. 10 min), washed with H_2O , and passed through a NaHCO_3 - Na_2SO_4 column (1 cm of each layered in a 19 mm i.d. column). The eluant was evaporated to ca. 10 ml and continued with the Al_2O_3 column in Step A.

Step C. CHLOROPHENOLS, HIGH ORGANIC MATTER SOILS, AND OTHER SAMPLES DIFFICULT TO CLEAN UP. After completing parts A and B of the cleanup procedure, the eluant from the Al_2O_3 column was evaporated just to dryness, cooled to ca. 0°C on an ice bath, and 10 ml of 1:1 (v/v) HNO_3 : H_2SO_4 mixture was added. The mix was gradually warmed to room temperature and added to 50 ml of ice water in a separatory funnel after 15 min. The beaker was rinsed with 5 $\times$ 10 ml of hexane. The combined hexane rinses were

shaken for 1 min, washed with H_2O , and the hexane was drained through a NaHCO_3 - Na_2SO_4 column. The volume was adjusted for analysis.

Quantitation was made on a 5% OV-225 column using electron-capture gas chromatography (ec-gc). Analysis was made with temperature programming from 230 to 260°C at 4°C/min and a 16-min hold at the final temperature.

Confirmation Techniques for Dioxins. If a positive response was found by ec-gc, the *p* value was determined for the dioxin between hexane and acetonitrile on a portion of the extract. The *p* value equals final concentration in hexane, initial concentration in hexane (Beroza and Bowman, 1965). Another portion of the extract was irradiated for 16 hr under a sun lamp (maximum output, 310 nm) at a distance of 30 cm since TCDD and the lower dioxins are destroyed under these conditions.

The remainder of the extract was condensed for confirmation on different gc columns using different detectors. The columns preferred were 1:1 (w/w) mix of 1.5% OV-17 plus 2.0% QF-1 and 5% UCW-98, both on Chromosorb W, 80-100 mesh. Flame ionization and microcoulometric detection were with these detectors. The ultimate confirmation was provided by a Perkin-Elmer Model 270 gc-mass spectrometer (gc-ms). There was very little fragmentation since the parent peak (*m/e* 322) was the parent ion and *m/e* 257 and 194 were the only fragments over 20% in relative abundance for the tetradoxin.

Two-dimensional tlc was used for cleanup and partial confirmation occasionally. The plate was developed first in acetonitrile followed by benzene. The spots were visualized under a uv lamp and were scraped off, extracted, and assayed by gc if desired. Infrared spectrometry was also used to confirm the identity of various chlorodioxins.

RESULTS AND DISCUSSION

Relative retention times of 13 dioxin isomers and a few selected chlorinated hydrocarbon insecticides are presented in Table I. The values were determined isothermally at

220°C. Because of the large value ($rt_a = 46.31$) for the octa-CDD, the 5% OV-225 column was normally used with temperature programming from 230 to 260°C at a rate of 4°C/min and a final temperature hold. Under these conditions, analysis was completed in about 40 min. The use of the three recommended columns (5% OV-225, 1.5% OV-17 plus 2% QF-1, and 5% UCW-98) prevented confusion of some chlorinated hydrocarbon insecticides in environmental samples with dioxins because relative retention times were sufficiently different.

ρ Values for dioxins are presented in Table II. Values for four specific isomers (2,7-, 2,3,7-, 2,3,7,8-, and 1,2,3,4,6,7,8,9-chlorodibenzo-*p*-dioxins) are given. Other values are for mixtures of isomers and were determined using total peak area or peak height. R_f values for all dioxin isomers by tlc in acetonitrile were 0.82 ± 0.08 and in benzene were 0.80 ± 0.02 . The di-, tri-, tetra-, and hexadioxin were not separated from each other under our test conditions, but were separated from most interfering impurities in formulations. Care must be taken not to overload the spots or the material appears to stay on the origin. This behavior is presumably due to the low solubility of dioxins in most solvents.

In a further confirmatory step, dioxins were destroyed by uv light. The lower isomers (<4 Cl) were completely destroyed in 16 hr by irradiation (Crosby *et al.*, 1971) at concentrations less than 0.2 ppm in hexane. Nondestruction of the suspected di-, tri-, or tetrachlorodioxin is sufficient evidence that the peak is not a chlorodioxin. The higher the degree of chlorination, the more resistant the dioxins were to irradiation. Only 20% of the octa-CDD was destroyed in 16 hr at concentrations of about 0.3 ppm.

A total of 129 pesticide samples were analyzed for dioxins. Table III presents the occurrence and content of the dioxins in all samples. TCDD (tetra-) was found primarily in 2,4,5-T samples. Only one sample of silvex contained more than 0.5 ppm of TCDD. Detectable amounts of hexa-CDD were found in four 2,4,5-T samples, one 2,4-D, and two other samples other than the chlorophenols. Hepta- and octa-CDD also occurred in four other samples apiece in addition to their presence in chlorophenols. Most samples were made before 1970 and may not represent current production materials.

The TCDD concentration in selected pesticides is also presented in Table III. Twenty-two of 42 2,4,5-T samples collected contained less than 0.5 ppm of TCDD. Of the 20 samples containing more than 0.5 ppm of TCDD, 15 were obtained for the yearly survey of one manufacturer. The samples were from 1966–1970, with four samples usually collected each year. There was a tenfold drop in TCDD

Table II. ρ Values for Chlorodibenzo-*p*-dioxins in a 1:1 (v/v) Acetonitrile:Hexane Solvent System*

Isomer	ρ Value	std dev
2,7-CDD	0.76	± 0.03
2,3,7-CDD	0.86	± 0.03
2,3,7,8-CDD	0.51	± 0.06
hexa-CDD	0.94	± 0.03
hepta-CDD	0.90	± 0.05
octa-CDD	0.90	± 0.05

* Ratio of peak height in hexane solution before equilibration with hexane-saturated acetonitrile to peak height in hexane after equilibration.

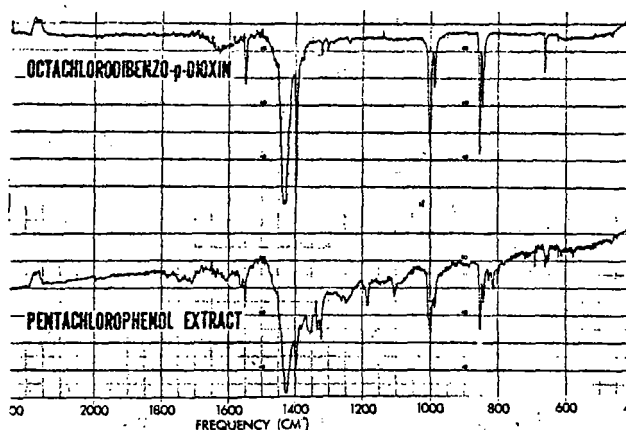


Figure 2. Infrared spectra of authentic octachlorodibenzo-*p*-dioxin and a cleaned-up pentachlorophenol extract. The material was incorporated in KBr and the spectra made on a Perkin-Elmer 625 infrared spectrometer

content by this manufacturer between 1968 and 1969. However, their technical 2,4,5-T still contained 2–3 ppm of TCDD in 1970. Four of the five remaining samples containing over 0.5 ppm of TCDD also came from this same manufacturer and were received from the PRD laboratories. Recent (1970) samples from another manufacturer contained <0.5 ppm of TCDD. No other samples tested had over 0.5 ppm of TCDD. Any sample could contain one or more different chlorodioxins.

The higher polychlorodibenzo-*p*-dioxin content of the same selected pesticides is also presented in Table III. Four samples of 2,4,5-T contained greater than 0.5 ppm of the hexa isomers. No higher dioxins were found in silvex or dicamba

Table III. Number and Content of Polychlorodibenzo-*p*-dioxins in Selected Pesticides

Pesticide	ppm of -chlorodibenzo- <i>p</i> -dioxin										No. of samples contam- inated	Total no. of samples tested	
	tetra		<10	hexa		<1000	hepta		<1000	octa			
	<10	<100		<100	<100		<10	<100		<10			<100
2,4,5-T	7 ^a	13	3	1	0	ND ^b	...	...	ND	...	...	23	42
Silvex	1	0	ND	...	...	ND	...	...	ND	...	...	1	7
2,4-D (-DB,-DP)	ND	...	1	0	0	ND	...	...	ND	...	...	1	28
Dicamba	ND	...	ND	...	...	ND	...	...	ND	...	...	0	8
Chlorophenol													
tri-	ND	...	4	0	0	1	0	0	2	0	0	4	6
tetra-	ND	...	1	1	0	1	2	0	1	2	0	3	3
penta-	ND	...	0	7	0	0	4	6	0	4	6	10	11
Others	ND	...	1	0	1	3	0	1	3	1	0	7	24

* Any sample may contain one or more different dioxins. ^b ND = <0.5 ppm of any one chlorodioxin.

and only one sample of 2,4-D contained measurable amounts of the hexa isomers. The hexa isomers present presumably were there because of tetrachlorophenol impurities which condensed with each other. Higher dioxins were found in some samples of several other pesticides, including erbon, tetradifon, ronnel, sesone, and DMPA.

The dioxin content of the 20 chlorophenol samples is also presented in Table III. No TCDD was detected in any sample at levels above 0.5 ppm. However, the higher dioxins were plentiful. Trichlorophenol contained only small amounts of hexachlorodibenzo-*p*-dioxin and no sample contained over 10 ppm. Tetrachlorophenol contained less than 100 ppm of hexa-, hepta-, and octachlorodibenzo-*p*-dioxins, while six of 20 pentachlorophenol samples contained over 100 ppm of the hepta- and octachlorodibenzo-*p*-dioxin isomers. An infrared spectrum of a pentachlorophenol extract is presented in Figure 2. It is quite obvious that the extract contains octachlorodibenzo-*p*-dioxin when the spectrum is compared to the standard octachlorodibenzo-*p*-dioxin spectrum. Analysis by ec-gc indicated both hepta- and octachlorodioxins were present in the sample. This may account for the peak broadening. The reason for the high amounts of dioxin in chlorophenol is probably due to heat treatment during synthesis. As chlorination of phenol proceeds past the dichlorophenol stage, heat must be supplied in order to keep the reaction mixture in a melt condition. Since heat is being supplied in the presence of chlorophenols, the formation of higher chlorinated dibenzo-*p*-dioxins might

be expected. The rate and time of melt heating probably governs the formation and amounts of the various dioxin isomers. If the chlorination temperature is raised too high too quickly, the lower chlorinated dioxins may be formed since the lower chlorophenols would be present and available for reaction with each other. If the temperature is raised too high after nearly all chlorophenol is in the penta form, octachlorodibenzo-*p*-dioxin would be the predominant impurity formed.

In conclusion, TCDD has been present at levels above 0.5 ppm in the past, but was less than 0.5 ppm in the current production samples examined. Higher chlorodioxins are present predominantly in chlorophenols with the highest amounts present in pentachlorophenol. Thirty-eight percent of all samples examined contained at least one chlorodioxin, with many containing more than one.

LITERATURE CITED

- Beroza, M., Bowman, M. C., *J. Ass. Offic. Anal. Chem.* 48, 358 (1965).
 Cantrell, J. S., Webb, N. C., Mabis, A. J., *Acta Crystallogr.* B25, 150 (1969).
 Courtney, K. D., Gaylor, D. W., Hogan, M. D., Falk, H. L., Bates, R. R., Mitchell, I., *Science* 196, 864 (1970).
 Crosby, D. G., Wong, A. S., Plimmer, J. R., Woolson, E. A., *Science* 173, 748 (1971).
 Schultz, K. H., *Arbeitsmedizin-Socialmedizin-Arbeitshygiene* 3, 25 (1968).

Received for review August 19, 1971. Accepted November 15, 1971.

MAR 2 1980

EXHIBIT B

FINAL Report

THE ex. B

MAR 2 1980

ANALYSIS OF TECHNICAL GRADE PESTICIDES FOR TCDD AT PARTS-PER-BILLION LEVEL

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ABSTRACT

Gulf South Research Institute (GSRI) analyzed 69 technical grade pesticide batch samples (Silvex; 2,4,5-trichlorophenoxyacetic acid; an organophosphate; 2,4,5-trichlorophenol; and a chlorinated phenolic antiseptic), 31 replicate samples of these batch samples, and 41 quality control samples for total tetrachlorodibenzo-p-dioxin (TCDD). The samples were extracted with hexane and the extracts were dried, concentrated, and chromatographed on alumina. Samples were qualitatively and quantitatively analyzed by gas chromatography/mass spectrometry (GC/MS) in the selective ion monitoring (SIM) mode. Certainty of TCDD detection was assessed via summation of six qualitative criteria. Based on this assessment, the data were rated positive, doubtful, or nondetectable. For those authentic samples in which detection was judged positive, levels of total TCDD ranged from nondetectable to 30 ppb; quality control samples contained up to 3984 ppb. The use of high efficiency capillary column GC/MS is recommended for future TCDD investigations.

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ACKNOWLEDGMENT

The cooperation of Mr. Ronald F. Thomas, Supervisory Chemist, Benefits and Field Studies Division, Environmental Protection Agency, in sampling design and shipment of the samples to Gulf South Research Institute is appreciated. The assistance of Ms. Patricia Ott, Project Officer, is gratefully acknowledged. The assistance of Dr. Mary M. McKown, GSRI senior analytical chemist, throughout the project performance is appreciated.

SECTION 1

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) may be formed as undesirable trace impurities in the production of technical grade chlorophenols. The latter are employed extensively as pesticides, as wood preservatives and as starting materials for a series of other products. Through careful control over conditions in the pesticide manufacturing process, only minimal quantities of PCDDs are formed, but at temperatures above 200°C a condensation of chlorophenols may occur with formation of increasing quantities of PCDDs (1).

Several PCDDs have been found to be highly toxic and possibly carcinogenic and are stable in biological systems (2). Theoretically, a total of 75 different PCDDs exist, including 22 tetrachlorodibenzo-p-dioxins. The toxicological properties of positional isomers of the same dioxin may vary significantly. Since the 2,3,7,8-tetrachlorodibenzo-p-dioxin (Figure 1) is extremely toxic (3), TCDDs deserve special consideration. Although the synthetic route for TCDD formation during chlorophenol manufacture favors 2,3,7,8-TCDD, other tetrachlorodibenzo-p-dioxin isomers may also be formed (4).

Under the aegis of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental Protection Agency (EPA) has set maximum TCDD contamination limits in technical grade hexachlorophene at 100 ppb (0.1 ppm) in order to reduce human and environmental exposure. The purpose of the GSRI research effort was to determine levels of TCDD present in technical grade pesticide samples submitted by EPA.

In view of the inherent toxicity of TCDD, special precautions in handling are required during analysis. Concentration by several orders of magnitude is sometimes necessary, and an ultrasensitive detection method is preferred. A combination of gas chromatography and mass spectrometry has been shown to be an excellent method for detecting TCDDs at very low levels (5). Identification of individual TCDD isomers requires an initial, high efficiency separation such as glass capillary gas chromatography (GC) prior to mass spectrometric (MS) analysis. Low resolution GC columns, i.e., packed columns, provide a convenient means for TCDD isomer summation (total TCDD) by GC/MS.

The present study has focused on determination of total TCDD in technical grade samples by low resolution gas chromatography/mass spectrometry. Sample preparation included addition of ³⁷Cl 2,3,7,8-TCDD to assess

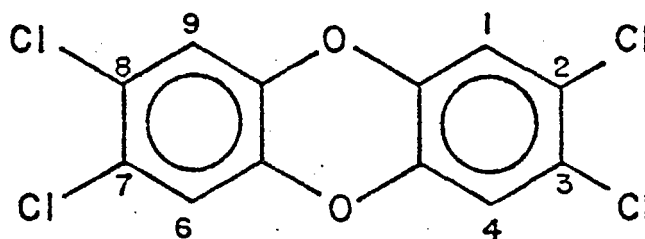


Figure 1. Structure of 2,3,7,8-TCDD.

dioxin recovery, solvent extraction, and cleanup on alumina. GC/MS analyses were carried out using a packed column; the standard addition injection technique was used. Selective ion monitoring (SIM) and complete mass spectral data were obtained. Six qualitative criteria for TCDD detection were developed incorporating the precision of the analytical measurement system. The project quality assurance protocol included internal quality control samples (precision and accuracy) and quality control charting.

SECTION 2

CONCLUSIONS

The results of low resolution gas chromatographic/mass spectrometric analysis of 141 samples (including quality controls and authentic pesticide samples containing unknown quantities of TCDD) for total tetrachlorodibenzo-p-dioxin have indicated levels ranging from nondetectable to 30 ppb for 69 authentic pesticide batch and 31 replicate samples and nondetectable to 3984 ppb for 41 quality control samples. TCDD was not detected (detection limit 10 ppb) in 66 percent of the authentic samples, detection was doubtful in 9 percent and positive in 25 percent. Whereas TCDD could not be detected in a majority of authentic samples, positive samples contained 10-30 ppb total TCDD.

SECTION 3

RECOMMENDATIONS

The investigation of total tetrachlorodibenzo-p-dioxin levels in compounds for whose synthesis 2,4,5-trichlorophenol is used should be continued. In view of the known toxicity of the 2,3,7,8-TCDD isomer and incomplete separation of the isomers using packed columns, future investigations should be performed using high efficiency gas chromatography (capillary column)/mass spectrometry. These data are of major importance from the point of view of environmental protection.

In spite of the eminent need for standardized methods for TCDD analysis in technical grade pesticide formulations and environmental samples, such procedures are still lacking. The establishment of standardized methods would permit substantial progress in baseline and on-going studies. Such methods should be directed toward improved sample preparation (to reduce interferences) and improved resolution/detection of the 2,3,7,8-TCDD isomer, with the consideration in mind that such methods should be suitable for use by moderately well equipped laboratories.

SECTION 4

EXPERIMENTAL PROCEDURES

SAFE HANDLING IN THE LABORATORY

Disposable rubber gloves were worn while handling samples and solutions, and all evaporations were performed in a hood. Waste materials were stored in a capped steel drum labeled TCDD WASTE. Personnel associated with the project had periodic physical examinations.

GENERAL PROCEDURES

After taking custody of samples furnished by the EPA, TCDD residues were identified and quantified using a three-stage analytical protocol including sample preparation, mass spectral data acquisition, and data analysis.

TCDD Standards

TCDD standards in benzene were supplied by Dr. Aubry Dupuy, Pesticide Monitoring Laboratory, EPA. The 2,3,7,8-TCDD standard concentration was 500 picograms (pg)/microliter (μ l). Concentration of the ^{37}Cl labeled 2,3,7,8-TCDD standard was 480 pg/ μ l. Labeled TCDD was used to determine dioxin recovery through the entire extraction/cleanup procedure. Nonlabeled TCDD was used to quantify dioxin levels in the samples.

Sample Preparation

Ethanol, hexane, benzene, carbon tetrachloride, and methylene chloride were nanograde (pesticide) quality; inorganic salts, acids and bases were ACS grade. Distilled water was prepared by filtering through a mixed bed ion exchanger, activated carbon, and distilling in an all glass still. Aluminum oxide Woelm neutral, activity grade I, was used for column chromatography.

The procedure used in these analyses is a modification of that provided by EPA in the contract. In general, it involved caustic KOH saponification of the pesticide followed by extraction of the neutral TCDD into hexane. The hexane solution was cleaned up by extracting several times with sodium hydroxide solution and sulfuric acid, followed by chromatography on alumina. The eluate was concentrated for GC/MS analysis. Details of the procedures follow.

A 1 g quantity of sample was transferred quantitatively to a 500 ml separatory funnel containing 60 ml of pesticide grade ethanol. Exactly 48 ng of ^{37}Cl labeled 2,3,7,8-TCDD was added with a microsyringe and the mixture swirled. Four milliliters of 40 percent KOH was added and the mixture immediately shaken. The room temperature digestion was allowed to proceed for 30 minutes, with occasional swirling. Two-hundred milliliters of distilled water was added to dissolve the precipitate formed upon base addition, and the resulting solution was extracted with three 100 ml portions of nanograde hexane. The combined hexane extracts were washed sequentially with three 100-ml volumes of 1N NaOH, one 80-ml volume of distilled water, and 95 percent H_2SO_4 until no further color appeared in H_2SO_4 . A final washing with 80 ml distilled water completed the extraction.

The hexane extract was filtered through a 15 mm x 20 cm column of granular anhydrous Na_2CO_3 followed by concentration in a Kuderna-Danish evaporator to a volume of about 4 ml. The anhydrous Na_2CO_3 had been purified by pre-extraction with nanograde CH_2Cl_2 in a Soxhlet extractor and drying at 100°C in a vacuum oven. The concentrate was reduced to 0.5 ml under a stream of UHP or ultra high purity nitrogen (Matheson, Rutherford, N.J.). The residue was reconstituted in 2 ml nanograde hexane and chromatographed through a 4.5 cm column. The column consisted of activated alumina over which a 1/4 inch layer of purified granular Na_2SO_4 was placed. The column was preconditioned by washing with 5 ml of CH_2Cl_2 , followed by removal of residual CH_2Cl_2 by blowing dry N_2 through the column, and heating for 48 hours at 240°C . The sample was eluted from the column with 6 ml CCl_4 followed by 4 ml of CH_2Cl_2 . The carbon tetrachloride layer was discarded and the CH_2Cl_2 fraction evaporated under UHP N_2 to 20 μl . The concentrate was reconstituted to 2 ml with hexane, evaporated to 20 μl , and rediluted with hexane to 2 ml. The final extract volume was concentrated to 200 μl . The three evaporations were necessary to remove residual CH_2Cl_2 .

The extract was divided equally into two glass ampules. Ampules were flame sealed, and the extract volume noted by marking the liquid level. After coding the ampules were stored in a freezer at -10°C .

Samples were processed in "sets". Each set of samples consisted of internal and EPA supplied quality control (QC) and pesticide samples. Generally each set of samples contained two quality control samples and four pesticide samples processed from left to right as indicated below:

... $\overline{A_i \quad X_j \quad X_{j+1} \quad X_{j+2} \quad X_{j+3} \quad B_k}$...

where A_i = ith observation of the accuracy
 QC sample (for example, pesticide sample #0093, furnished by EPA, accepted value for TCDD = 2.3 ppm).
 X_j = the jth EPA pesticide sample (unknown TCDD level),
 and B_k = kth observation of the zero level TCDD QC sample (solvent blank spiked only with ^{37}Cl TCDD, 48 ng).

QC and EPA sample numbers for each paired extract were coded with a numerical suffix: one ampule, -2 (for immediate GC/MS assay) and the other, -1 (for backup analysis). EPA sample numbers ranged from 2 to 9 digits. Replicate samples in this report are denoted by an "I" (initial), "D" (duplicate), "T" (triplicate), or "Q" (quadruplicate) after the sample batch number. The series sample number (e.g., 148920) represents one source of technical grade material; the -1, -2, -3, etc., following the series sample numbers each represents a different batch sampled from that source.

TCDD and ^{37}Cl TCDD standards were transferred to Mininert (Supelco) valve vials. A back-up seal in the Teflon valve had been removed and the assembly prewashed. Standards were coded and frozen until utilized.

Mass Spectral Data Acquisition

The low resolution gas chromatograph/mass spectrometer used was a Hewlett-Packard (HP) 5985 GC/MS in the electron ionization (EI) source configuration. The control unit displayed the ion current (peak area) on a Tektronix 4012 CRT computer terminal, and hard copies were provided by the Tektronix 4631 hard copy unit.

Generally spectrometer operating conditions were as follows: column 3 ft x 2 mm ID glass; packing 3 percent OV-11 on 100/120 mesh Supelcoport; column temperature 200°C for 1 minute to 240°C at 6°C per minute; injection heater temperature 250°C; jet separator interface temperature 260°C; ion source temperature 150°C; carrier gas, helium 32 ml/min; electron impact source 70 eV; emission 0.3 mA; electron multiplier 2.6 KV; mass range 150-330 amu; scan time 3 ms/amu. The mass spectrometer was tuned to monitor three molecular ions at m/e values of 320, 322, and 328. Retention time of TCDD was approximately 3.6 minutes under the above conditions.

The GC/MS protocol for TCDD measurement was designed to permit simultaneous data acquisition for: ^{37}Cl TCDD recovery, TCDD detection limit, TCDD quantification, and confirmation via mass spectra (molecular ion abundances). In this procedure, sample extracts and TCDD standards were thawed to room temperature. Ampules containing sample extract were broken above the liquid level and firmly positioned in a plastic block. TCDD solutions were always handled under a hood, and liquid and solid wastes were transferred daily to the TCDD waste drum.

A 10 μl Hamilton 701N microsyringe was used to inject sample and standard aliquots into the GC/MS. GC column performance and MS linearity were checked by co-injecting the following mixtures of standards, one injection per mixture in the sequence tabulated:

1 μl native TCDD + 1 μl	^{37}Cl TCDD
2 μl native TCDD + 2 μl	^{37}Cl TCDD
1 μl native TCDD + 1 μl	^{37}Cl TCDD
2 μl native TCDD + 2 μl	^{37}Cl TCDD

Data for each injection were displayed on the CRT computer terminal. Peaks ($m/e = 320, 322$ and 328) corresponding to the TCDD retention time region were integrated using a HP-21MXE data system and results displayed. Collected data for each injection were recorded on a Tektronix 4610 hard copy unit.

Quality control and pesticide samples were run in the same order in which the extracts were prepared. Co-injection of mixtures, one injection per mixture, was as follows:

COMPOSITION OF ANALYSIS SET
(μl co-injected)

Injection Number	Sample Volume (μl)	TCDD Native Standard	³⁷ Cl TCDD Standard
1	2	+	0
2	2	+	2
3	2	+	0
4	2	+	1

During the TCDD retention time-span the m/e 320, 322 and 328 peak areas were integrated, and a photocopy prepared for each injection (see sample hard copies in Appendices). Background noise level was integrated from the baseline adjacent to the m/e 322 peak. The ion current chromatogram from injection 1 was used for noise determination if the 322 peak was present; the chromatogram from injection 2 was used if the 322 peak was not found. A photocopy of the noise level was prepared.

Hard copies from injections 1 and 3 were inspected to determine if both m/e 320 and 322 peaks were found for both injections. A sample meeting these criteria was subjected to GC/MS mass fragmentation confirmation by evaporating the extract under N_2 to about 10 μl . Two microliters of the concentrated extract was injected. Both mass spectrum and absolute abundances for m/e values were obtained and hard copies prepared (see sample copies in Appendices).

Data Analysis

Integrated peak areas for TCDD standard solutions were subjected to linear regression analysis ($y = \text{peak area}$; $X = \mu\text{l}$ injected). Correlation coefficient (r), slope (s), intercept (i), and the ratio i/s were computed for $m/e = 320, 322$, and 328 . Instrumental sensitivity, expressed as area/pg, was computed for m/e 320 and 328 by dividing " s " by 500 (TCDD standard concentration in pg/ μl) and 480 (³⁷Cl TCDD standard concentration in pg/ μl). An average 320/322 ratio was computed from the (2 + 0 + 0) injections. Pertinent data were plotted on a QC chart to monitor instrument performance. Expressed as percent, $i/s \times 100$, average values were $< \pm 5$ percent. Large negative values, i.e., 20 percent, could be indicative of TCDD adsorption or degradation in the system. In any case, this problem was usually rectified by changing the GC column.

Peak areas for the method blank (QC), accuracy (QC), and EPA samples were tabulated on data sheets, designed exclusively for this project, along with other pertinent information. A sample data sheet is shown in Appendix A; the method for computing percent ³⁷Cl TCDD recovery, TCDD sample concentration, detection limit, and results of the confirmation analysis are also illustrated. Note that reported TCDD levels are corrected for recovery.

The contract specification for ³⁷Cl TCDD recovery in the pesticide samples was 50 to 120 percent, inclusive. If the initial GC/MS recovery assay was outside the specified range, the backup aliquot of the original extract was assayed. If the average of the two recovery values was within the specification range, contract requirements were satisfied. If the average recovery was outside the specification range, the sample was re-extracted and assayed one additional time. Confirmation of TCDD detection by recording complete mass spectra was optional at TCDD contamination levels <10 ppb and a contract requirement at ≥10 ppb.

The standard addition technique was not successful with samples containing large quantities (parts-per-million) of TCDD because the amount of TCDD standard co-injected was insignificant compared to the TCDD level in the pesticide extract. Determination of regression slope and intercept for TCDD quantifications with such samples was variable due to poor correlations. An alternative GC/MS protocol was used for these samples. An external calibration curve was constructed by injecting varying amounts of the TCDD standard into the GC/MS. The resultant linear regression equation for the standard, in terms of picograms rather than microliters injected was:

$$y = Xs + i \quad (1)$$

where y = peak area (m/e = 322)
 s = slope (area/pg TCDD)
 i = intercept
 X = pg TCDD injected

Rearranging Eq. (1):

$$X_p = (y_p - i)/s \quad (2)$$

where X_p = pg TCDD in the pesticide sample extract and y_p = average m/e 322 area for a 2 µl sample extract. TCDD concentration (ppm) in the sample is equal to:

$$\text{ppm} = X_p \frac{(200 \text{ µl sample extract volume})}{(2 \text{ µl injected into GC/MS})} / (R \cdot \text{sample weight} \cdot 10^4)$$

where R is the percentage recovery of ³⁷Cl TCDD.

SECTION 5

RESULTS AND DISCUSSION

QUALITY ASSURANCE

Several aspects of this project were monitored for quality assurance. These included (1) taking custody of standards and samples, (2) materials and reagents used in the analysis, (3) standard procedures, (4) calibrations, (5) record keeping, and (6) quality control charts. Chain of custody records were transmitted to GSRI along with samples and standards. Sample receipt was acknowledged by dating, signing, and returning a copy of the chain of transfer record to EPA. Discrepancies in labeling or replacement requests were noted on the transfer record.

Materials and reagents used in the analysis in general were ACS or pesticide grade. New lots of material were tested for TCDD interference by subjecting them to the analytical protocol. The sample extraction and clean-up procedure described in Section 4 was used throughout the project, without modification, and therefore constituted a standard operating procedure. Generally, the GC/MS protocol followed the standard procedure outlined in the previous section; certain modifications, i.e., changes in column temperature programming, were necessary to overcome interferences and refine the method.

The GC/MS instrument was tuned daily prior to analyses. GC/MS performance and linearity standards bracketed 5-6 successive analyses. Record keeping included an instrumental preventive maintenance log. GC/MS data and computer file reference number, date of extraction, and the sample weight were recorded in this log for each sample assayed.

Sample processing accountability was an important part of record keeping. Results were compiled on a master sample status form (see Appendix B) in the order samples were received. Results were also compiled on a cumulative data form (Appendix C) in the order samples were assayed. Cumulative quality control charts were useful indicators of whether or not the measurement process was in control. The charts showed trends or runs, sudden shifts in the mean, increased variability and often indicated the nature of a problem.

Figures 2 through 4 show quality control data for GC/MS performance, method blank, and accuracy of TCDD determinations. Control chart analysis of graphs A, B, C, and D (Figure 2) shows systematic variations with probable causes. A series of increasing and decreasing values (graphs B and D, observation numbers 1-6 and 29-34) was obtained after the instrument

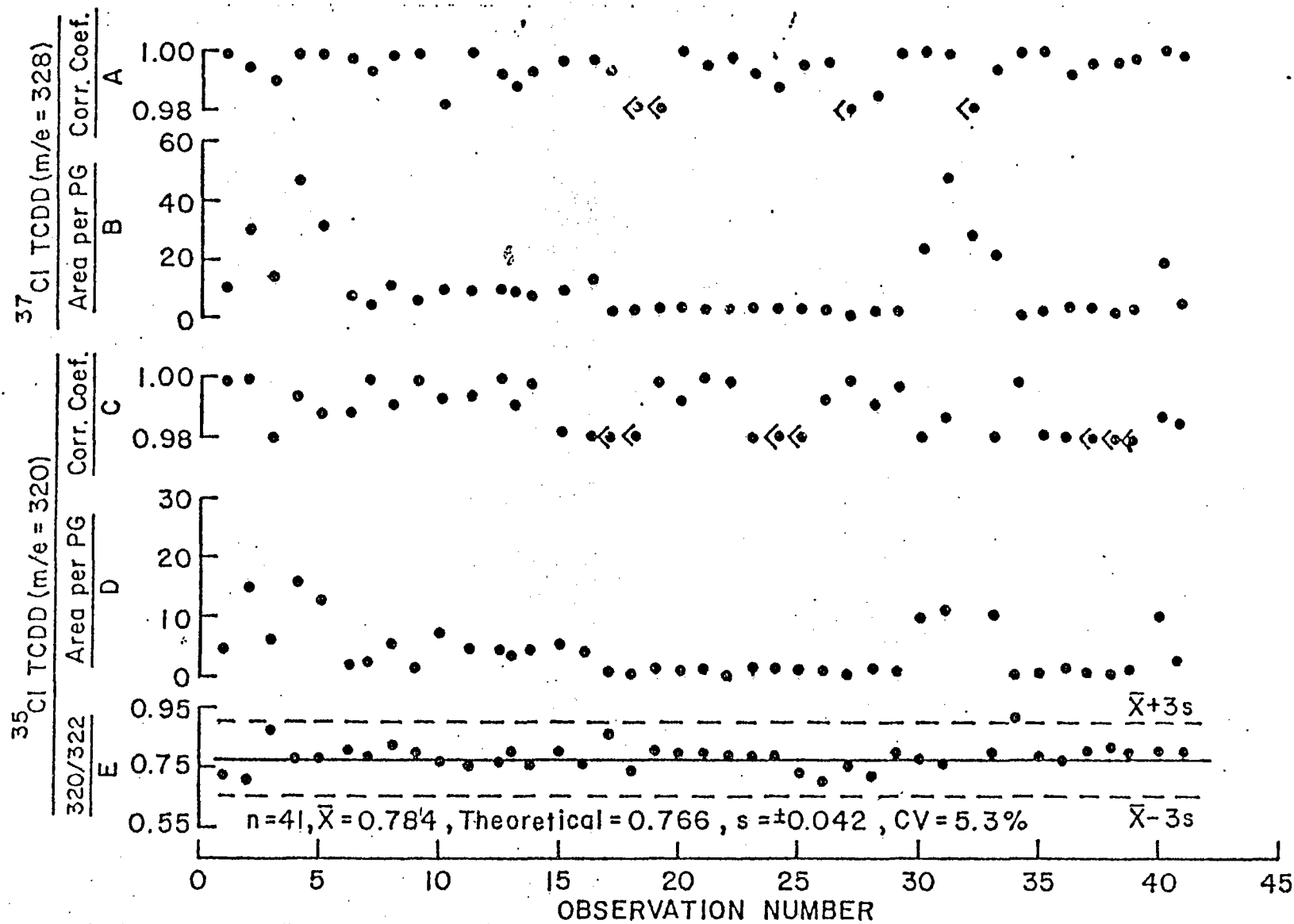


Figure 2. Quality control chart for GC/MS performance (Sample: ^{37}Cl TCDD and ^{35}Cl TCDD standards).

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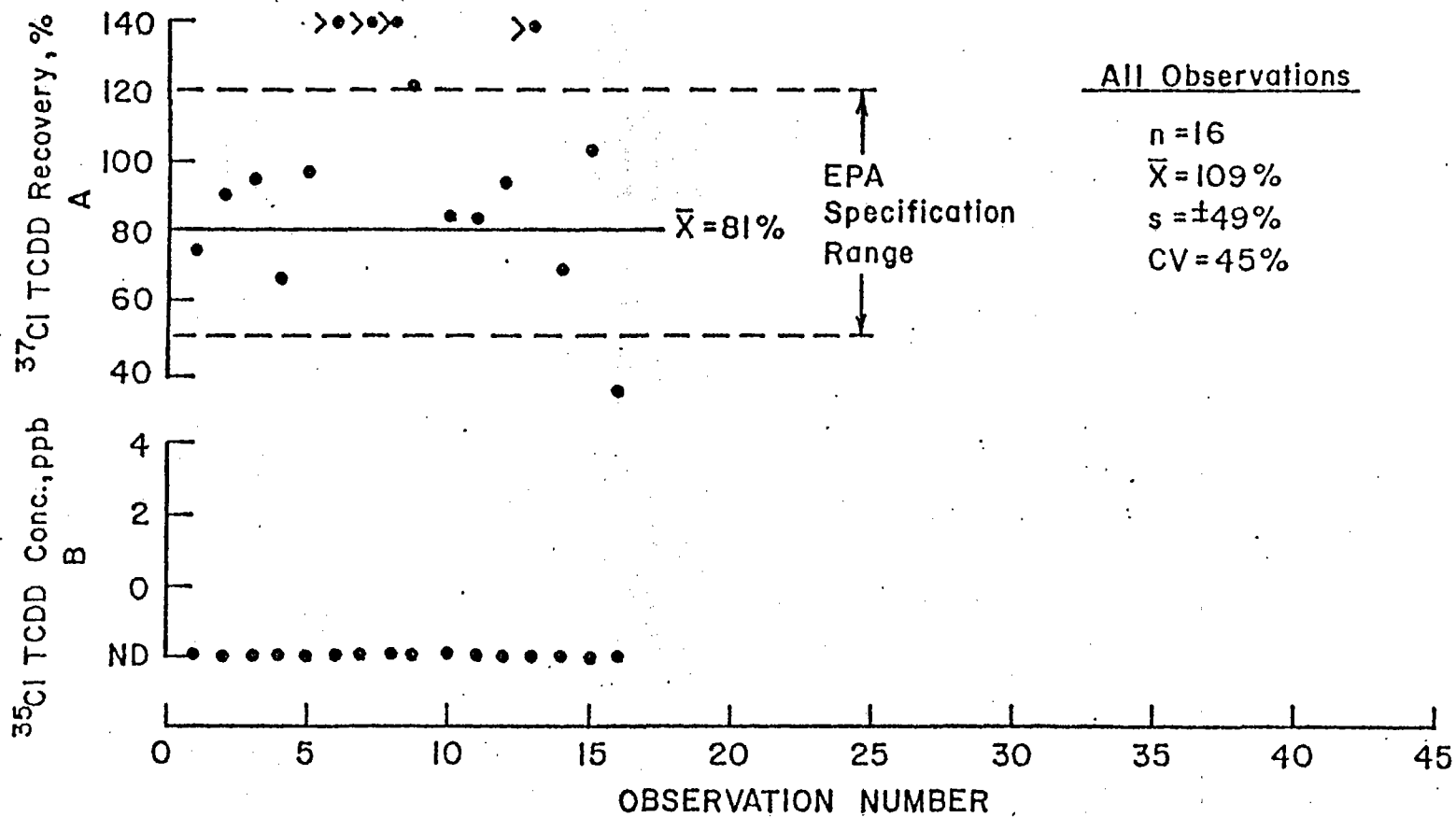


Figure 3. Method blank quality control chart (Sample: solvent blank with added ^{37}Cl TCDD).

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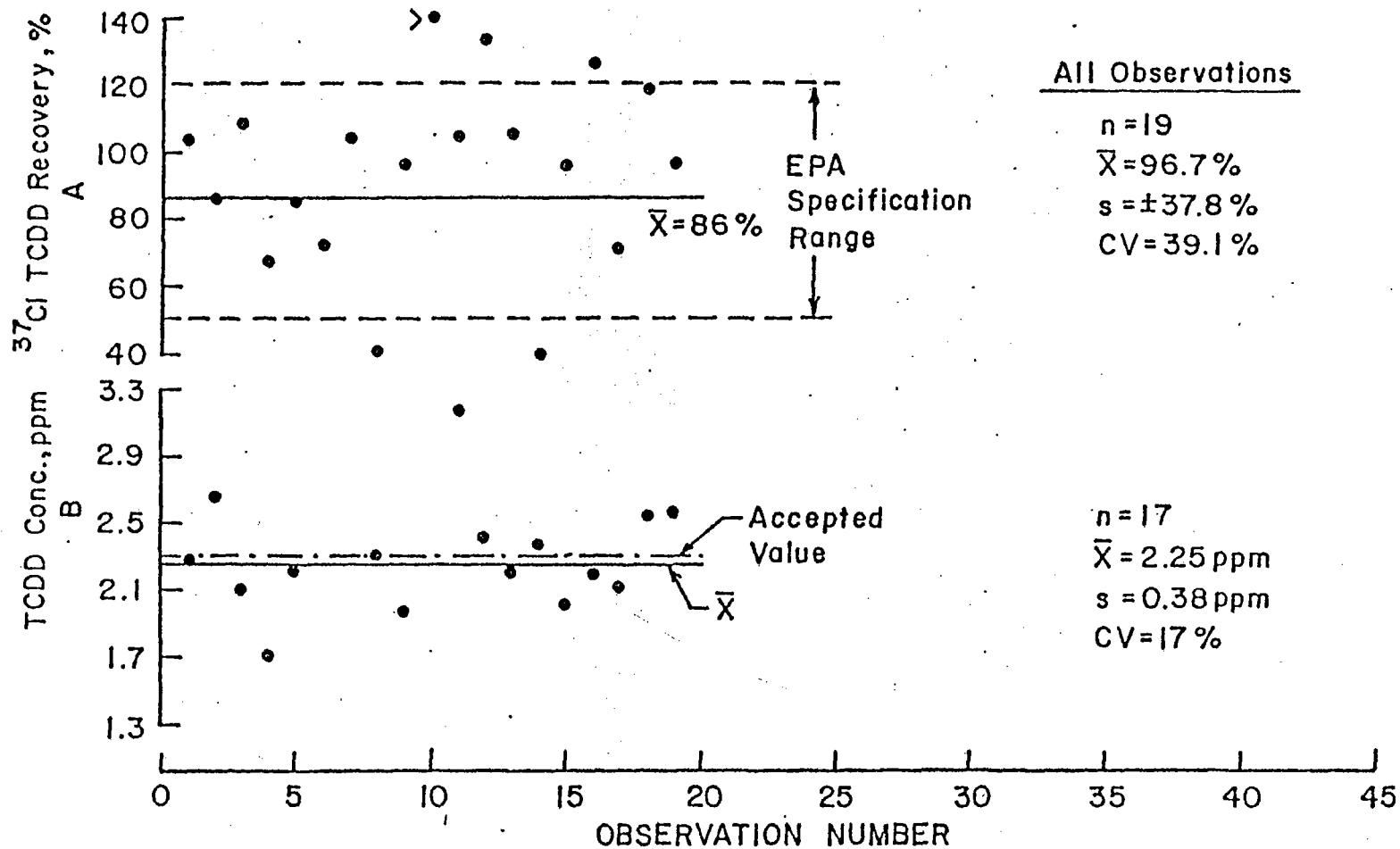


Figure 4. Accuracy quality control chart (Sample: EPA #0093).

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was shut down and the ion source cleaned. Observation numbers 18-29 and 35-39 show decreased sensitivity prior to source maintenance. Ion current chromatograms for observation 21 are shown in Appendix D. Concomitant with lower sensitivity, runs of 2 to 4 observations (graphs A and C) were observed with regression correlation coefficients <0.980 . Nonetheless, graph E shows that only 1 observation out of 41 was outside the arbitrarily chosen ± 3 standard deviation limits for 320/322 ion current peak areas. The theoretical ratio of 0.766 (6) compares favorably with the observed mean value ($\bar{X} = 0.784$, percent difference = + 2.3 percent). The conclusion was that the measurement process (320/322 ratio) was in control for 97.6 percent of the observations is based on Duncan's (7) criteria that define statistical control to be any point within 3 standard deviation limits. No limits were placed on A-D graphs.

Figures 3 and 4 show the method blank (solvent spiked only with ^{37}Cl labeled TCDD) and accuracy (EPA reference pesticide sample #0093) quality control charts with EPA recovery specification range. Mean recovery observations falling within EPA specifications were 81 and 86 percent, respectively. The EPA accepted value for total TCDD concentration in the pesticide reference sample is 2.3 ppm (8). A mean of 2.25 ± 0.38 ppm was obtained, with a coefficient of variation of 17 percent. No TCDD was observed in any method blank.

Several recovery observations were outside the specification range. A run of out-of-specification values (Figure 3, curve A, observations 6-8) was due in part to a partially clogged jet separator. Cleaning the jet separator rectified the problem. Random observations outside specification limits probably reflect overall variance of the total procedure.

A majority of out-of-specification recovery values were on the high side, i.e. >120 percent, probably because the upper one-sided tolerance of the analytical process is only 20 percent above the theoretical recovery value of 100 percent; the corresponding lower one-sided tolerance is 2.5 times as great, i.e., 50 percent.

Criteria for a Positive Sample

Qualitative criteria developed for the detection of TCDD in the pesticide extract matrix were: (1) a recovery of ^{37}Cl TCDD between 50 and 120 percent, inclusive; (2) both m/e 320 and 322 peaks existing at the TCDD retention time region; (3) well defined m/e 320 and 322 peaks whose retention time exactly equals that of TCDD standards; (4) signal-to-noise ratio of at least 2.5/1 at m/e 322; (5) ratio of m/e 320/322 peak areas in the proper isotopic proportion; and (6) TCDD confirmation by molecular ion spectra. Data for the first five requirements were obtained by selective ion monitoring at m/e 328, 322, and 320; data for TCDD confirmation were generated by recording spectra in the m/e range 150-350 and computer tabulation of absolute abundances of 119-131 m/e. The range of interest was m/e 320-328; the wide range facilitated background measurement. Pertinent information explaining these binary (positive/negative) decision making processes is shown in Table 1.

Criterion #1 was based solely on recovery specifications in the contract. Criteria 5 and 6 incorporate variance associated with the measurement system. Statistical limits imposed are shown in Table 1. The theoretical isotopic abundance ratio of m/e 320/322/324/326 relative to 322 is 0.766/1.000/0.489/0.001 (6). Criterion #6 was divided into three subcriteria (Table 1); eight binary permutations of the subcriteria are possible as shown in Table 2. Overall criterion #6 test characteristic was arbitrarily chosen from the three subcriteria. The rationale for subcriteria decision making correlates with isotopic abundance ratios. Greater emphasis was given to 6a (m/e 320/322) since these two m/e values are the largest. More precise ratio measurements are anticipated with 6a rather than the smaller 324/322 ratio for 6b or 6c. Table 3 shows the evaluation of the certainty of TCDD detection based on summation of qualitative criteria characteristics. Criteria 1 through 4 were absolute or necessary for determining if TCDD was detected except for the D (duplicate), T (triplicate) and Q (quadruplicate) reruns of original samples. Detection limits and mass confirmation spectra were not required for the latter.

Appendices D-I include representative hard copy data for GC/MS instrumental performance and TCDD detection.

Sample Data

A wide variety of pesticide samples were analyzed for total TCDD residues. Submitted EPA samples (unknowns and quality control samples) were analyzed on a blind basis. The two sample types were visually and experimentally indistinguishable. EPA provided decoding information only after completion of the assays and reporting of the TCDD data. Table 4 summarizes the decoding information. Generally, a 6-digit sample code represents a series of pesticide samples of the same generic class; individual samples were denoted by a hyphenated (in some cases) suffix made up of numbers and/or letters. For example, 137832-5D relates to the duplicate determination of the fifth subsample in the chlorinated phenolic antiseptic 137832 series. Each subsample represents a different batch of the manufactured technical grade pesticides.

Based on EPA decoding information, replicate sample data, if available, were collated according to sample batch number. After first combining replicate information with the appropriate first-run results, a TCDD detection judgement was made for each batch sample, regardless of the number of replicates assayed. These judgements were either positive, negative, or doubtful. For example, if the first run (I) sample of batch number 1234 was positive (e.g., 1234I is +), the duplicate (D) run negative (1234D is -), and the triplicate (T) run questionable (1234T is doubtful), then the judgement was that TCDD detection was doubtful in sample 1234. On the other hand, if a sample batch were judged TCDD positive, regardless of how many replicates were run, total TCDD level found was reported, including replicate data and the average value, if appropriate.

A total of 69 authentic pesticide batch samples were assayed. TCDD was detected in 17 batch samples (25 percent); TCDD detection was doubtful in 6 samples (9 percent); and TCDD was not detected in 46 batch samples (66

percent). Tables 5-12 summarize data for TCDD in various types of authentic pesticide samples. In those instances where all or most batch samples were positive for a given series, tabulation of mean value, standard deviation, and coefficient of variation is based on positive sample data. NA (not applicable) refers to information not available or not requested. For example, qualitative detection criteria #4 and #6 were not required for -D, -T, or -Q samples. Data for EPA quality controls are shown in Table 13.

Variation of TCDD detection judgements versus EPA sample type is shown in Table 14. For the authentic batch samples TCDD was nondetectable (detection limit 10 ppb) in 100 percent of the 2,4,5-trichlorophenol samples analyzed (n, number of batch samples assayed = 16). For comparative purposes, batch samples of 2,4,5-T, the butoxyethyl ester of 2,4,5-T, and the isooctyl ester of 2,4,5-T are grouped under 2,4,5-T as shown below.

2,4,5-T SAMPLE TYPE (16 BATCHES)				
Type	Number of Batches	Number of Batches/(percent)		
		Positive	Doubtful	Nondetectable
2,4,5-T	3	1/(33.3)	1/(33.3)	1/(33.3)
butoxyethyl ester of 2,4,5-T	10	9/(90)	1/(10)	0
isooctyl ester of 2,4,5-T	3	0	3/(100)	0
	16	10/(63)	5/(31)	1(6)

Overall, detection of TCDD was positive in 63 percent (n=10) of the batch samples, doubtful in 31 percent (n=5), and nondetectable in 6 percent (n=1). Specifically, detection of total TCDD was positive in 90 percent (n=9) of the butoxyethyl ester of 2,4,5-T batch samples. In contrast, detection of TCDD was judged equally positive, doubtful and nondetectable in all (n=3) of the 2,4,5-T batch samples, and TCDD detection was questionable in all (n=3) of the isooctyl ester of 2,4,5-T batch samples.

Eight Silvex type batch samples were assayed. TCDD detection was positive in 86 percent (n=6) of the butoxypropyl ester of Silvex and questionable in the remaining sample (n=1) of this type. Only one batch of isooctyl ester of Silvex was assayed; the TCDD detection judgement was positive. One-hundred percent of both the chlorinated phenolic antiseptic (n=22) and the organophosphate (n=7) batch samples gave negative TCDD detection characteristics.

Summarizing the TCDD detection judgements for authentic pesticide batch samples in terms of decreasing percentages, 66 percent were nondetectable, 25 percent positive, and 9 percent doubtful. Likewise, judgements for the 20 different EPA QC samples analyzed were 40 percent nondetectable, 30 percent doubtful, and 30 percent positive.

Variation of total TCDD level found versus sample type for positive samples is shown in Table 15. All authentic pesticide batch samples for which TCDD detection was judged positive contained total TCDD in the range 10-30 ppb. By contrast, total TCDD content of EPA quality controls varied from a low range of 31-99 ppb to a high of 3000-3499 ppb.

Precision of TCDD determinations in the EPA quality controls is shown in Table 16. Relative standard deviation ranged from 2.6 percent to 83 percent. Standard deviation range for authentic unknown replications was 3.1 percent to 52 percent, as shown in Table 17.

The prevalence of doubtful TCDD detection judgements in authentic pesticide samples and EPA quality controls suggests the need for improvements in the measurement system. A significant number of doubtful detection instances are related to apparent TCDD levels <10 ppb; in addition, TCDD confirmation was not a contract requirement at these low levels. Mean detection limit was $4.7 \text{ ppb} \pm 3.5 \text{ ppb}$ (standard deviation). Mean value plus two standard deviations = 11.7 ppb; thus questionable TCDD detection at the 10 ppb level can be expected. Therefore, any result found below 10 ppb in authentic samples was reported as nondetectable (ND). Actual values below 10 ppb in the quality controls are tabulated in Table 13 to demonstrate the inaccuracy in reporting such data. The qualitative criteria were used to define the judgement below 10 ppb (i.e., positive, doubtful, or negative).

Problems encountered in the project - low and high recoveries, unreliability of standards, inability to distinguish matrix problems from instrumental ones, variability in machine sensitivity, changes in chromatography, and interferences - contributed to observable variations in relative standard deviation and detection limits. Extracts yielded interferences of varying severity from other substances depending upon the efficiency and reproducibility of the cleanup procedure and sample type. Varying interferences also affected ^{37}Cl TCDD recovery. For example, excellent recoveries were obtained for samples 148921-3, 148921-3D, and 148921-4D. Zero percent recoveries were obtained with 148921-3T and 148921-4T. Internal quality control samples in the same sample sets (pertaining to extraction) gave excellent recoveries.

Low recoveries resulting from interferences might be prevented by employing a second Al_2O_3 cleanup column or other modification in the extraction procedure. Recoveries higher than the theoretical value were anticipated when m/e 328 peaks co-eluted with ^{37}Cl -TCDD.

Interfering m/e 320 and 322 peaks co-eluting with TCDD contributed to variability in calculated TCDD levels. In some instances peaks eluting prior to or immediately after TCDD prevented accurate peak interpretation.

Application of high resolution gas chromatography should help to overcome problems with interferences.

Problems with standard reliability included irreproducibility of lots and concentration upon standing. TCDD standards were provided by EPA and lots were checked against each other to identify suspect solutions. TCDD standards were provided in benzene solvent. Aliquots of the standards were transferred to small vials sealed with Teflon-lined rubber septums. Standards were withdrawn with a 10 μ l microsyringe. Repeated insertions of the microsyringe needle (four insertions were required per sample assay) resulted in formation of holes in the septum. Benzene evaporated through the porous septum and concentration of the TCDD standard increased. An attempt was made to solve the problem by adding a small amount of mercury to the standard vials to seal punctures in the septum. Between injections, vials were stored in an inverted position which prevented benzene evaporation. However, after a few days the solution turned cloudy, indicating instability of the standard solutions. The problem was rectified by using Mininert vials as outlined in the experimental section.

Variability in machine sensitivity contributed to variable TCDD detection limits and regression correlation coefficients. Approximately one hour was required to complete the four GC/MS runs needed to construct the regression line. Drift in GC/MS sensitivity and specificity over this period contributed to a poorer correlation coefficient with increased variability in reported TCDD recovery and quantification.

Distinguishing matrix problems from instrumental ones was difficult. Replicate recovery assays of the same extract provided data to elucidate problems. Poor precision was indicative of instrumental problems. High precision combined with recoveries outside the specification range suggested matrix or extraction problems.

Total TCDD was judged nondetectable in over 50 percent of the authentic pesticide batch samples assayed. Extending the lower working range of the measurement system seems desirable. This task might be accomplished by further cleanup of the extract, evaporation of the extract to a smaller volume, and employing high efficiency gas chromatography/mass spectrometry.

Application of the more tedious standard addition technique to these analyses appears justified over external calibration. Variations in the linear regression slope were observed for some samples, which may be indicative of matrix effects. The standard addition technique also tends to compensate for instrument drift. Therefore, more accurate data would be anticipated with the standard addition approach than with external working curve techniques.

REFERENCES

1. Blair, E.H. Chlorodioxins-Origin and Fate. Preface, Advances in Chemistry Series #120, American Chemical Society, Washington, D.C., 1973. p. 141.
2. Schwetz, B.A., J.M. Norris, G.L. Sparschu, V.K. Rowe, P.J. Gehring, J.L. Emerson, and C.G. Gerbig. Toxicology of Chlorinated Dibenzo-Para-Dioxins. Environ. Health Perspect. 5:87-99, 1973.
3. IARC (WHO). Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, 15, 1977. 354 pp.
4. Rappe, C., H.R. Buser, and H.P. Bosshardt. Identification and Quantification of Poly-Chlorinated Dibenzo-p-Dioxins (PCDDs) and Dibenzo-Ferans (PCDFs) in 2,4,5-T-Ester Formulations and Herbicide Orange. Chemosphere, 7(5): 431-438, 1978.
5. Firestone, D. The 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Problem: A Review. In: Ecological Bulletins (Stockholm), 27:39-52, 1978.
6. Beynon, J.H. Mass Spectrometry and its Applications to Organic Chemistry. D. Van Nostrand Co., Princeton, N.J. 1960. 640 pp.
7. Duncan, A.J. Quality Control and Industrial Statistics, 4th ed., Richard D. Irwin, Inc., Homewood, Ill., 1974. p. 207.
8. Personal Communication, Mr. Ronald Thomas, Environmental Protection Agency, Beltsville, Md., 1978.

TABLE 1. QUALITATIVE CRITERIA EXPLANATION

No.	Criteria	Explanation
1	50% $\leq$ R $\leq$ 120%	If initial R value exceeds specifications backup extract is assayed. If R still out-of-specification, sample is extracted and assayed one additional time.
2	existence of both m/e 320 and m/e 322	If only one of two peaks exists, sample listed as ND, not detected.
3	well defined m/e 320 and 322 peaks at TCDD retention time	A change in slope from negative to positive if the TCDD peak is a shoulder on a contaminating peak.
4	m/e 322 integrated peak area at least 2.5 times greater than baseline integrated noise	If less than 2.5, sample listed as ND, not detected
5	m/e 320/322 isotopic ratio, selected ion monitoring	Control limits set by mean value $\pm$ 3 standard deviations as determined by quality control data obtained with EPA reference pesticide sample #0093.
6	m/e range 320-326, absolute abundances, molecular ion spectra	Divided into three subcriteria: (a) m/e 320/322 control limits set by mean value $\pm$ 3 standard deviations as determined by quality control data obtained with EPA reference pesticide sample #0093. (b) m/e 324/322 control limits set by mean value $\pm$ 3 standard deviations as determined by quality control data obtained with EPA reference pesticide sample #0093. (c) Existence of m/e 320, 322, 324, and 326 in absolute abundance table.

TABLE 2. DEPENDENCE OF CRITERION #6 OVERALL BINARY CHARACTERISTIC ON
SUBCRITERIA RESULTS

Overall Criterion #6 Characteristic	Permutation		
	6a	6b	6c
- (negative)	-	-	-
	-	+	-
	-	-	+
	-	+	+
+ (positive)	+	+	+
	+	+	-
	+	-	+
	+	-	-

TABLE 3. TCDD DETECTION JUDGEMENT BASED ON
QUALITATIVE CRITERIA CHARACTERISTICS

Qualitative Criteria No.						Judgement
1	2	3	4	5	6	
For First-Run Samples:						
+	+	+	+	+	+	TCDD detected
+	+	+	+	-	+	
+	+	+	+	-	-	TCDD detection questionable
+	+	+	+	+	-	
Any or all negative ^a				NA ^b	NA	TCDD not detected
For Duplicate (-D), Triplicate (-T), or Quadruplicate (-Q) Samples:						
+	+	+	NA	+	NA	TCDD detected if also detected in first-run sample
						TCDD detection question- able if first-run sample also questionable or not detected.
+	+	+	NA	-	NA	TCDD detection question- able regardless of first-run judgement
Any or all negative (-)			NA	+	NA	TCDD not detected regardless of first- run judgement

^aQualitative criteria 1 through 4 all absolute or necessary
for TCDD detected (positive) judgement.

^bNA = not applicable.

TABLE 4. VARIOUS EPA SAMPLES ANALYZED FOR TOTAL TCDD

Sample Series	Number of Batch Samples	Pesticide Sample Type (Generic Name)
<u>AUTHENTIC PESTICIDES</u>		
137401	1	2,4,5-T ^a
137402	1	
137403	1	
137832	14	chlorinated phenolic
137834	8	antiseptic
137833	2	2,4,5-trichlorophenol
137835	4	
148917	5	
148918	5	
141996	1	isooctyl ester of 2,4,5-T
141998	2	
141997	1	isooctyl ester of Silvex ^b
148919	10	butoxyethyl ester of 2,4,5-T
148920	7	butoxypropyl ester of Silvex
148921	7	organophosphate insecticide
<u>QUALITY CONTROLS</u>		
229617		
226617		
225511		
222841		
222455		
220777		
93-01		
14-01		
0093		
1-01		
305		
304		
303		
90		
11		
4		
3 W		
3 X		
3 Y		
3 Z		

^a2,4,5-T is 2,4,5-trichlorophenoxyacetic acid.

^bSilvex is 2,4,5-TP

TABLE 5. TCDD DATA FOR AUTHENTIC 2,4,5-TRICHLOROPHENOL
(16 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl. ^a	ND	
137833	-1	95	4.6	+	-	NA	NA	NA	NA			✓	ND
	-2	79	3.5	+	-	NA	NA	NA	NA			✓	ND
137835	-1	102	3.7	+	-	NA	NA	NA	NA			✓	ND
	-2	104	5.0	+	-	NA	NA	NA	NA			✓	ND
	-3	82	9.3	+	-	NA	NA	NA	NA			✓	ND
	-4	89	2.1	+	-	NA	NA	NA	NA			✓	ND
148917	-1	89	2.6	+	-	NA	NA	NA	NA			✓	ND
	-2	69	1.9	+	+	+	+	+	NA			✓	ND
	-3	100	2.5	+	-	NA	NA	NA	NA			✓	ND
	-4	80	8.8	+	-	NA	NA	NA	NA			✓	ND
	-5	86	2.5	+	-	NA	NA	NA	NA			✓	ND
148918	-1	51	1.8	+	+	+	+	+	NA			✓	ND
	-2	93	5.6	+	-	NA	NA	NA	NA			✓	ND
	-3	96	2.6	+	-	NA	NA	NA	NA			✓	ND
	-4	95	5.0	+	-	NA	NA	NA	NA			✓	ND
	-5	61	1.7	+	-	NA	NA	NA	NA			✓	ND

^aDtfl. = Doubtful

TABLE 6. TCDD DATA FOR AUTHENTIC 2,4,5-T (2,4,5-TRICHLOROPHENOXYACETIC ACID)
(3 Batch Samples).

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)	Average Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND		
137400	-137401	71	2.4	+	+	+	+	+	NA			✓	ND	18
	-137402									✓				
	-137402I	68	5.2	+	+	+	+	-	+	✓			17	
	-137402D	105	NA	+	+	+	NA	+	NA	✓			18	Dtfl.
	-137403										✓			
	-137403I	71	4.2	+	+	+	+	+	+	✓ ^a			14	
	-137403D	58	NA	+	-	NA	NA	NA	NA			✓	ND	

^aProbably false positive since the duplicate sample (137403D) was ND.

TABLE 7. TCDD DATA FOR AUTHENTIC BUTOXYETHYL ESTER OF 2,4,5-T
(10 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)	Average Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND		
148919	-1													21
	-1I	80	6.8	+	+	+	+	+	+	✓			23	
	-1D	114	NA	+	+	+	NA	+	NA	✓			19	
	-2									✓				23
	-2I	67	2.5	+	+	+	+	+	+	✓			25	
	-2D	99	NA	+	+	+	NA	+	NA	✓			21	
	-3	104	8.0	+	+	+	+	+	+	✓			24	
	-4	82	6.4	+	+	+	+	+	+	✓			29	
	-5									✓				23
	-5I	89	4.3	+	+	+	+	+	+	✓			22	
	-5D	114	NA	+	+	+	NA	+	NA	✓			23	
	-6									✓				25
	-6I	108	3.0	+	+	+	+	+	+	✓			28	
	-6D	78	NA	+	+	+	NA	+	NA	✓			22	
	-6T	72	1.1	+	+	+	+	+	+	✓			21	
	-7										✓			Dtfl.
	-7I	53	2.0	+	+	+	+	+	-		✓		33	
	-7D	111	NA	+	+	+	NA	+	NA		✓		19	
	-8									✓				22
	-8I	117	1.8	+	+	+	+	+	+	✓			14	
	-8D	116	NA	+	+	+	NA	+	NA	✓			30	
	-9									✓				22
	-9I	97	3.1	+	+	+	+	+	+	✓			23	
	-9D	70	NA	+	+	+	NA	+	NA	✓			23	
	-9T	99	4.5	+	+	+	+	+	+	✓			19	
	-10									✓				23
	-10I	61	3.5	+	+	+	+	+	+	✓			27	
	-10D	104	NA	+	+	+	NA	+	NA	✓			19	
Statistical information for TCDD positive batch samples:													7	
													mean total TCDD (ppb)	22.7
													standard deviation (ppb)	1.3
													coefficient of variation (%)	5.7

TABLE 8. TCDD DATA FOR AUTHENTIC ISOOCYTL ESTER OF 2,4,5-T
(3 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)	Average Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND		
141996														Dtfl.
	-141996I	103	2.0	+	+	+	+	-	+		✓		15	
	-141996D	61	NA	+	+	+	NA	+	NA		✓		30	
	-141996T	78	5.9	+	+	+	+	+	+	✓ ^a			21	
	-141996Q	103	NA	+	+	+	NA	-	NA		✓		27	
141998														
	-1										✓			Dtfl.
	-1I	110	2.5	+	+	+	+	+	+	✓			28	
	-1D	73	NA	+	+	+	NA	-	NA		✓		13	
	-2										✓			Dtfl.
	-2I	108	2.2	+	-	NA	NA	NA	NA			✓	ND	
	-2D	79	1.5	+	+	+	+	+	+	✓ ^a			27	

^a Other replicate(s) doubtful or nondetectable, therefore, probably not dioxin-contaminant or interferences present.

TABLE 9. TCDD DATA FOR AUTHENTIC BUTOXYPROPYL ESTER OF SILVEX
(7 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery Cl37TCDD	Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)	Average Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND		
148920	-1	71	4.4	+	+	+	+	+	+	✓			21	19
	-2									✓				
	-2I	83	2.3	+	+	+	+	+	+	✓			19	
	-2D	115	NA	+	+	+	NA	+	NA	✓			17	19
	-2T	83	6.3	+	+	+	+	+	+	✓			22	
	-3									✓				
	-3I	66	5.5	+	+	+	+	+	+	✓			21	Dtfl.
	-3D	77	NA	+	+	+	NA	+	NA	✓			17	
	-4										✓			
	-4I	67	1.0	+	+	+	+	-	NA		✓		20	21
	-4D	113	NA	+	+	+	NA	+	NA		✓		20	
	-5									✓				
	-5I	97	5.2	+	+	+	+	+	+	✓			24	22
	-5D	79	NA	+	+	+	NA	+	NA	✓			17	
	-5T	66	7.2	+	+	+	+	-	+	✓			23	
	-6	103	0.9	+	+	+	+	+	+	✓			12	22
	-7									✓				
	-7I	74	5.6	+	+	+	+	+	+	✓			22	
	-7D	111	NA	+	+	+	NA	+	NA	✓			21	

Statistical information for TCDD positive batch samples:

n	4
Mean total TCDD (ppb)	20.3
Standard deviation (ppb)	1.5
Coefficient of variation (%)	7.4

TABLE 10. TCDD DATA FOR AUTHENTIC ISOOCYL ESTER OF SILVEX
(1 Batch Sample)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND	
141997	141997	81	9.7	+	+	+	+	+	+	✓			20

TABLE 11. TCDD DATA FOR AUTHENTIC CHLORINATED PHENOLIC ANTISEPTIC
(22 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND	
137832	-1	83	16	+	-	NA	NA	NA	NA			✓	ND
	-2	77	12	+	-	NA	NA	NA	NA			✓	ND
	-3											✓	ND
	-3I	102	8.9	+	-	NA	NA	NA	NA			✓	
	-3D	68	NA	+	-	NA	NA	NA	NA			✓	
	-4											✓	ND
	-4I	50	1.8	+	-	-	-	-	NA			✓	
	-4D	61	NA	+	-	NA	NA	NA	NA			✓	
	-5											✓	ND
	-5I	91	4.0	+	-	NA	NA	NA	NA			✓	
	-5D	79	NA	+	-	NA	NA	NA	NA			✓	
	-6	90	3.9	+	-	NA	NA	NA	NA			✓	ND
	-7	84	9.4	+	-	NA	NA	NA	NA			✓	ND
	-8	93	2.2	+	-	NA	NA	NA	NA			✓	ND
137834	-9	68	8.4	+	-	NA	NA	NA	NA			✓	ND
	-10	64	3.6	+	-	NA	NA	NA	NA			✓	ND
	-11	78	2.3	+	-	NA	NA	NA	NA			✓	ND
	-12	74	4.4	+	-	NA	NA	NA	NA			✓	ND
	-13	96	2.7	+	-	NA	NA	NA	NA			✓	ND
	-14	68	2.6	+	-	NA	NA	NA	NA			✓	ND
	-1	93	7.1	+	-	NA	NA	NA	NA			✓	ND
	-2	111	2.4	+	-	NA	NA	NA	NA			✓	ND
	-3	91	1.9	+	-	NA	NA	NA	NA			✓	ND
	-4	78	3.0	+	-	NA	NA	NA	NA			✓	ND
	-5	100	1.7	+	-	NA	NA	NA	NA			✓	ND
	-6	93	2.3	+	-	NA	NA	NA	NA			✓	ND
	-7	117	4.3	+	-	NA	NA	NA	NA			✓	ND
	-8	102	1.9	+	-	NA	NA	NA	NA			✓	ND

TABLE 12. TCDD DATA FOR AUTHENTIC ORGANOPHOSPHATE
(7 Batch Samples)

EPA Series Number	EPA Batch Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)
				1	2	3	4	5	6	Pos.	Dtfl.	ND	
148921	-1	51	9.9	+	-	NA	NA	NA	NA			✓	ND
	-2	110	2.9	+	-	NA	NA	NA	NA			✓	ND
	-3											✓	ND
	-3I	100	5.2	+	-	NA	NA	NA	NA			✓	
	-3D	100	NA	+	-	NA	NA	NA	NA			✓	
	-3T	0	NA	-	-	NA	NA	NA	NA			✓	
	-4											✓	ND
	-4I	111	2.2	+	-	NA	NA	NA	NA			✓	
	-4D ^a	95	NA	+	+	+	NA	+	NA		✓		
	-4T	0	NA	-	-	NA	NA	NA	NA			✓	
	-5	78	1.5	+	-	NA	NA	NA	NA			✓	ND
	-6	69	4.5	+	-	NA	NA	NA	NA			✓	ND
	-7	93	9.3	+	-	NA	NA	NA	NA			✓	ND

^aProbably false positive due to contamination or interferences present, since replicate samples were ND.

TABLE 13. TCDD DATA FOR QUALITY CONTROLS
(20 Different QC Samples; 41 Assays)

EPA QC Sample Number	Percent Recovery C137TCDD	Limit of Detection (ppb)	Qualitative Criteria						Judgement			Total TCDD (ppb)	Average Total TCDD (ppb)
			1	2	3	4	5	6	Pos.	Dtfl ^a	ND		
222455													ND
222455I	92	3.1	+	+	+	+	+	+	✓		✓	3.6	
222455D	76	NA	+	-	NA	NA	NA	NA			✓	ND	
220777													Dtfl.
220777I	71	2.5	+	+	+	+	+	+	✓	✓		39	
220777D	119	NA	+	+	+	NA	+	NA	✓			45	
226617													Dtfl.
226617I	95	4.9	+	+	+	+	+	+		✓		23	
226617D	116	NA	+	+	+	NA	+	NA		✓		6	
225511													Dtfl.
225511I	76	5.9	+	+	+	+	-	+	✓			74	
225511D	77	NA	+	+	+	NA	+	NA	✓			43	
229617													ND
229617I	58	3.6	+	+	-	+	-	NA		✓		7	
229617D	82	NA	+	-	NA	NA	NA	NA			✓	ND	
1-01													212
1-01I	101	12.7	+	+	+	+	+	+	✓			236	
1-01D	61	NA	+	+	+	NA	+	NA	✓			126	
1-01T	94	NA	+	+	+	NA	-	NA	✓			274	
11													81
11I	80	0.7	+	+	+	+	+	+	✓			80	
11D	84	NA	+	+	+	NA	+	NA	✓			83	
11T	100	NA	+	+	+	NA	+	NA	✓			79	
3W	77	9.9	+	-	NA	NA	NA	NA			✓	ND	
3Y													ND
3YI	59	4.9	+	+	-	+	-	+			✓	ND	
3YD	83	NA	+	-	NA	NA	NA	NA			✓	ND	
3X													87
3XI	83	2.4	+	+	+	+	-	+	✓			56	
3XD	92	NA	+	+	+	NA	+	NA	✓			124	
3XT	89	NA	+	+	+	NA	+	NA	✓			82	
93-01	115	5.5	+	+	+	+	+	+	✓			1620	
4													3627
4I	113	13	+	+	+	+	+	+	✓			3270	
4D	90	NA	+	+	+	NA	+	NA	✓			3984	
14-01													ND
14-01I	83	3.1	+	+	+	+	+	NA		✓		5.7	
14-01D	107	NA	+	-	NA	NA	NA	NA			✓	ND	
14-01T	89	NA	+	+	+	NA	+	NA		✓		9.6	
3Z													ND
3ZI	68	2.5	+	-	NA	NA	NA	NA			✓	ND	
3ZD	106	NA	+	-	NA	NA	NA	NA		✓		ND	
222841													ND
222841I	89	2.0	+	+	+	+	+	NA		✓		2.2	
222841D	76	NA	+	-	NA	NA	NA	NA			✓	ND	
0093	97	82	+	+	+	+	+	+	✓			2250	
90													Dtfl.
90I	88	2.8	+	+	+	+	+	+	✓			85	
90D	60	NA	+	+	+	NA	-	NA		✓		57	
303													Dtfl.
303I	79	2.1	+	+	+	+	+	-		✓		16	
303D	81	NA	+	+	+	NA	+	NA		✓		18	
304													ND
304I	98	1.2	+	+	+	+	+	+	✓			9.6	
304D	72	NA	+	+	+	NA	+	NA	✓			7.1	
305													Dtfl.
305I	116	14	+	+	+	+	+	+	✓			1610	
305D	94	NA	+	+	+	NA	+	NA	✓			1732	

^aReplicate doubtful or ND or other reruns suggest probably not dioxin-contaminant or interferences present.

TABLE 14. VARIATION OF TCDD DETECTION JUDGEMENT VERSUS EPA SAMPLE TYPE

Sample Type	Number Batch Samples Analyzed	Number/(Percent) of Batch Samples Versus Detection Judgement		
		Positive	Doubtful	Nondetectable
AUTHENTIC PESTICIDE BATCH SAMPLES				
2,4,5-Trichlorophenol	16			16/100
2,4,5-T	3	1/(33.3)	1/(33.3)	1/(33.3)
Butoxyethyl Ester of 2,4,5-T	10	9/(90)	1/(10)	
Isooctyl Ester of 2,4,5-T	3		3/(100)	
Butoxypropyl Ester of Silvex	7	6/(86)	1/(14)	
Isooctyl Ester of Silvex	1	1/(100)		
Chlorinated Phenolic Antiseptic	22			22/(100)
Organophosphate	<u>7</u>	<u> </u>	<u> </u>	<u>7/(100)</u>
TOTALS	69	17(25)	6/(9)	46/66
QUALITY CONTROLS				
Twenty different QC Samples Assayed		6/(30)	6/(30)	8/(40)

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TABLE 15. VARIATION OF TOTAL TCDD LEVEL VERSUS SAMPLE TYPE FOR POSITIVE SAMPLES

Sample Type	Number Batch Samples Positive for TCDD	TCDD Range (ppb)									
		10-30	31-99	100-499	500-999	1000-1499	1500-1999	2000-2499	2500-2999	3000-3499	3500-3999
		Number Batch Samples/(Percent)									
AUTHENTIC PESTICIDE BATCH SAMPLES											
2,4,5-T	1	1/(18)									
Butoxyethyl Ester of 2,4,5-T	9	9/(100)									
Butoxypropyl Ester of Silvex	6	6/(100)									
Isooctyl Ester of Silvex	1	1/(100)									
QUALITY CONTROLS											
Six different QC Samples Positive for TCDD		2/(32.8)	1/(16.8)			1/(16.8)	1/(16.8)	1/(16.8)		1/(16.8)	

TABLE 16. COMPARISON OF REPLICATE RESULTS FOR EPA QUALITY CONTROL SAMPLES

Sample	Total TCDD Concentration (ppb)			Statistical Analysis		
	Run No. 1	Run No. 2(-D)	Run No. 3(-T)	Average (ppb)	Standard Deviation (ppb)	Relative Standard Deviation (%)
1-01	236	126	274	212	77	36
11	80	83	79	81	2.1	2.6
3Y	ND	ND	NA	NA	NA	NA
3X	56	124	82	87	34	39
4	3270	3984	NA	3627	504	14
14-01	5.7 ^a	ND	9.6 ^a			
3Z	ND	ND	NA			
222841	2.2 ^a	ND	NA			
222455	3.6	ND	NA			
220777	39	45	NA	42	4.2	9.4
226617	23 ^a	6 ^a	NA	15	12	83
225511	74	43	NA	59	22	37
229617	7 ^a	ND	NA			
90	85	57 ^a	NA	71	20	28
303	16 ^a	18 ^a	NA	17	1.4	8.3
304	9.6	17.1	NA	8.4	1.8	21
305	1610	1732	NA	1671	86	5.2

^aQualitative criteria judgement: TCDD detection doubtful.

TABLE 17. COMPARISON OF REPLICATE RESULTS FOR AUTHENTIC PESTICIDE BATCH SAMPLES

Type Sample	Batch Sample	Total TCDD Concentration (ppb)				Statistical Analysis		
		Run No. 1	Run No. 2 (-D)	Run No. 3 (-T)	Run No. 4 (-Q)	Average (ppb)	Standard Deviation (ppb)	Relative Standard Deviation (%)
2,4,5-T	137402	17	18			18	0.71	4
	137403	14	ND					
Chlorinated Phenolic Antiseptic	137832-3	ND	ND					
	137832-4	ND	ND					
	137832-5	ND	ND					
Isooctyl Ester of 2,4,5-T	141996 ^a	15	30	21	27	23	7	30
	141998-1	28	13 ^a			21	11	52
Butoxyethyl Ester of 2,4,5-T	148919-1	23	19			21	2.8	14
	148919-2	25	21			23	2.8	13
	148919-5	22	23			23	0.71	3.1
	148919-6	28	22	21		24	3.8	15.8
	148919-7	33 ^a	19 ^a			26	9.9	38
	148919-8	14	30			22	11	51
	148919-9	23	23	19		22	2.3	10.3
	138919-10	27	19			23	5.7	25
Butoxypropyl Ester of Silvex	148920-2	19	17	22		19	2.5	13.2
	148920-3	21	17			19	2.8	15
	148920-4	20 ^a	20 ^a			20	-	-
	148920-5	24	17	23		21	3.8	18.1
	148920-7	22	21			22	0.71	3.3
Organophosphate Formulations	148921-3	ND	ND					
	148921-4	ND	14 ^a					

^aQualitative criteria judgement: TCDD detection doubtful.^bA third sample of 148921-3 and 148921-4 was also run but not reported due to a 0 percent recovery.

Appendix A. Data Sheets for Sample 148919-5-2

ANALYSIS OF TECHNICAL GRADE PESTICIDES FOR TCDD

Data Sheet

Sample No. 148919-5-2 Date Received 2/8/78 Date of GC/MS Analysis 8/2/78
 Date and Type of Extraction/Cleanup 7/18/78 1 HEXANE - NaOH - H₂SO₄ - Al₂O₃
 Sample Weight 1.0084g Sample Volume 2 μ l Total Extract 200 μ l
 GC Conditions: Column 3' OV-11, 3% Injector Temperature 250°C Column Temperature 210(3)°C
 Carrier Gas and Flow Rate UHP He 1 32 ml/min (6) 260°C

GC/MS Data

μ l Co-Injected			Peak Area			
Sample	³⁵ Cl-TCDD	³⁷ Cl-TCDD	m/e			Ratio
			320	322	328	320/322
2	0	0	<u>269</u>	<u>338</u>	<u>1146</u>	<u>0.796</u>
2	1	2	<u>828</u>	<u>1060</u>	<u>3547</u>	<u>X</u>
2	0	0	<u>219</u>	<u>289</u>	<u>1023</u>	<u>0.758</u>
2	2	1	<u>1436</u>	<u>1906</u>	<u>2471</u>	<u>X</u>
Corr. (r)=			<u>0.9993</u>	<u>0.9991</u>	<u>0.9971</u>	
Slope. (s)=			<u>594.1</u>	<u>791.7</u>	<u>1245</u>	
Intcp. (i)=			<u>241.8</u>	<u>304.4</u>	<u>1112</u>	
i/s =			<u>0.406</u>	<u>0.385</u>	<u>0.893</u>	
Avg. Ratio =			<u>0.777 ± 0.027</u>			

ANALYSIS OF TECHNICAL GRADE PESTICIDES FOR
148919-5R1-2 TCDD
Results

RECOVERY

^{37}Cl -TCDD Std. Conc.(pg/ μl) = 480 % Recovery(R)
Added to Sample(ng) = 48 (I) $R = \frac{\text{II}}{\text{I}} \times 100 = \underline{89} \%$
Recovered(ng):
i/s x 48 = 42.8 (II)

QUANTIFICATION

^{35}Cl -TCDD Std. Conc.(pg/ μl) = 500

	<u>320</u>	<u>322</u>	
Amount in Extract(ng) = i/s x <u>50</u>	<u>20.3</u>	<u>19.3</u>	(III)
Amount in Sample(ng) = III / R	<u>22.8</u>	<u>21.7</u>	(IV)
Sample Conc.(ppb) = IV / g Sample	<u>22.6</u>	<u>21.5</u>	

Avg. Conc. (ppb) = 22.1 $\pm$ 0.78

LIMIT OF DETECTION

N/A _____ A ✓

m/e = 322

L.D., 2.5(S/N) = 4.3 ppb

CONFIRMATION ANALYSIS (Fragmentation Pattern Recorded)

N/A _____ A ✓

Result Positive ✓

Result Negative _____

Appendix B. Master Sample Status Form

Master Sample Status For 328-884-11

[illegible]

page _____

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Appendix C. Cumulative Tabulated Analytical Data Form

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Page_____

Appendix D. GC/MS Performance Ion Current Chromatograms

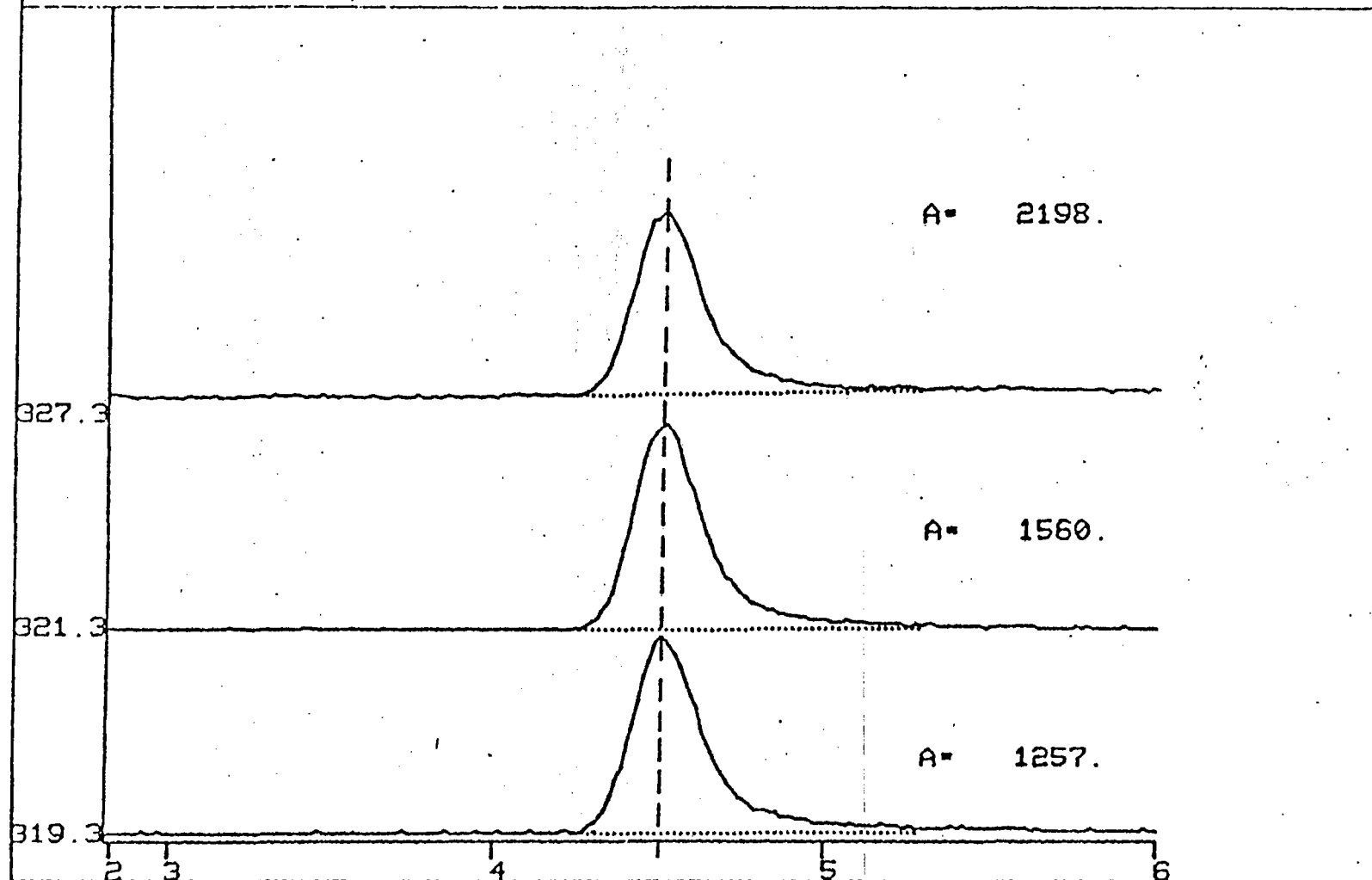
(Observation #21)

OBSERVATION #21 DATA

ul co-injected			m/e/ Peak area		
³⁵ Cl TCDD		³⁷ Cl TCDD	320	322	328
2	+	2	1257	1560	2198
1	+	1	583	735	1073
2	+	2	1203	1612	2317
1	+	1	576	722	1176
Corr (r)			0.9999	0.9990	0.9952
Slope (s)			680.5	857.5	1133
Intercept (i)			-101	-129	-8.5
s/500 or 480			1.36	1.71	2.36
Avg. 320/322 = 0.795					

2UL TCDD NATIVE CL35 +CL37 STDS CHECK
31JULY78, 0011, 1 OF 4

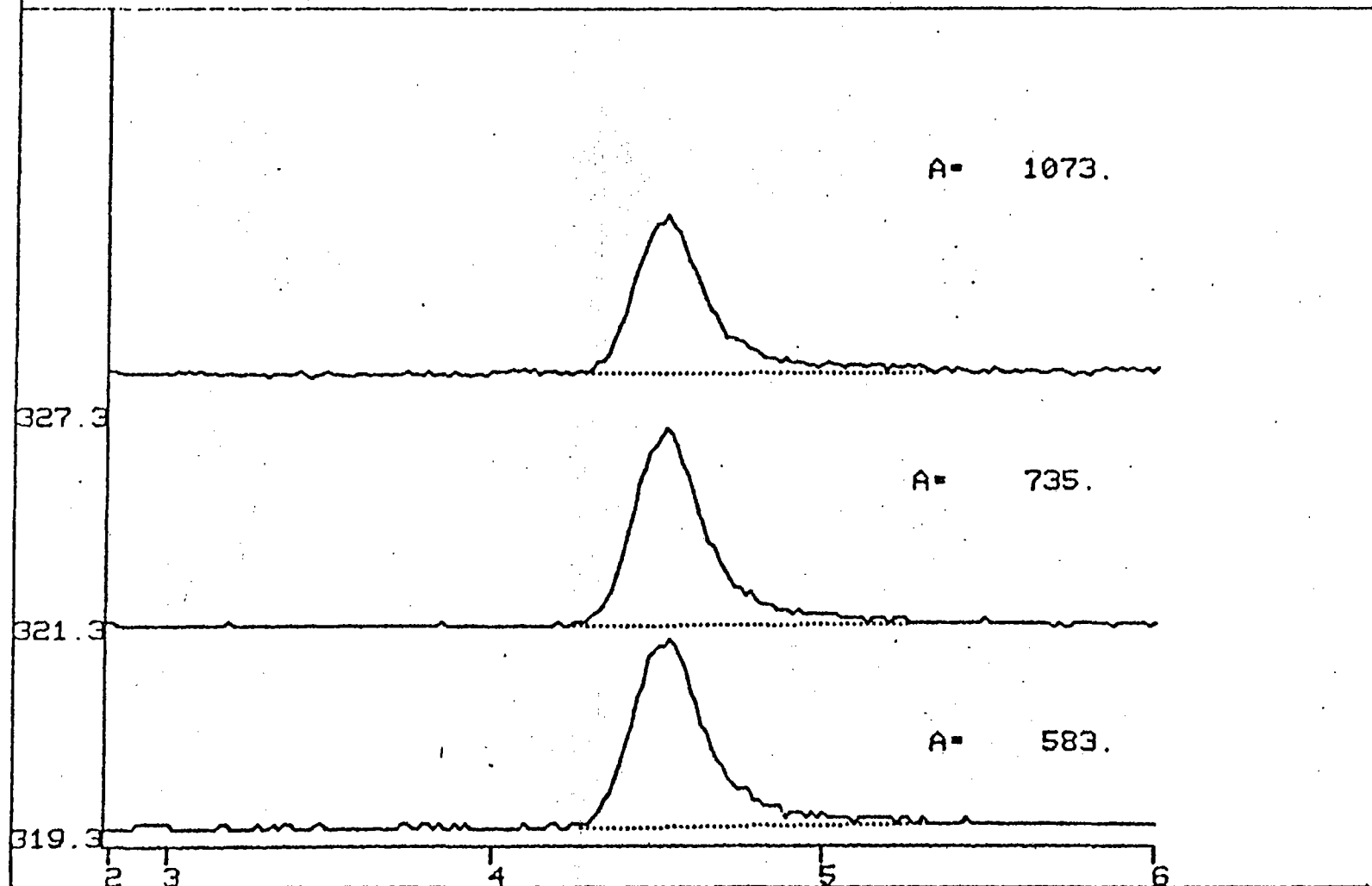
FRN 21079
1ST SC/PG 1
X= 1 00 Y= 1.00



21710

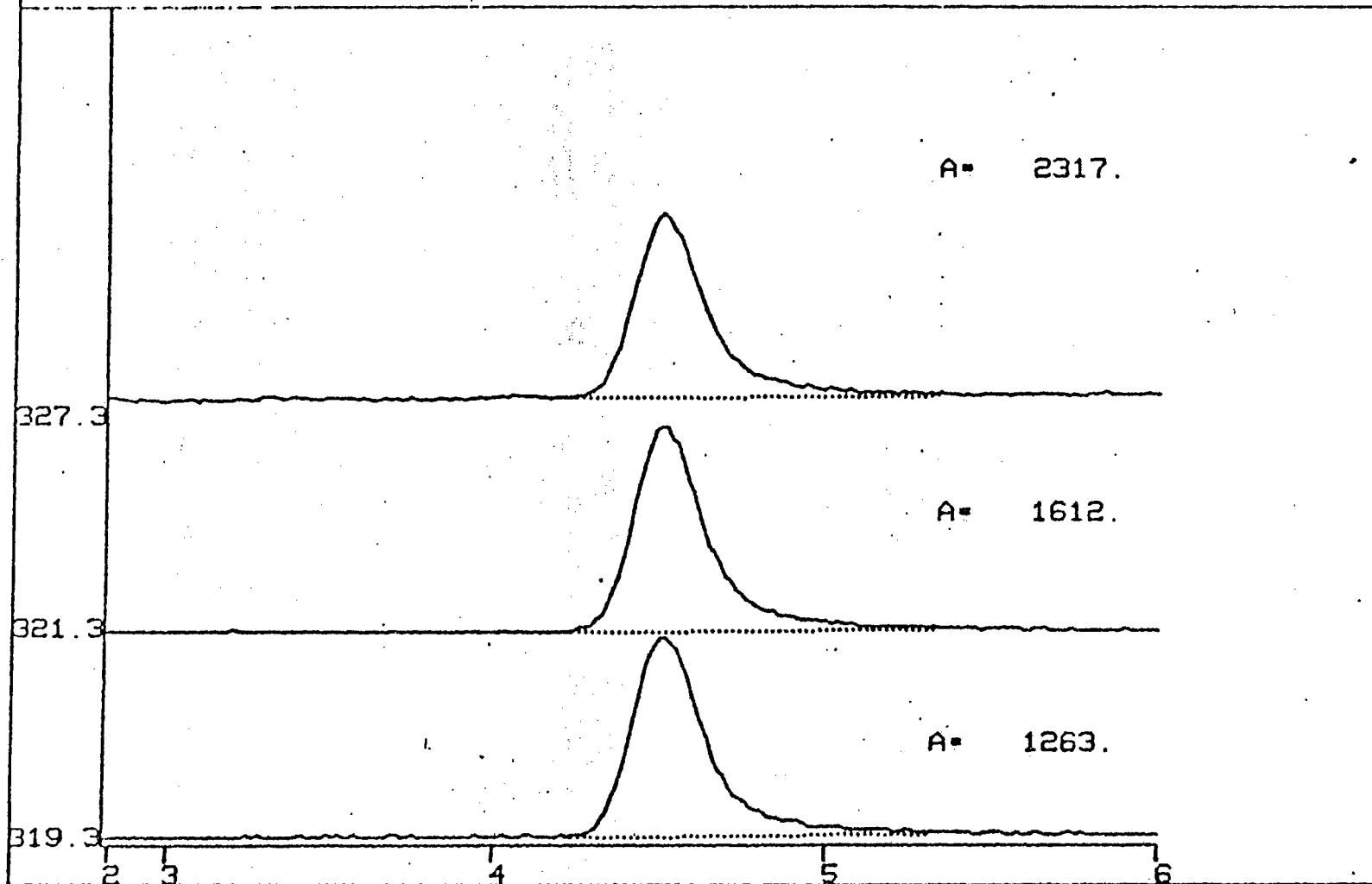
10
** SELECTED ION CHROMATOGRAM **
1UL NATIVE TCDD CL35 +1UL CL37 STDS CHECK
31JULY78, 0011. 2 OF 4

FRN 21080
1ST SC/PG. 1
X= 1.00 Y= 1.00



2UL TCDD NATIVE CL35 +CL37 STDS CHECK
31JULY78, OV11, 3 OF 4

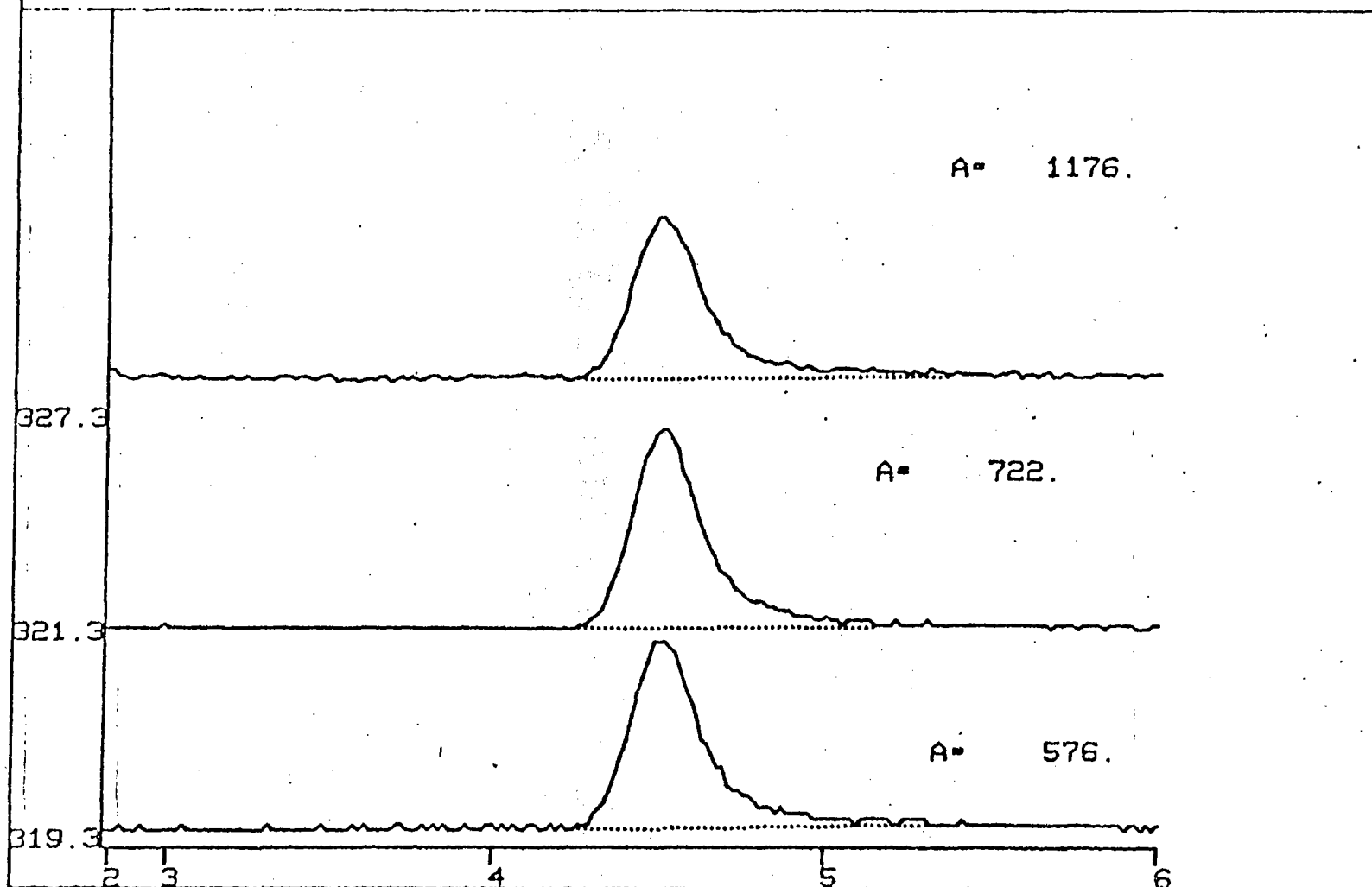
FRN 21081
1ST SC/PG: 1
X= 1.00 Y= 1.00



21712

** SELECTED ION CHROMATOGRAM **
1UL TCDD NATIVE CL35 +CL37 STDS CHECK.
31JULY78, QU11, 4 OF 4

FRN 21082
1ST SC/PG, 1
X= 1.00 Y= 1.00



21713

Appendix E. Ion Current Chromatograms for
TCDD Detected in a Pesticide Sample

(EPA Sample #4)

QUALITATIVE CRITERIA

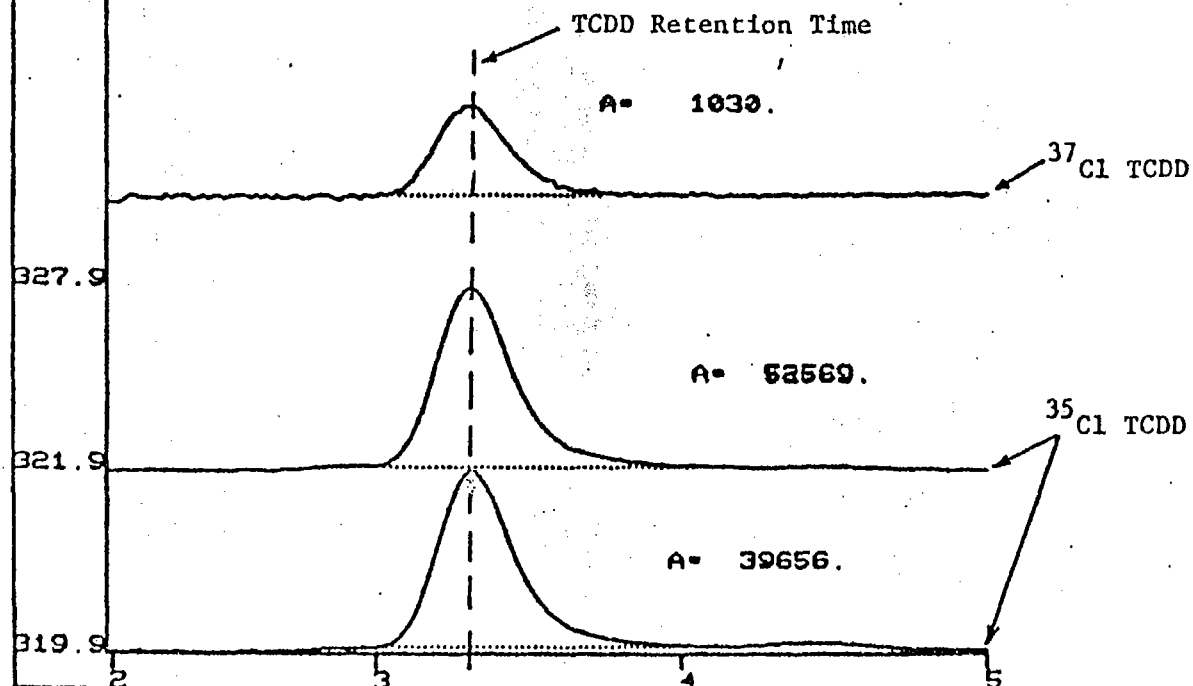
1	2	3	4	5	6
+	+	+	+	+	+

JUDGEMENT: TCDD detected in the sample
because all six criteria were positive.

4R-2. 2+ 0+0
15AUG78. KMRG

** SELECTED ICH CHROMATOGRAM **

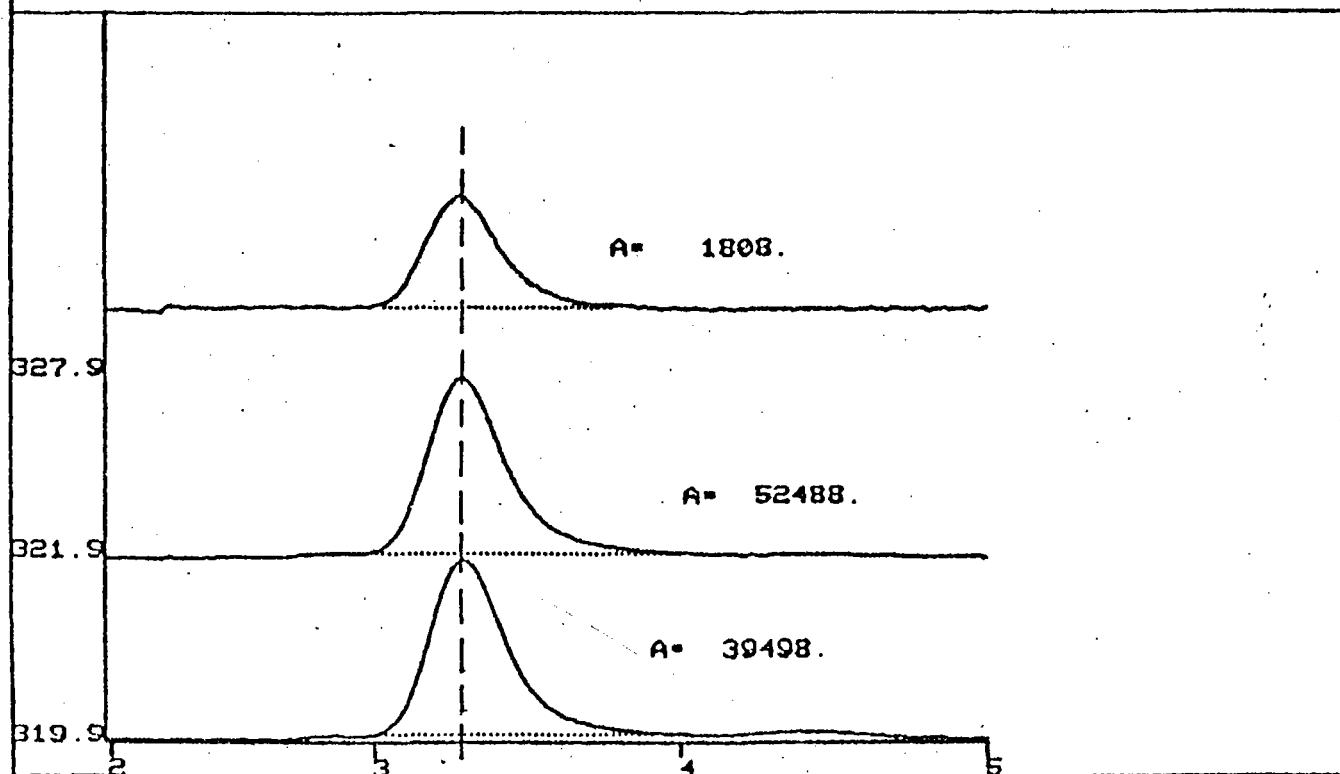
FAN 21533
1ST SC/PQ. 1
X= 1.00 Y= 1.00



4R-2, 2+2+1
15AUG78, KRG

** SELECTED ION CHROMATOGRAM **

FRN 81507
1ST SC/PO. 1
X= 1.00 Y= 1.00



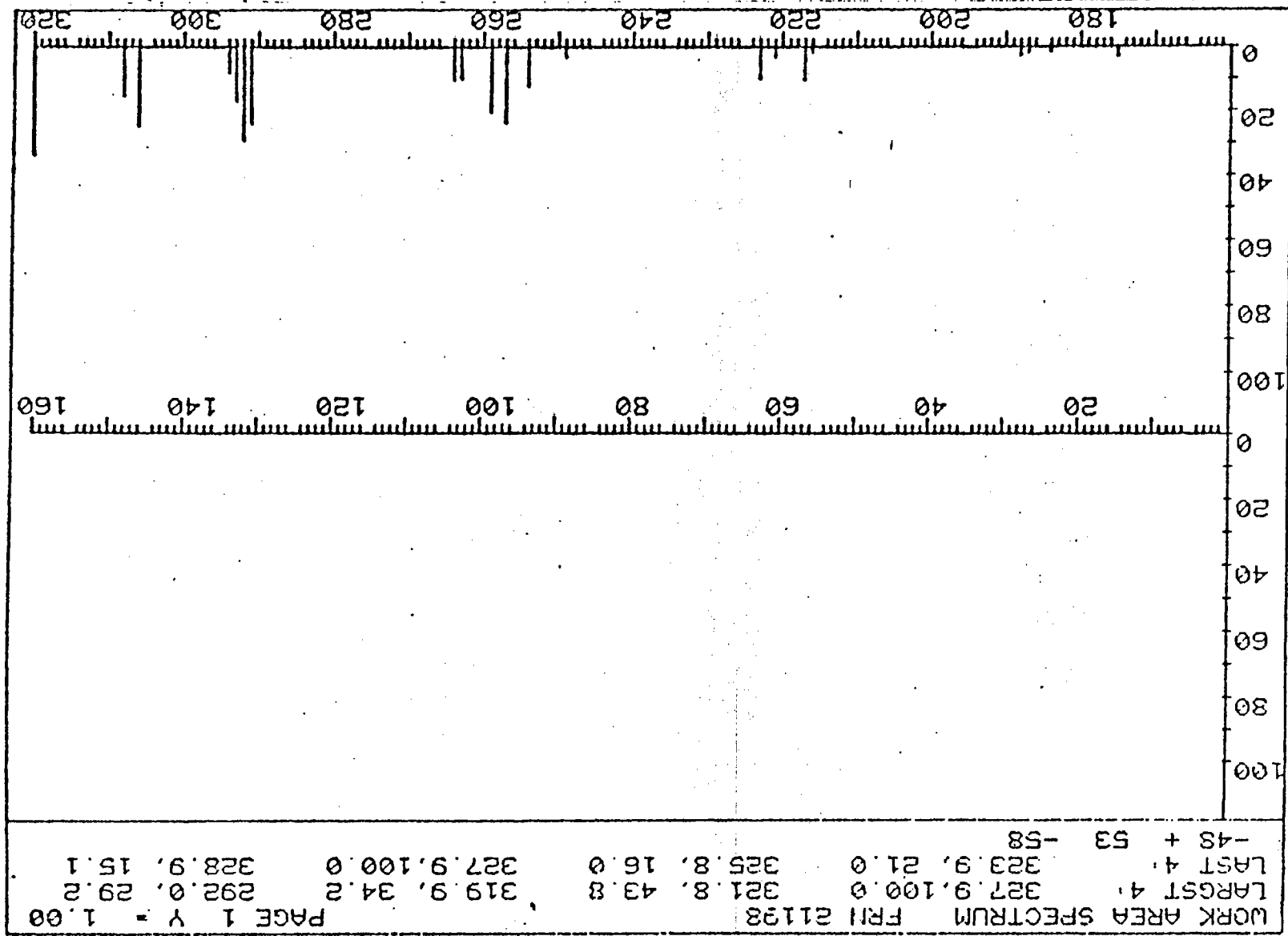
52

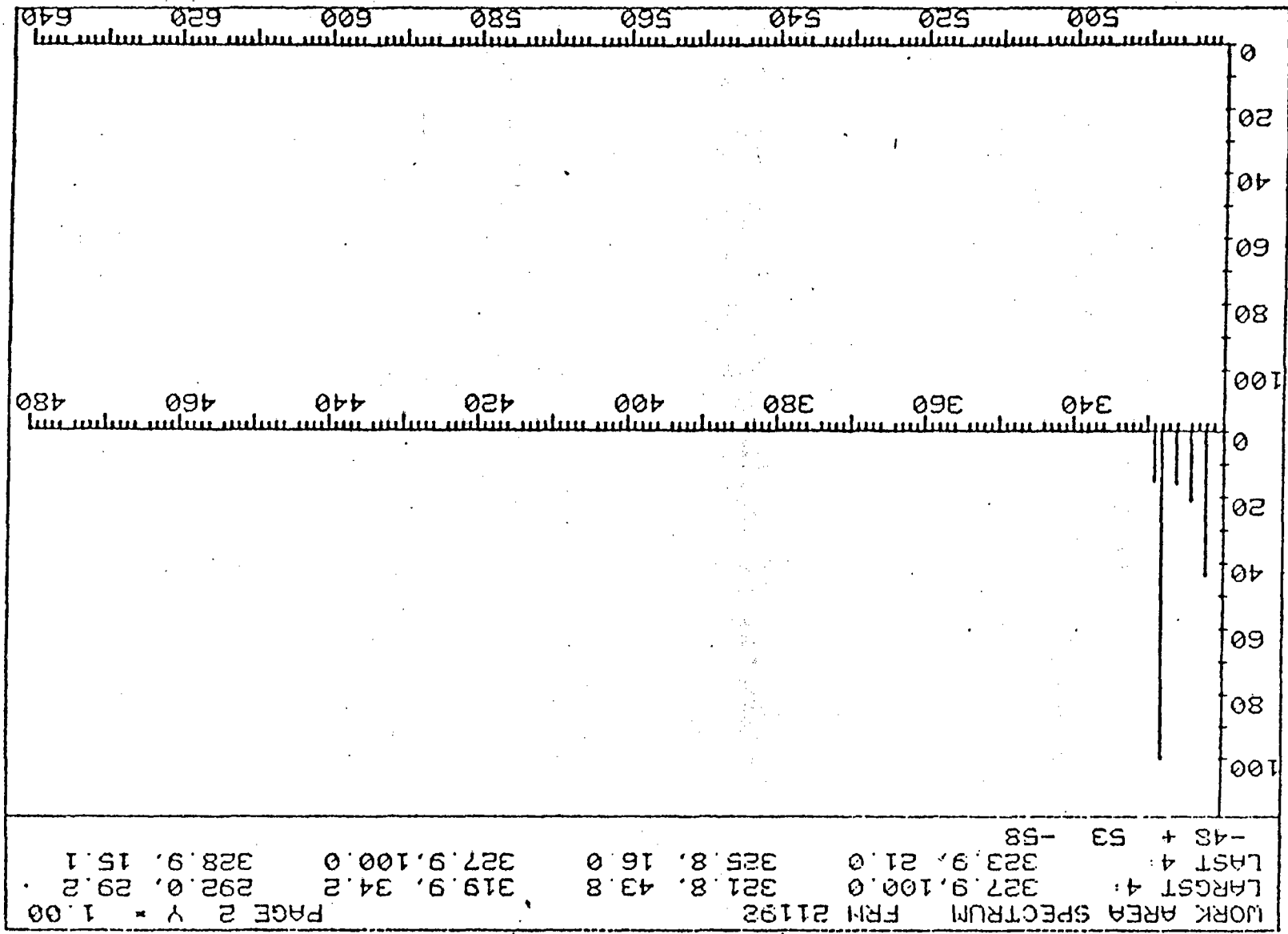
21716

Appendix F. Mass Spectrum for TCDD Detected
in a Pesticide Sample

(EPA Sample #148919-5)

NOTE: Existence of m/e lines at 320, 322, 324 and 326
indicative of line at m/e 328 due to ³⁷Cl TCDD.





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Appendix G. Mass Abundance Tabulation for TCDD
Detected in a Pesticide Sample

(EPA Sample #90)

NOTE: Mass abundances at m/e 320, 322, 324 and 326
indicative of TCDD.

FRN 21643 SPECTRUM 47 RET. TIME

>PAUSE

MASS	ABUND	MASS	ABUND
247	15.	271	8.
248	11.		
249	19.	272	11.
250	11.	273	14.
251	15.	274	135.
251	15.	275	81.
252	8.	276	23.
253	45.	277	11.
254	22.	278	8.
255	40.	279	8.
256	15.	280	6.
257	59.	281	40.
		282	13.
259	473.	283	15.
260	130.	284	6.
261	41.	285	12.
262	21.		
263	28.	287	12.
264	8.	288	26.
264	7.	289	11.
264	7.	290	26.
265	9.	291	15.
266	8.	292	11.
267	19.	293	21.
268	10.	294	8.
269	15.	295	7.
270	7.	296	4.

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4.7

MASS	ABUND	MASS	ABUND
297	4.	319	7.
297	3.	319	7.
298	4.	320	22.
299	7.	321	10.
		322	29.
300	3.	324	14.
300	3.	325	9.
301	5.	326	7.
302	5.		
303	7.	328	38.
304	5.	329	10.
305	4.	330	5.
306	8.	331	15.
307	11.	332	11.
307	10.	333	9.
308	9.	334	5.
309	13.	334	4.
310	5.	336	4.
311	7.	336	4.
312	4.	337	6.
313	9.	337	6.
		338	6.
314	6.	339	4.
315	45.	340	2.
316	19.	341	7.
317	53.		
318	18.	342	4.

<CONT>

Appendix H. Ion Current Chromatograms for
Nondetectable TCDD in a Pesticide Sample

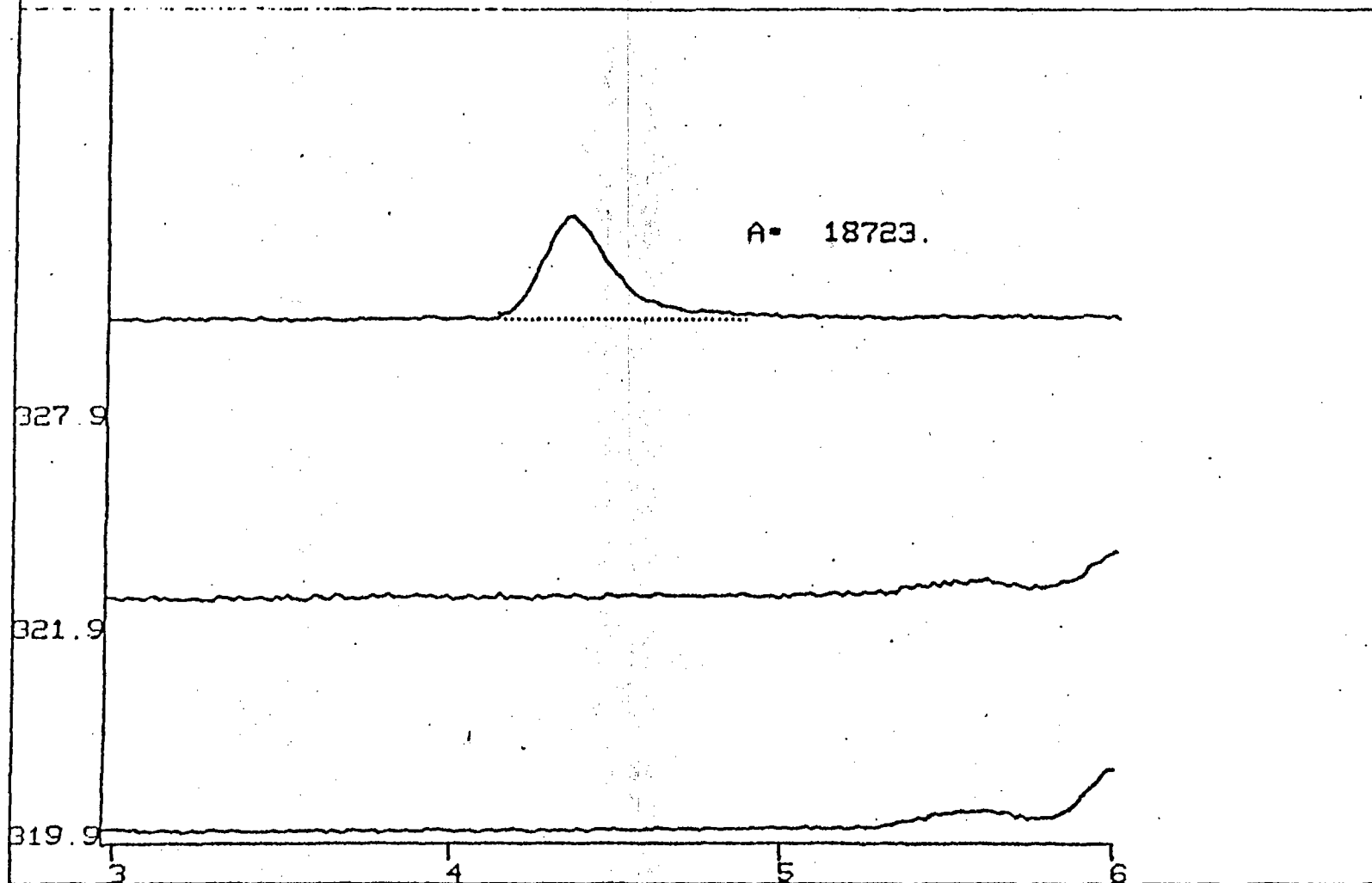
(EPA Samples #137834-3 and 3YD)

QUALITATIVE CRITERIA						
Sample	1	2	3	4	5	6
137834-3	+	-	NA	NA	NA	NA
3YD	+	-	NA	NA	NA	NA

JUDGEMENT: TCDD not detected in the sample because criteria #2
was negative due to absence of both m/e 320 and 322 peaks.

2000UL FEST EXTR TODD 137834-3 + QULS STANDARD
05JUN78. 00-11. 210(10240(6). 1 OF 4

FRN 21243
1ST SC/PG: 54
X= 1.00 Y= 1.00



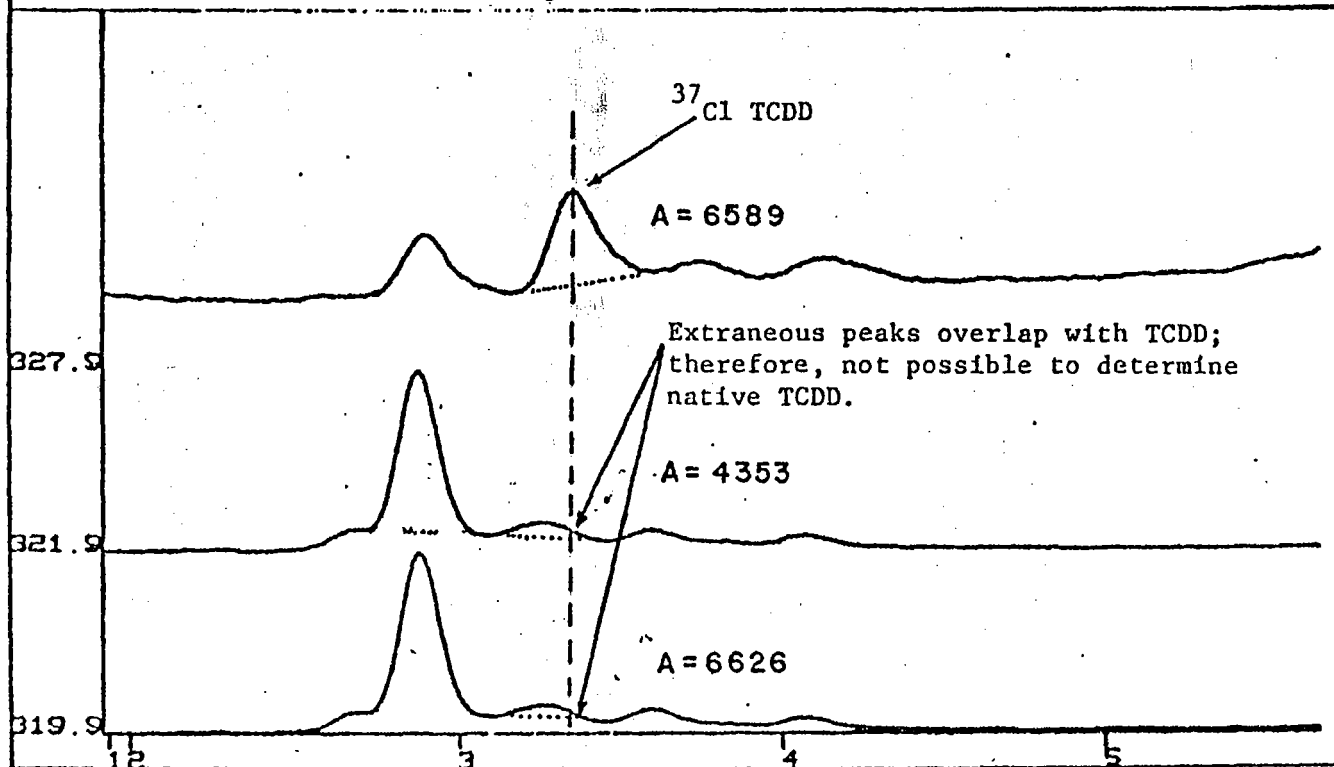
21723

FRN 21244
1ST SC/PG: 1
X= 1.00 Y= 1.00



21724

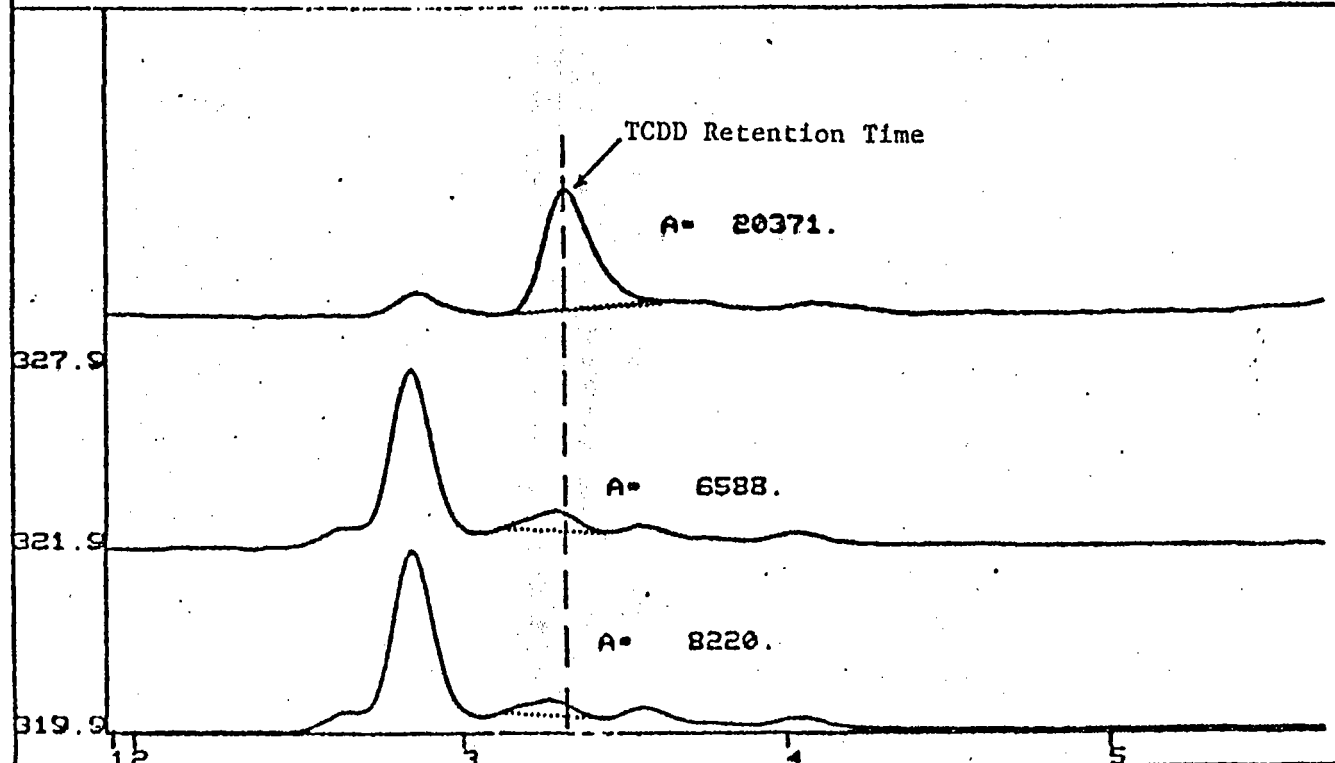
** SELECTED ICH CHROMATOGRAM **
 2/200UL PEST EXT 3V-D-2 + 3 SUL SOLV NO STDs 80SEPT78 16T SC/PQ 1
 20SEPT78 0V11 21011262010 1 OF 4
 X= 1.00 Y= 1.00



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21725

** SELECTED ION CHROMATOGRAM **
 2\200UL PEST EXT 3Y-D-2 + 2UL CL37 & 1 UL CL35 TCDD 20SEP78SC/PQ. 1
 20SEP78 OV11 210(1)262310 X= 1.00 Y= 1.00
 2 OF 4



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Appendix I. Ion Current Chromatograms for Questionable
TCDD Detection in a Pesticide Sample

(EPA Sample #90D)

QUALITATIVE CRITERIA					
1	2	3	4	5	6
+	+	+	NA	-	NA

JUDGEMENT: TCDD detection questionable
because criteria #5 was negative due to
m/e ratio out-of-specification.

10

** SELECTED ION CHROMATOGRAM **

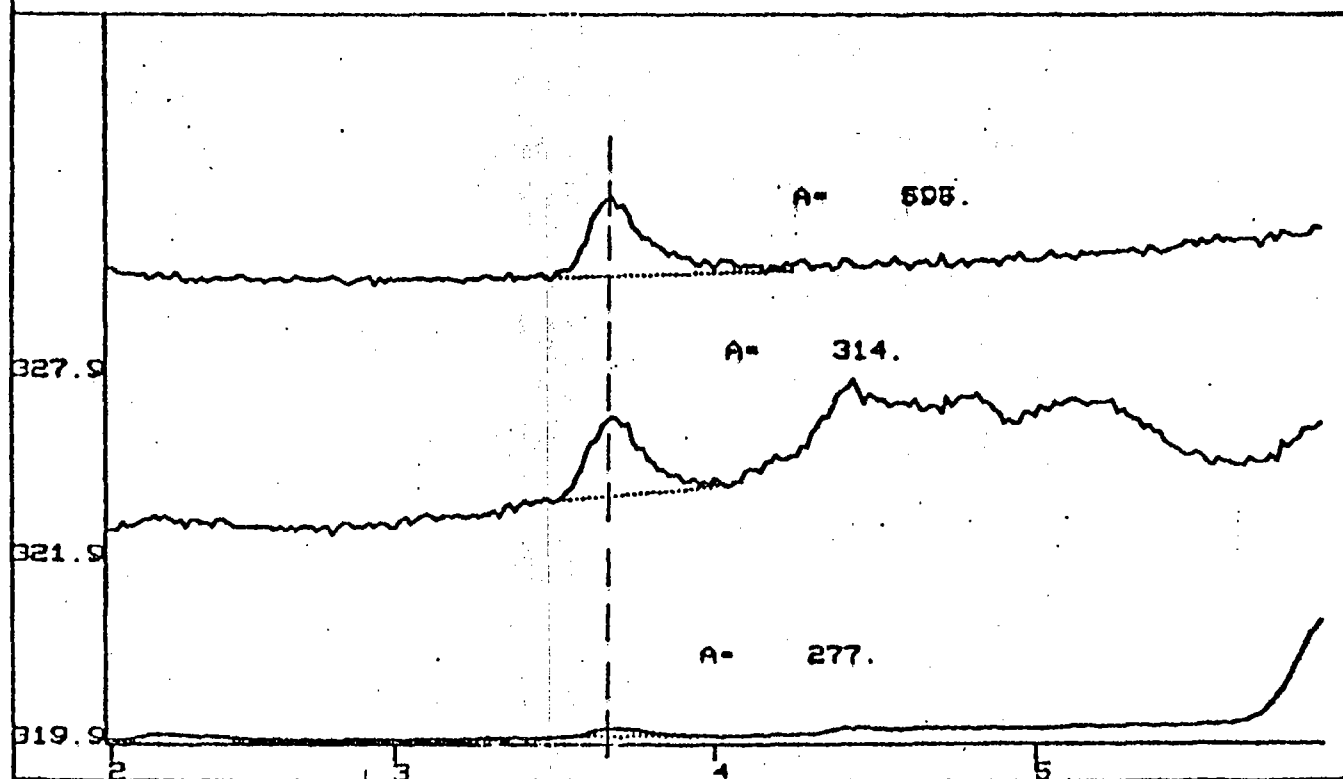
2/200UL PEST EXTR 90-D-2 + OULS STDS (1 OF 4)

01SP78, HP5985, 0V11, 210(1)250(10)

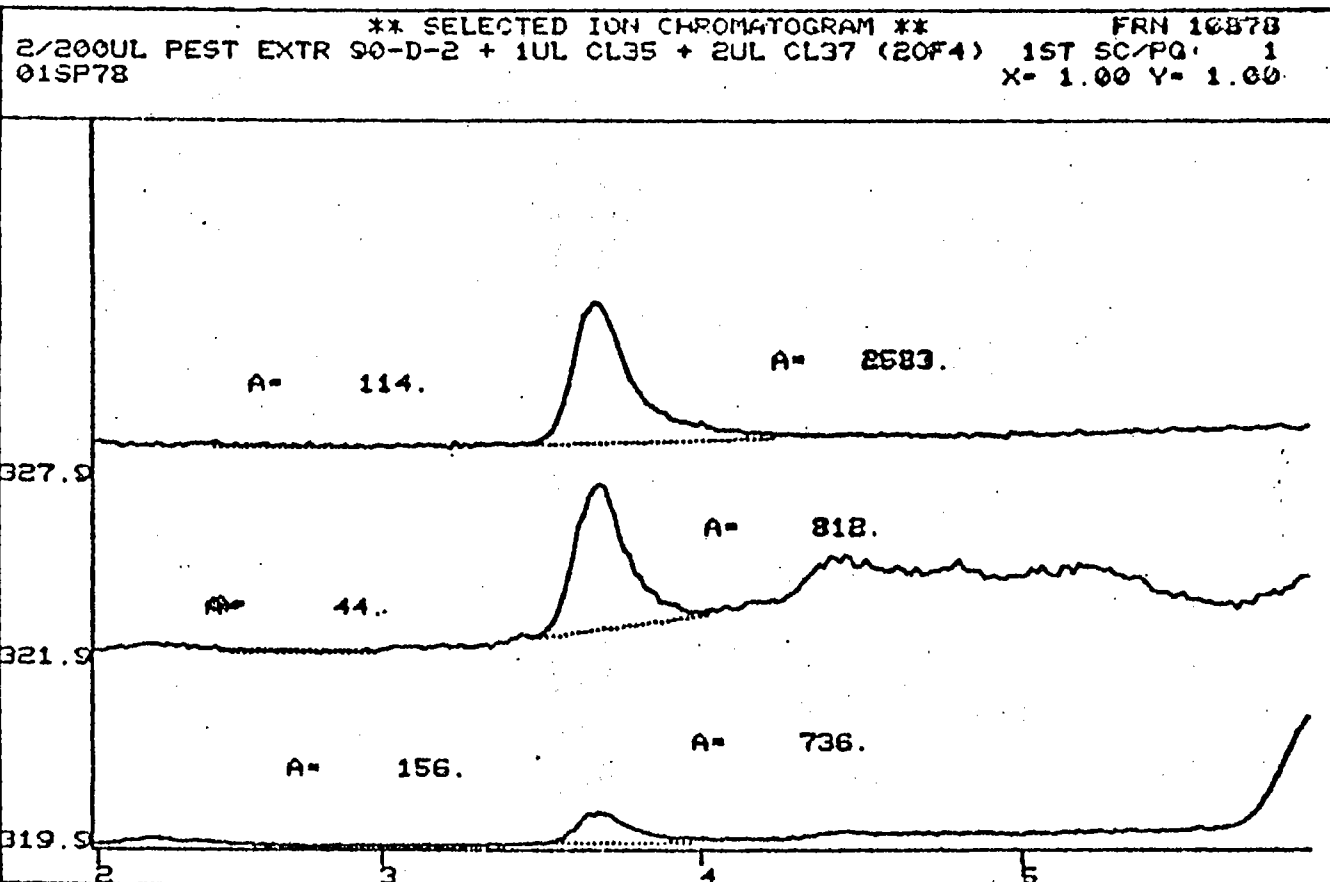
FRN 10B77

1ST SC/PQ. 1

X= 1.00 Y= 1.00



21728



65

21729

MAR 2 1980

EXHIBIT C

CHROM. 7193

DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-1,4-DIOXIN AT PARTS PER BILLION* LEVELS IN TECHNICAL-GRADE 2,4,5-TRICHLOROPHENOXYACETIC ACID, IN 2,4,5-T ALKYL ESTER AND 2,4,5-T AMINE SALT HERBICIDE FORMULATIONS BY QUADRUPOLE MASS FRAGMENTOGRAPHY

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(Received November 1st, 1973)

SUMMARY

A fast, highly selective gas chromatographic method was developed for the determination of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (TCDD) in technical 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and also in 2,4,5-T alkyl ester and amine salt herbicide formulations using quadrupole mass fragmentography. The method is faster, more sensitive and has a higher specificity than previously reported methods using conventional gas chromatography. It requires minimal or no sample clean-up. The content of TCDD found in batches of 2,4,5-T produced from 1967 to 1973 by one manufacturer showed a sharp decrease after 1970 from 500 to 50 ppb.*

INTRODUCTION

Polychlorinated dibenzo-1,4-dioxins, especially 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (TCDD), are extremely toxic^{1,2}, teratogenic³ and mutagenic⁴ compounds. Owing to their stability in biological systems^{5,6} and their potential for accumulation through food chains⁷, they present a threat to man, animals and the environment. In order to prevent environmental contamination with TCDD and thus to prevent irreparable biological damage, the further use of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and other chemically related pesticides possibly contaminated with TCDD must therefore depend on the purity of the active ingredient. The Swiss Federal Research Station at Wädenswil, as the authority for the licencing of agricultural pesticides in Switzerland, established a specification for a maximum content of 50 ppb* of TCDD in 2,4,5-T. Similar specifications for TCDD in 2,4,5-T have been adopted in other countries. There has been a considerable international effort in the development of methods^{1,6,8-11} to enable manufacturers and government laboratories to control the purity of these products. As a contribution, this paper describes a simple method that permits the rapid and accurate determination of

* Throughout this article the American (10⁹) billion is meant.

TCDD at the required parts per billion level in technical-grade 2,4,5-T and its formulations. Mass spectrometry is widely used for pesticide analysis¹². But mass fragmentography so far has been used mainly for the analysis of drugs and their metabolites in biological systems¹³⁻¹⁵. We assume that this technique will also gain more and more acceptance in the fields of pesticide residue analysis and formulation control.

EXPERIMENTAL

Reagents

Methanol, diethyl ether and ethyl acetate were obtained in puriss quality and light petroleum (b.p. 40-65°) and lithium hydroxide in purum quality from Fluka (Buchs, Switzerland). Aluminium oxide (basic, cationotropic) from Woelm (Eschwege, G.F.R.) was used as received.

Reference compound

2,3,7,8-Tetrachlorodibenzo-1,4-dioxin (TCDD) was obtained from Stickstoffwerke Linz (Linz, Austria) and its purity was checked by gas chromatographic (GC), mass spectrometric (MS) and micro-elemental analysis. It was found to be suitable for use as a standard without further purification. Standard solutions of TCDD in ethyl acetate were prepared at concentrations of 1 and 10 µg/ml. The safety precautions to be taken when handling this highly toxic compound have been stressed elsewhere¹¹.

Instrumentation

A Finnigan Model 1015D quadrupole gas chromatograph-mass spectrometer was used to obtain conventional mass spectra on the reference compound. A GC column was interfaced with the mass spectrometer via a venting system and a glass jet separator. The glass column (1.5 m × 2 mm I.D.) was packed with 3% silicone OV-225 on Chromosorb W AW-DMCS. The column had been previously treated with silylation reagent. The helium carrier gas flow-rate was adjusted to 25 ml/min. The injection port temperature was 250°, the column temperature 230° and the separator temperature 250°. Mass spectra were recorded at 70 eV. The ion energy used was approximately 10 V and the electron multiplier voltage 3 kV. A scan time of 2 sec was found to be suitable for an *m/e* range of 35-550.

For mass fragmentography, the instrument was operated in a similar fashion. With the instrument set at unit resolution, the ion intensities at *m/e* 320 for TCDD were monitored (single ion detection). This adjustment was made using the precision mass meter of the instrument. The signals were recorded on a regular 10-mV recorder using an additional 0.16-Hz filter.

A second, similarly treated glass column with an auxiliary T-port at approximately one third of the column length from the inlet was used for back-flushing possible late-eluting, interfering substances. This column was packed in the first portion (50 cm) with 3% Carbowax 20M on Chromosorb W AW-DMCS; and in the second portion (100 cm) with 3% silicone OV-225. The column was installed in the gas chromatograph with helium carrier gas lines connected to both the inlet port and the auxiliary port. Additionally, venting lines were mounted for the inlet port and

auxiliary port. The carrier gas and venting lines could be individually switched on and off by toggle valves outside the chromatograph. Operation of this column at 240° was done with constant pressure (2.0 atm) rather than constant flow-rate in order to achieve efficient back-flushing. The total retention time for TCDD on both this composite column and on the first conventional column was between 5 and 6 min.

Sample preparation

A sample preparation based on the work of Edmunds *et al.*¹¹ was used. Twenty grams of sample were dissolved or suspended in 150 ml methanol and 25 ml of 5 N lithium hydroxide solution were added. The mixture was refluxed for 20 min and then cooled to room temperature, and 500 ml of water and 200 ml of light petroleum were added and the mixture was vigorously shaken. After separation of the phases, the organic phase was dried over anhydrous sodium sulphate. A portion of about 100 ml was concentrated under vacuum on a rotary evaporator (maximum temperature 50°) to about 10 ml. An aliquot was usually taken because some formulations formed emulsions that were difficult to separate.

The concentrate was transferred to an alumina column (45 ml of basic aluminium oxide in a 50 cm × 20 mm I.D. column). The column was eluted with 100 ml of light petroleum, 50 ml of 5% diethyl ether and then by 100 ml of 25% diethyl ether in light petroleum. These eluates were discarded. TCDD was eluted with 150 ml of diethyl ether and the solution was concentrated to approximately 10 ml. After transfer to a suitable receiver, it was further concentrated to about 0.2 ml with a gentle stream of nitrogen. Care was taken never to allow the sample to be evaporated completely to dryness. The samples, made up to 1.0 ml with ethyl acetate, were now ready for GC-MS analysis.

A shortened procedure was used for technical 2,4,5-T or 2,4,5-trichlorophenol by omitting the refluxing and the clean-up steps. To a 20-g sample, dissolved in 150 ml of methanol, 500 ml of water and 25 ml of 5 N lithium hydroxide solution were added. After addition of 200 ml of light petroleum and vigorous shaking, the organic phase was separated, dried and an aliquot concentrated on the rotary evaporator. Again, final concentration was carried out with a gentle stream of nitrogen. The samples, made up to volume (1.0 ml) with ethyl acetate, were then analyzed by GC-MS.

Samples that contained TCDD at levels above 100 ppb were re-analyzed by mass fragmentography after being methylated with diazomethane according to the micro-method described by Schlenk and Gellerman¹⁶. Methylation was carried out in 10% methanol-diethyl ether solution.

Recovery

Recovery experiments were carried out by adding differing amounts of TCDD, ranging from 0.1 to 10 µg, to the hydrolysis reagents, to technical 2,4,5-T, 2,4,5-trichlorophenol or to formulations. These fortification levels correspond to 5-500 ppb for technical materials and 50-5000 ppb for 10% 2,4,5-T formulations.

Determination

For quadrupole mass fragmentography, the mass spectrometer was used to

monitor at unit resolution the molecular ion for TCDD at m/e 320. The precision mass meter was adjusted by slowly evaporating a few nanograms of TCDD from the direct insertion probe.

A standard curve was prepared by injecting differing amounts of TCDD (0.5–100 ng) on the GC column and plotting peak heights *versus* amount injected. Quantitation of TCDD in samples was then achieved by comparing their peak heights with the standard curve.

In order to allow injection of up to 50 μ l of solution, a venting period of 1 min was used to prevent overloading the MS vacuum system with solvent vapour. Samples prepared by the shortened procedure were analyzed using the dual-column system. An initial venting period of 1 min was used and back-flushing initiated after 4 min, which prevented early- and late-eluting components from entering and contaminating the separator and ion source. This timing period was previously determined by a few standard injections so as to ensure that none of the TCDD is lost through venting and back-flushing.

Confirmation of the identity of TCDD in samples with a suspected content higher than 100 ppb was carried out by MS. A larger aliquot, corresponding to approximately 100 ng of TCDD, was injected and complete mass spectra were recorded. Five mass spectra taken at 15-sec intervals bracketing the retention time of TCDD were sufficient. The spectra were evaluated by comparison of the mass peaks in the region m/e 200–500 of scans obtained before, during and after elution of suspected TCDD. The ion intensities of m/e 320, 322 and 324 (M^+ , four chlorine atoms) and 257 and 259 ($M^+ - COCl$, three chlorine atoms) were compared with that of a TCDD standard. In addition, the spectra were checked for the absence of chlorine-containing higher molecular ions that could possibly form fragments at these m/e values.

In addition, samples containing large amounts of TCDD were reassessed by mass fragmentography after methylation in order to observe any reduction of the TCDD content caused by this treatment.

RESULTS AND DISCUSSION

The MS data for TCDD indicate intense molecular ions with the characteristic clustering due to chlorine isotopes at m/e 320, 322 and 324. The only other ions of some intensity in the higher mass range are at 257 and 259 ($M^+ - COCl$). Quadrupole mass fragmentography was carried out by single ion detection of the ion at m/e 320. This ion was used rather than the more intense ion at m/e 322 because of possible interference of PCBs. In addition, column bleeding from silicone OV-225 showed a minor signal at m/e 322.

The response of the Finnigan 1015D quadrupole mass spectrometer proved to be sufficiently stable over a suitable period of time to allow the use of an external standard for quantitation with acceptable precision. The peak height of the response was found to be linear in the range 0.5–100 ng of TCDD injected. A detection limit of better than 0.5 ng could easily be achieved without any attempt to maximize the response. This corresponds to a level of approximately 1 ppb based on a 20-g sample of technical material. This detection limit was found to be sufficient for

the present work. For the samples investigated, a single GC peak was usually obtained.

Back-flushing and venting the dual-column system was achieved by switching the carrier gas stream and vent by toggle valves outside the heated zones. Such a system has been described by Deans¹⁷ as early as 1965 and more recently by Warner *et al.*¹⁸. The system allowed the back-flushing of unwanted late-eluting components and prevented them from entering the second, final GC column. With the same system, early-eluting components are vented and do not contaminate the separator and ion source. It is of importance in pesticide analysis that the components of interest are in contact only with glass and not with any metallic parts such as valves. Only components with retention times in a narrow range are determined. Of course, this system is applicable only when a few components are of interest. The use of a first column to cut out unwanted components results in an efficient clean-up and simplifies sample preparations prior to GC analysis. Such systems are easily built, easy to operate and suitable for routine application and automation. In combination with single ion detection, this leads to a high specificity of detection. We are sure that such techniques will gain an increasingly wider application in pesticide analysis.

Recovery experiments for TCDD added to the reagents and carried through all the steps of the procedure ranged from 40% at the 0.1 μg level to 80–100% at the 1–10 μg level. Addition of similar amounts of TCDD to 2,4,5-T and 2,4,5-trichlorophenol led to recoveries of 80–100% at levels corresponding to 50–500 ppb. Recoveries in both 2,4,5-T ester and amine salt formulations were lower (60–80%).

Some results for the content of TCDD in 2,4,5-T are reported in Table I. The samples listed represent the production of a German manufacturer over a period of 6 years. We were told after this investigation that the sample with code 12.8.70 and a content of *ca.* 2000 ppb TCDD was not of German but of American origin.

TABLE I

TCDD CONTENT IN SAMPLES OF 2,4,5-T OBTAINED FROM A GERMAN MANUFACTURER

Sample code	TCDD content (ppb)	
	Complete procedure	Simplified procedure
7.8.67	300	240
17.7.70	390, 490	520
12.8.70	1840	1950
12.11.70	420	580, 640
12.12.70	50	60
14.9.71	80	60, 70
22.9.72	n.a.*	40
18.9.73	n.a.*	80
Dowicide 2 (2,4,5-trichlorophenol)	2	<3

* Not analysed.

The first column in Table I lists the results obtained according to the original procedure, while the second column lists the results obtained by the shortened procedure.

The results indicate acceptable agreement for duplicate samples and also for the two procedures. The results show that the content of TCDD in technical 2,4,5-T for this manufacturer ranged from 300 to 600 ppb prior to 1971. Since then, the TCDD content decreased to 40–80 ppb, which is about compatible with the 50 ppb specification valid in Switzerland. As a comparison, a single result for a sample of 2,4,5-trichlorophenol (Dowicide 2) is included, which shows a content of TCDD of about 2 ppb.

In addition, a comparison of the results listed in Table I indicates that refluxing samples with methanolic lithium hydroxide does not increase the TCDD content. The simplified method results in a very fast procedure for the analysis of TCDD in technical 2,4,5-T and 2,4,5-trichlorophenol and is suitable for routine analysis in quality control.

Table II lists some typical results obtained by analyzing 2,4,5-T formulations taken from commercial sources in Switzerland in June, 1973. These formulations were analyzed using the complete procedure with refluxing and alumina column clean-up. The results indicate TCDD contents of 20–70 ppb relative to 2,4,5-T. Again, the results are acceptable with our specification.

TCDD in samples with a suspected content above 100 ppb could be confirmed in all instances by MS. Confirmation of TCDD by complete MS analysis on these samples was preferred to multiple ion detection on three or four prominent ions. Scanning the whole mass range from m/e 35 to 550 in fixed intervals before, during and after elution of the suspected TCDD allowed the confirmation and identification of TCDD in all those samples, although no GC peak could usually be observed on the total ion monitor, because of a multitude of other components that were eluted from the column at about the same retention time as TCDD. These components

TABLE II

TCDD CONTENT IN 2,4,5-T FORMULATIONS TAKEN FROM COMMERCIAL SOURCES IN SWITZERLAND IN JUNE, 1973

Results are given in ppb relative to 2,4,5-T.

<i>Formulation</i>	<i>Sample</i>	<i>TCDD content</i>
Ester	Manufacturer I (15% 2,4,5-T)	50
	Manufacturer II (45% 2,4,5-T)	20
Amine salt	Manufacturer III (12% 2,4,5-T)	10, 20
	Manufacturer IV (9% 2,4,5-T)	50, 70
	Manufacturer V (9% 2,4,5-T)	20, 50

could be identified by MS as polychlorinated biphenyls, dibenzofurans and diphenyl oxides.

The presence of polychlorohydroxydiphenyl ethers as possible precursors of TCDD could not be substantiated. This was observed by Rappe and Nilsson¹⁹ for octachlorodibenzo-1,4-dioxin. Our finding is based on the fact that with the samples examined in this study, no significant reduction of the apparent TCDD content occurred after methylation of the neutral part.

REFERENCES

- 1 N.I.E.H.S. Conference on Chlorinated Dibenzodioxins and Dibenzofurans, April 2-3, 1973, Research Triangle Park, N.C., U.S.A., *Environ. Health Perspect.*, 5 (1973).
- 2 Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency, Government Printing Office, Washington, D.C., May, 1971.
- 3 K. D. Courtney, D. W. Gaylor, M. D. Hogan, H. L. Falk, R. R. Bates and I. Mitchell, *Science*, 168 (1970) 864.
- 4 J. P. Seiler, *Experientia*, 29 (1973) 622.
- 5 R. H. Stehl, R. R. Papenfuss, R. A. Bredeweg and R. W. Roberts, unpublished results.
- 6 P. C. Kearney, *Joint Meeting on Pesticides, United Kingdom, Canada and U.S.A., Washington, D.C., November, 1970.*
- 7 J. Verrett, *Statement before the U.S. Senate Sub-Committee on Energy, Natural Resources and the Environment, April 15, 1970.*
- 8 D. Firestone, J. Ress, N. L. Brown, R. P. Barron and J. N. Damico, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 85.
- 9 W. B. Crummett and H. R. Stehl, *Environ. Health Perspect.*, 5 (1973) 15.
- 10 K. S. Brenner, K. Müller and P. Sattel, *J. Chromatogr.*, 64 (1972) 39.
- 11 J. W. Edmunds, D. F. Lee and C. M. L. Nickel, *Pestic. Sci.*, 4 (1973) 101.
- 12 F. J. Biros, *Residue Rev.*, 40 (1973) 1.
- 13 C.-G. Hammar, B. Holmstedt and R. Ryhage, *Anal. Biochem.*, 25 (1968) 532.
- 14 C.-G. Hammar, B. Holmstedt, J.-E. Lindgren and R. Tham, *Advan. Pharmacol. Chemother.*, 7 (1969) 53.
- 15 J. M. Strong and A. J. Atkinson, Jr., *Anal. Chem.*, 49 (1972) 2287.
- 16 H. Schlenk and J. J. Gellerman, *Anal. Chem.*, 32 (1960) 1412.
- 17 D. R. Deans, *J. Chromatogr.*, 18 (1965) 477.
- 18 C. R. Warner, M. C. Johnson, D. G. Prue and B. T. Kho, *J. Chromatogr.*, 82 (1973) 263.
- 19 C. Rappe and C. A. Nilsson, *J. Chromatogr.*, 67 (1972) 247.

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- (29) S. J. W. Price and A. F. Trotman-Dickenson, *Trans. Faraday Soc.*, **54**, 1630 (1958).
- (30) J. W. Robinson, R. Garcia, G. Hindman, and P. Slevin, *Anal. Chim. Acta*, **69**, 203 (1974).
- (31) G. E. Parris and F. E. Brinckman, *Environ. Sci. Technol.*, **10**, 1128 (1976).
- (32) J. J. Ritter and N. K. Adams, *Anal. Chem.*, **48**, 612 (1976).
- (33) G. L. Everett, T. S. West and R. W. Williams, *Anal. Chim. Acta*, **70**, 291 (1974).
- (34) D. J. Johnson, B. L. Sharp, T. S. West, and R. M. Dagnall, *Anal. Chem.*, **47**, 1234 (1975).
- (35) G. Torsi and G. Tessari, *Anal. Chem.*, **48**, 1318 (1976).

RECEIVED for review September 10, 1976. Accepted November 17, 1976. Contributions from the National Bureau of Standards are not subject to copyright. G.E.P. was supported

by a National Research Council Post-doctoral Research Associateship at NBS during the completion of this work. We acknowledge with thanks the continued encouragement and partial financial support of the U.S. Naval Ship Research and Development Laboratory (Annapolis), Maryland and the NBS Office of Air and Water Measurement. Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Automated Cleanup of Herbicides by Adsorption Chromatography for the Determination of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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An automated system is described which repeatedly uses a low-cost silica column for chemical cleanup. Its use in separating and collecting trace levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from esters of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in commercial herbicide preparations is demonstrated. Previously, chemical cleanup by column adsorption chromatography required a newly-packed column for each sample. More than 400 runs have been achieved on a single column to date. System accuracy, as determined by checking against identical samples manually prepared, is excellent. The standard deviation for the same ester, analyzed 36 times, was 0.002 ppm at an average TCDD concentration of 0.026 ppm. There is no measurable cross-contamination from sample to sample.

Chemical cleanup by column adsorption chromatography has become a widely used technique, particularly in the area of pesticide residue analysis. Many different adsorbents have found application, notably Florisil, magnesium oxide, alumina, charcoal, and silica gel (1). The procedure with such columns has been to discard the packing after a single use. In order to reuse an adsorption column for chemical cleanup, the surface must be "renewed" by removing the polar molecules which comprise the bulk of the sample. While manual regeneration of an adsorption column would be impracticably slow, automatic regeneration makes repeated use of an adsorption column possible.

Automation is well-known in liquid column chromatography, particularly in amino acid analysis by ion exchange chromatography (2-4). Perhaps less well-known are automated peptide (5) and sugar (6) chromatography systems. More recently Small et al. have devised schemes for automated ion exchange chromatography utilizing a novel technique which allows for universal ion detection conductimetrically (7).

While a number of advances have occurred in the development of automated ion-exchange chromatography employing "clean" samples, development of automated cleanup systems employing reusable columns has lagged. A semi-automatic apparatus for purifying amino acids from non-amino acid components such as salts and protein pre-

cipitants has been developed recently by Blanshard et al. (8). The purification, which takes place on a cation exchange column, precedes normal automated amino acid determination. Their two-hour procedure includes a regeneration of the resin with HCl to remove bound cations prior to introduction of a new sample.

The use of automated gel permeation chromatography for the separation of pesticides from lipids and oils has been successfully applied by Tindle and Stalling (9, 10). Gel permeation can effect a satisfactory separation for these samples because many pesticides have molecular weights in the range of 200-400, while most lipids fall between 600 and 1500. An evaluation of their GPC unit was conducted by Griffith and Craun (11). They reported that cleanup efficiency was superior to that obtained with acetonitrile partitioning, although in some cases an ensuing cleanup on Florisil was necessary prior to gas chromatographic determination. This instrument is now available in commercial form (12). Stalling et al. have recently expanded their system to permit combined exclusion/carbon bed cleanup (13, 14).

Three recent review articles on automated pesticide residue analysis describe no additional automated cleanup units utilizing column chromatography (15-17). However, since the completion of the present work, one paper has appeared which describes the regeneration of a liquid-liquid chromatographic column (18). The authors report that an analytical column packed with Bio-Sil A coated with sulfuric acid is made reusable by successively passing 1:1 chloroform-cyclohexane, 0.1 N sulfuric acid in 50% ethanol, and chloroform-cyclohexane between analyses. It is necessary that a new precolumn be packed for each analysis, but the analytical column lasts from 10-30 runs.

We sought to automate the separation and collection of trace levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a toxic impurity formed in the manufacture of esters of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), from commercial herbicide preparations. As the molecular weight of the ester used most in this study is 370 and that of TCDD 320, GPC would not be expected to resolve these two compounds satisfactorily. Reported here is an automated adsorption chromatographic system which successfully effects the separation.

Principle of the Method. The ester of 2,4,5-T used

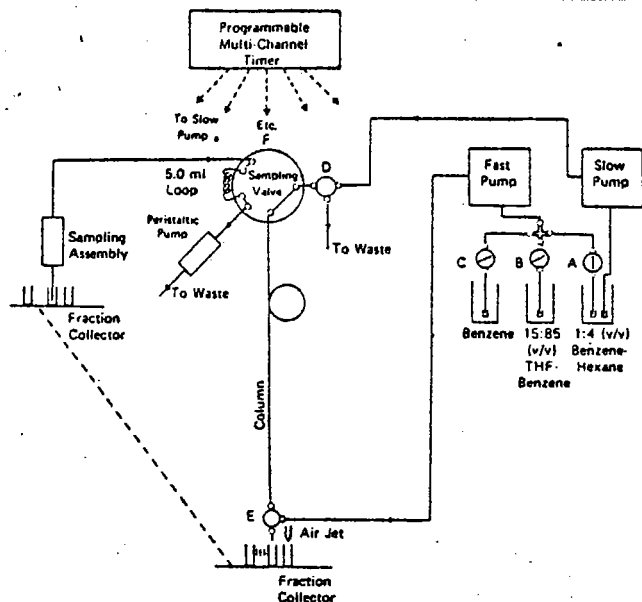


Figure 1. Schematic of automated system. See text for details.

throughout most of this study was the 1-isobutoxy-2-propyl (designated PiB-propylene glycol isobutyl). To separate TCDD from the PiB ester of 2,4,5-T on silica gel, a solution of 1:4 (v/v) benzene-hexane may be used (19). Under these conditions the TCDD passes through the column essentially unretained while the more polar ester is held up on the packing. To prepare the column for another sample, the adsorbed molecules must be removed from the silica surface.

This is accomplished by eluting with a more polar solvent combination—15:85 (v/v) tetrahydrofuran (THF)-benzene. The THF is then removed with benzene followed by a short re-equilibration with 1:4 benzene-hexane prior to application of the next sample.

EXPERIMENTAL

Description of System. The system is constructed of commercially available components except for a few easily-machined parts. A schematic diagram is shown in Figure 1. (Also see Figure 2.)

The column is constructed of 316 stainless steel tubing and has a single turn; it measures 1 m X 5 mm. It is packed dry with 100-200 mesh "high-purity" silica gel (Curtin Scientific, No. 531-178). As outlined in the previous section, three different solvent combinations are pumped through the column. Their delivery is controlled by 2-way valves A, B, and C (Altex, 201-00; pneumatic actuators, Altex, 201-12). After sample application, the 1:4 benzene-hexane elutes TCDD while the ester is retained on the column. Approximately 20 ml of the 1:4 benzene-hexane eluent is pumped at a flow rate of 1 ml/min (Slow Pump, Milton Roy, Model 396-31) to maximize the separation of TCDD and ester, and occurs in the forward direction through the column, as shown by the arrow. To remove the ester from the silica surface ca. 100 ml of 15:85 THF-benzene is passed through the column in the reverse direction at a flow rate of 4 ml/min (Fast Pump, Milton Roy, Model 396-89). Switching between the forward and reverse flow directions is accomplished with the aid of 3-way valves D (Altex, 201-01) and E (Valco, ACV-3HP). Backflushing is used to prevent ester from contacting the lower portion of the column. This serves two purposes: 1) minimizes coelution of components which may interfere in the ensuing determination of TCDD by gas chromatography-mass spectrometry (GC-MS), and 2) extends the column life by preventing the lower portion of the column from contacting the bulk of the sample. As the objective of this work is to reuse the same column for an extended period of time, the latter consideration is of prime importance. Our experiments have shown that the ester is retained on the top third of a freshly-packed column. Use of a long column should help prolong column life. As a final precaution to extend column life,

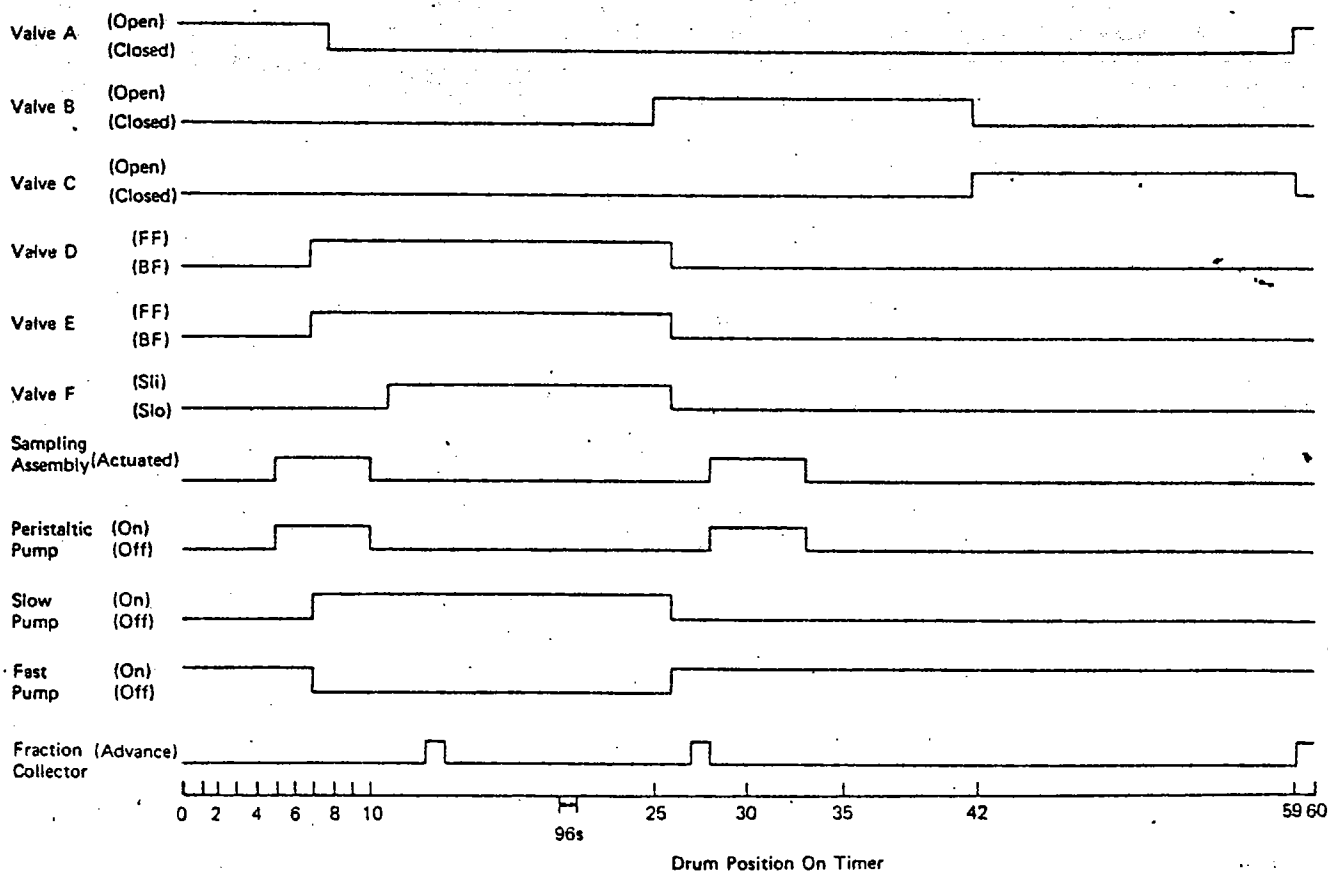


Figure 2. Timing diagram

FF = Switched for forward flow, BF = Switched for backflushing, Sli = Sample loop switched in, and Slo = Sample loop switched out

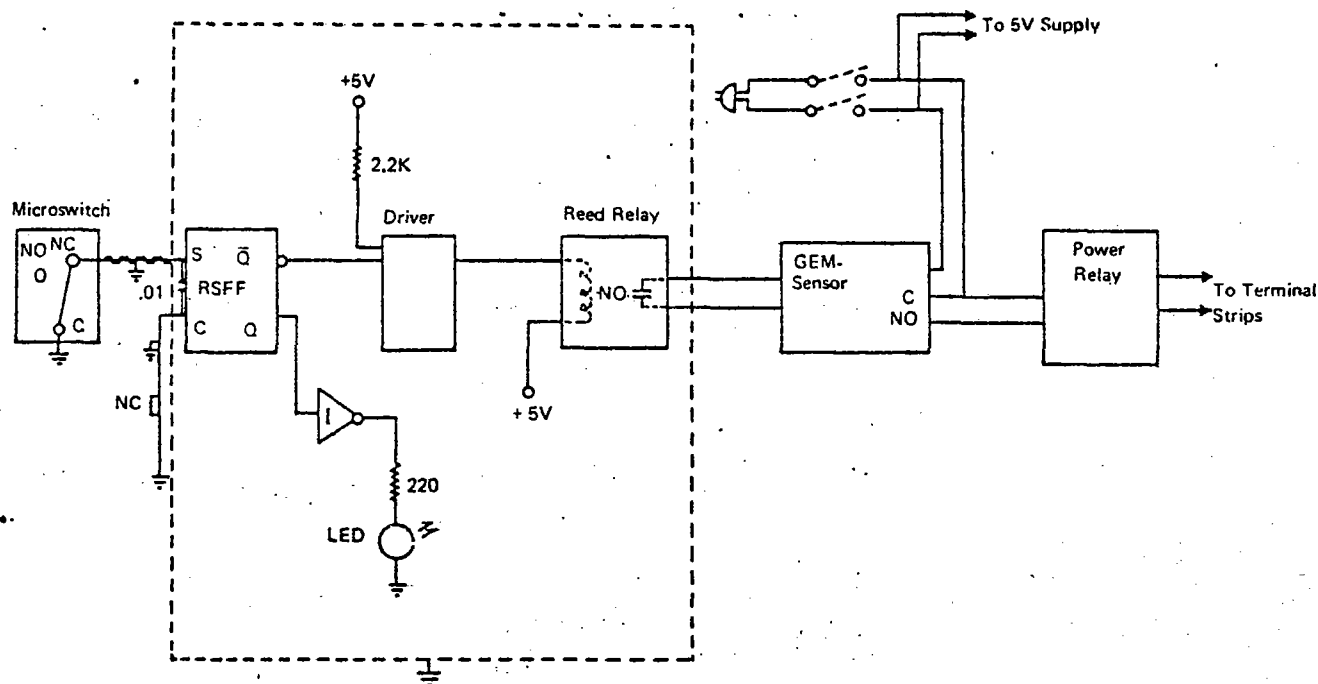


Figure 3. Schematic of start/stop circuitry

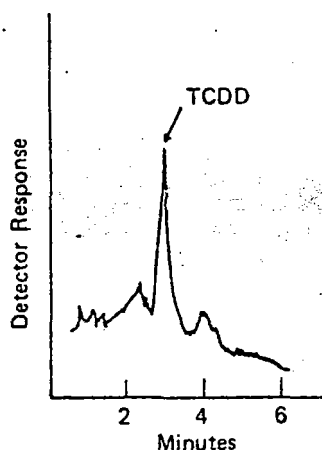


Figure 4. Typical mass chromatogram

THF is shaken with 5-Å molecular sieve (Linde) to minimize surface deactivation due to the introduction of water.

After the passage of 15:85 THF-benzene, 110 ml of benzene are passed. The purpose of benzene is to strip off the THF prior to introduction of the next sample. Following a reequilibration with 1:4 benzene-hexane at 4 ml/min, the next sample may be applied to the column.

The sampling system consists of a plunger fitted with a hypodermic needle, in combination with a Buchler peristaltic pump (Nuclear-Chicago) employing AutoAnalyzer tubing (Technicon Instrument Corp.). Sample introduction is accomplished with a Valco automatic sampling valve (AVSV-6-HP) with a 5.0-ml external loop attached. Sample and collection vials are suitably placed on a fraction collector (ISCO, Model 273) as shown in Figure 1. The solvents and volumes used in the elution scheme presented here were arrived at using TLC, column chromatography, and ultraviolet and NMR spectrometry.

The entire system, capable of sequentially processing ten samples, is under the control of a multichannel, mechanically-programmable timer (Sealectro, 092-286-5501-000). Each channel of the timer drum has 60 positions. In this system the drum is stepped once every 96 s by a $\frac{1}{2}$ rpm synchronous motor (Sealectro, 095-026-6525-000) so that an entire cycle (one sample processed) takes 96 min.

Several features of the timing diagram (Figure 2) are apparent from the previous discussion. The extra sampling time (positions 28-33) is for flushing out the viscous sample with solvent to prevent plugging

Table I. TCDD Concentration vs. Run No. for PiB Ester of 2,4,5-T

Run No.	ppm TCDD	Run No.	ppm TCDD
21	0.026	50	0.025
24	0.028	51	0.024
25	0.030	52	0.025
27	0.024	53	0.026
29	0.026	55	0.025
31	0.026	57	0.024
33	0.029	59	0.024
34	0.028	60	0.025
35	0.034	61	0.024
36	0.028	62	0.024
37	0.026	63	0.025
38	0.028	64	0.026
39	0.026	65	0.024
41	0.026	67	0.025
43	0.026	72	0.023
44	0.023	73	0.026
48	0.024	79	0.024
49	0.026	90	0.024

of the sample line and ensures against cross-contamination from one sample to the next. During positions 27-59, the collected TCDD is positioned under an air jet to evaporate the benzene-hexane solvent.

A protective feature of the apparatus is a simple start/stop circuit shown in Figure 3. The flip-flop shown is interposed between a microswitch and the power relay, and serves as a buffer in case of mechanical slippage of the shut-off assembly. The microswitch is closed by a pin located on the fraction collector reel when the timer reaches position 59 after the last sample has been processed. After loading the next batch of samples, one needs only to press the clear button to restart the system. A Light Emitting Diode (LED) indicates when samples are being processed. Proper circuit layout, routing of critical lead-in wire, and circuit shielding are essential to prevent false triggering of the digital circuitry. An additional protective feature is the inclusion of a pressure switch (Barksdale Valves, DIS-A80) which automatically shuts off power to the system in the event of a severe air leak.

The timer, start/stop circuit, air solenoids, terminal strips, and manifold for the pneumatic lines are all located in a control module. Because of the high flammability of the solvents, the solvent con-

Table II. Correlations of Automated and Manual Cleanups

Run No.	Sample	ppm 2,3,7,8-TCDD		Difference ^a
		Automated	Manual	
40	PiB ester of 2,4,5-T	0.017	0.014	0.003
42	PiB ester of 2,4,5-T	0.030	0.029	0.001
45	PiB ester of 2,4,5-T	0.020	0.021	-0.001
46	PiB ester of 2,4,5-T	0.032	0.032	0.000
47	PiB ester of 2,4,5-T	0.027	0.027	0.000
68	PiB ester of 2,4,5-T	0.028	0.029	-0.001
69	PiB ester of 2,4,5-T	0.023	0.023	0.000
75	PiB ester of 2,4,5-T	0.028	0.029	-0.001
76	PiB ester of 2,4,5-T	0.030	0.029	0.001
77	PiB ester of 2,4,5-T	0.056	0.056	0.000
80	PiB ester of silvex	0.036	0.034	0.002
81	PiB ester of 2,4,5-T	0.050	0.049	0.001
82,91	PiB ester of 2,4,5-T	0.092	0.091	0.001
83	PiB ester of 2,4,5-T	0.020	0.022	-0.002
84	PiB ester of 2,4,5-T	0.048	0.050	-0.002
85	Butoxypropyl ester of 2,4,5-T	0.008	0.008	0.000
96	PiB ester of 2,4,5-T	0.114	0.119	-0.005

^a Difference = Automated - Manual.

tainers are 0.5-gallon "safety" cans with flame arrestors. As a precaution against accumulation of flammable vapors in the event of a leak, points of sparking are purged with a light stream of air. Finally, since the collected TCDD is concentrated under a current of air, all components other than the control module are located inside a hood.

Operation of the System. Five-gram samples are weighed into No. 7 dram vials and diluted to 25 ml with 1:4 benzene-hexane. The vials are capped with aluminum foil, shiny side up, and placed on the inside track of the fraction collector reel. Up to ten sample vials may be placed on the reel. After the microswitch has been moved forward and clamped, the system is ready to be turned on for unattended operation. Prior to determination of TCDD by GC-MS, the collected dioxin is manually transferred from the No. 7 dram vial to a No. 1 dram vial, evaporated to dryness, and then brought to a final volume of 200 μ l with *o*-xylene. As the sample is applied to the column from a 5.0-ml loop, this makes for a fivefold concentration (1 g $\rightarrow$ 200 μ l).

Determination of TCDD. The extracts obtained from the automated unit were analyzed for TCDD as follows.

The concentration of TCDD was determined by GC-MS using a LKB 9000S. Injections of 3-5 μ l were made onto a 3 ft $\times$ 3 mm glass column packed with 3% OV-3 on Gas Chrom Q. The analysis conditions were as follows: carrier gas: helium at 35 cm³/min; temperatures: column, 230 $^{\circ}$ C; injector, 250 $^{\circ}$ C; separator, 270 $^{\circ}$ C; ion source, 270 $^{\circ}$ C; electron energy: 70 eV; accelerating voltage: 3.5 kV; trap current: 60 μ A.

A typical mass chromatogram is shown in Figure 4.

RESULTS AND DISCUSSION

Three factors were evaluated: 1) the number of times a column can be reused in a cleanup role; 2) system accuracy and precision; and 3) the degree of cross-contamination, if any, from one sample to the next.

Ninety-six runs were made in our initial study. Samples of the same PiB ester of 2,4,5-T were run intermittently from runs 21-90. The results are shown in Table I. The average TCDD content for these determinations was 0.026 ppm with a standard deviation of 0.002 ppm. Three separate manual cleanups of the same sample, performed per the method of Crummett and Stehl (19), also yielded an average dioxin concentration of 0.026 ppm. Nine blanks were interspersed among these runs; no TCDD was detected in the blanks with a detection limit of 0.005 ppm.

Sixteen samples of the PiB ester of 2,4,5-T were cleaned up by both the manual and automated methods and their dioxin levels determined. In addition, one each of a butoxy propyl ester of 2,4,5-T and a PiB ester of 2-(2,4,5-trichlorophenoxy) propionic acid (silvex) were compared. The TCDD concentrations found are shown in Table II along with the corresponding run number. The agreement is excellent for more

than 50 runs. At this point there was no indication of column degradation.

Since completing the initial study, we have run several other esters of 2,4,5-T. We have not accumulated the same extensive correlative data for these esters as reported for the PiB ester of 2,4,5-T. Once the performance of the column was demonstrated, esters differing only in the alcohol portion would be expected to chromatograph similarly.

Future Development. The demonstrated ability to repeatedly use a silica gel column for chemical cleanups opens up other possibilities. Immediately obvious is the substitution of other adsorbents such as alumina and Florisil where applicable. As with any analysis, certain advantages attend to its automation. Routine monitoring of production materials is simplified. In contrast to the 3 hours required to work up a sample manually using the procedure of Crummett and Stehl (19), the automated system does its work on up to 10 samples, overnight. Improved accuracy and precision as well as increased reliability result. When working with TCDD, the less chance of human contact with this toxic substance, the better. Also, from the human standpoint, when performing a routine analysis, repeated use of a single column eliminates the tedium of packing a new column for every sample.

We wish to emphasize the simplicity of the present system. As sequencing is under the control of a mechanically-programmed electromechanical timer, familiarity with computer programming and computer interfacing is not required, as would be the case if a mini- or microcomputer were used. If it is desired to alter the timing sequence for use with a different cleanup scheme, only a different timing drum is required for a quick changeover, or the actuators are repositioned for a slower changeover.

ACKNOWLEDGMENT

The authors thank Larry McFarland for the construction of the control module and Don Girardin for performing most of the manual cleanups. The authors are also grateful to their colleagues for much stimulating discussion.

LITERATURE CITED

- (1) W. P. McKinley, D. E. Coffin, and K. A. McCully, *J. Assoc. Off. Agr. Chem.*, **47**, 863 (1964).
- (2) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
- (3) K. Dus, S. Lindroth, R. Pabst, and R. M. Smith, *Anal. Biochem.*, **14**, 41 (1966).
- (4) G. Ertingshausen, H. J. Adler, and A. S. Reichler, *J. Chromatogr.*, **42**, 355 (1969).

- (5) D. J. Bennett and E. H. Creaser, *Anal. Biochem.*, **37**, 191 (1970).
- (6) A. R. Tschida and H. Markowitz, *Anal. Biochem.*, **26**, 337 (1968).
- (7) H. Small, T. S. Stevens, and W. C. Bauman, *Anal. Chem.*, **47**, 1801 (1975).
- (8) K. C. Blanshard, H. F. Bradford, P. R. Dodd, and A. J. Thomas, *Anal. Biochem.*, **67**, 233 (1975).
- (9) D. L. Stalling, R. C. Tindle, and J. L. Johnson, *J. Assoc. Off. Anal. Chem.*, **55**, 32 (1972).
- (10) R. C. Tindle and D. L. Stalling, *Anal. Chem.*, **44**, 1768 (1972).
- (11) K. R. Griffitt and J. C. Craun, *J. Assoc. Off. Anal. Chem.*, **57**, 168 (1974).
- (12) GPC 1001-Autoprep, Analytical Biochemistry Laboratories Inc., Columbia, Mo. 65201.
- (13) D. L. Stalling, J. Johnson, and J. N. Huckins, "Automated Gel Permeation-Carbon Chromatographic Cleanup of Dioxins, PCBs, Pesticides, and Industrial Chemicals", in "Environmental Quality and Safety, Supplement Vol. III, Pesticides Lectures of the IUPAC Third International Congress of Pesticide Chemistry" (Helsinki, July 1974), F. Coulston and F. Korte, Ed., G. Thieme Publ., Stuttgart, Germany, 1975, pp 12-18.
- (14) D. L. Stalling, Proc. Conf. Environ. Sensing Assessment, Las Vegas, Nev., Institute of Electrical and Electronic Engineers, Inc., USA Annals No. 75CH1004-1, New York, 1976, Section 7-5.
- (15) B. A. Karihuber and D. O. Eberle, *Anal. Chem.*, **47**, 1094 (1975).
- (16) H. A. McLeod, *J. Chromatogr. Sci.*, **13**, 302 (1975).
- (17) D. E. Ott, *Residue Rev.*, **55**, 1, 1975.
- (18) J. F. Morot-Gaudry, V. Fiala, J. C. Huet, and E. Jolivet, *J. Chromatogr.*, **117**, 279 (1976).
- (19) W. B. Crummett and R. H. Stehl, *Environ. Health Perspectives*, **15**, Sept., 1973.

RECEIVED for review October 8, 1976. Accepted December 2, 1976.

Characterization of Coal by Laser Pyrolysis Gas Chromatography

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Applications of laser pyrolysis gas chromatography (LPGC) to study the influence of composition of coals on the distribution of gaseous products are presented. LPGC provides a rapid method when used in conjunction with plasma stoichiometric analysis for determining the relative concentrations of carbon, hydrogen, and oxygen in coals. Pyrograms and correlations between experimental products and elemental compositions are presented.

Pyrolysis gas chromatography has been used to characterize coals of various rank (1-3). The highest pyrolysis temperature used in these studies was 1273 K, resulting in the evolution of large quantities of low molecular weight gases. Benzene and toluene are the major aromatic products (1). Changes in the chromatograms with coal rank were apparent (1, 2). The volatility of the organic compounds in the coal was enhanced by the addition of water to the coal samples before pyrolysis (1). Coal pyrolysis in nitrogen at temperatures of 623-773 K produced methane and ethane as major products with carbon monoxide, ethylene, and propane also formed (4).

An argon plasma torch reactor has also been used to study the rapid devolatilization of small coal particles (5). The weight loss increased with both increased heating rate and increased temperature. Heating rates from 10⁵-10⁶ K/s and a final temperature of 2000 K produced a greater loss of volatile matter than is obtained by proximate analysis. A microwave discharge in argon was used to study the gasification reactions of coal (6). The major products were H₂ and CO with the major hydrocarbon products being CH₄, C₂H₂, and C₂H₄. Also formed in smaller quantities were H₂O, CO₂, HCN, and higher molecular weight hydrocarbons up to C₈.

Rapid pyrolysis of coal has been achieved by having pulsed lasers irradiate the samples and using mass spectrometry for identification and quantitation (7-18). Laser pyrolysis gas chromatography (LPGC) has been used in studies of organic compounds and oil shales (19-21). Because of the rapidity of analysis, this technique was selected to use to characterize coal samples.

The method of free energy minimization was used in a computer program to calculate the gaseous product distribution for coal samples at temperatures from 2000 to 3500 K at a pressure of 2280 Torr (22). Correlations are presented of the experimental gaseous product compositions to the elemental composition of the coal. The comparison between the calculated product distributions at 3300 K and the experimental results agree for H₂, CO, and C₂H₂ to 5% by volume or better for the five coal samples.

EXPERIMENTAL

The coal samples were received as powders from Paul Weir Company, Chicago, Ill. Table I lists the composition of each sample. The samples were pelletized at 20 000 psi to form pellets of 1-2 mm thickness. The samples were sectioned and placed in quartz sample tubes of 6-mm o.d.

A Perkin-Elmer model 3920 gas chromatograph was used with a beta-induced luminescence detector (BILD) and the hydrogen flame ionization detector connected in series. The BILD detects components in the column effluent by measuring the quenching of the luminescence from trace levels of nitrogen in the helium carrier gas that is excited by beta's from a tritium foil. The design and response of this detector has been described (23). The quartz sample tube containing the pelletized sample was mounted on the injection port of the gas chromatograph. Helium carrier gas purged the system and carried the pyrolysis products onto the analytical column.

A pulsed ruby laser with an output of 2.6 joules in the normal-mode was used in these studies. The beam was focused on the sample surface in the quartz tubes. Only about 0.01 cm² of the sample was irradiated by the focused laser beam.

RESULTS AND DISCUSSION

Hydrogen sulfide and hydrogen cyanide have been reported as products from laser pyrolysis of coal (7, 11). Hydrogen sulfide and hydrogen cyanide have retention times similar to the retention times of water and acetylene, respectively, under the experimental conditions. The experimental peaks are reported as water and acetylene with recognition that hydrogen sulfide and hydrogen cyanide may be formed.

Equilibrium product distribution calculations were made for five coal samples for the elements carbon, oxygen, hydrogen, nitrogen, and sulfur. An initial calculation at 2280 Torr and 2500 K was made for 33 compounds: CH₄, C₂H₄, C₂H₂, C₂H, CH₃, CH₂O, HCO, CO, CO₂, H₂, H, OH, H₂O, C₂N₂, HCN, CS₂, HNCO, HNO, N₂, N, NO, NO₂, N₂O, NH, NH₂, NH₃, SN, SH, S₂, SO, SO₂, H₂S, and C₁₅. Nine species with insignificant concentrations were excluded from further consideration. They were OH, HNCO, HNO, N, NO₂, N₂O,

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MAR 2 1980

EXHIBIT E

MAR 2 1980

Ex. E

METHOD ML-AM 75-34

DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
IN 2,4,5-TRICHLOROPHENOXY ACETIC ACID AND RELATED
MATERIALS

The method herein described is applicable to:
2,4,5-T Acid, Silvex*Acid, Chlorinated Phenols,

and includes

Caustic Extractions
Silica Gel Cleanup
Ion Exchange Cleanup
Alumina Cleanup

*Trademark of The Dow Chemical Company.

ANALYTICAL METHOD

May 20, 1975

ML-AM-75-34

DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN 2,4,5-TRICHLOROPHENOXYACETIC ACID AND RELATED MATERIALS

1. Scope

This procedure is applicable to the determination of 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) and other related chlorinated materials in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T acid), 2,4-dichlorophenoxyacetic acid, and related acidic and phenolic materials and esters of these acids.

2. Principle

The neutral components of the sample are separated from the matrix. This is accomplished by extraction from caustic or by silica gel column chromatography, or by ion exchange column. The residue is concentrated and a portion examined by gas chromatography-mass spectrometry for species which have ions of the same mass, number of chlorine atoms, and retention time as TCDD.

3. Interferences

There are no known interferences when applied to 2,4,5-T acid, 2,4-D acid, 2,4,5-trichlorophenol, tetrachlorophenol, pentachlorophenol, and esters of these acids. Other isomers of tetrachlorodibenzo-p-dioxin are separated from TCDD on the chromatographic column and do not interfere.

4. Safety Precautions

2,4,5-T acid and related materials should be handled with the normal amount of caution. Avoid breathing sample dust or vapors. The preparation of standards of TCDD should be done so as to prevent exposure to the pure material and with extreme care to avoid spills. All glassware used should be well scrubbed and rinsed with acetone, benzene, or chloroform before returning to general laboratory use. Special care should be

exercised in their disposal. It is advisable to read "Summary of Safe Handling of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Laboratory", available from the Sample Coordinator, Ag-Organics Department, 9008 Building, The Dow Chemical Company, Midland, MI 48640.

Some of the other reagent used can be hazardous to handle also; follow the safe handling precautions recommended by the supplier.

5. Sampling

Samples should be taken and treated to insure homogeneity. If the product can be melted easily (not 2,4,5-T acid), minimum temperatures and times should be used. Grinding and blending of large pieces of material is recommended.

6. Apparatus

(a) Gas chromatograph-mass spectrometer (GC-MS): LKB-9000S (LKB Productor, Bromma, Sweden) or equivalent, with multiple ion detector.

(b) Gas chromatographic column for LKB: 1 m x 3 mm I.D. (glass) packed with 3% OV-3 on 80/100 Gas Chrom. Z.

(c) 50 cm x 16 mm I.D. glass chromatography columns with 250 ml reservoir.

7. Reagents

(a) 10% aqueous sodium hydroxide: weigh 100 g of ACS reagent grade sodium hydroxide into a 250 ml beaker and pour with swirling into 750 ml of distilled water in a liter volumetric flask. When solution is complete, dilute to volume.

(b) 1% aqueous sodium hydroxide: dilute 100 ml of 10% sodium hydroxide to one liter with distilled water.

(c) Benzene: ACS reagent grade; "distilled in glass" grade is desirable.

(d) Methanol: ACS reagent grade.

(e) Hexane: ACS reagent grade, "distilled in glass" grade is desirable.

(f) Silica Gel: High purity 100-200 mesh, used as received without activation.

(g) Ion exchange resin AG-21K brand chloride form, 50/100 mesh, available from Bio-Rad Laboratories, 32nd and Griffin Avenue, Richmond, CA 94804.

(h) Pure TCDD: available from Sample Coordinator, Ag-Organics Department, 9008 Building, The Dow Chemical Company, Midland, MI 48640.

8. Calibration

(a) Standard TCDD: Weigh 1-2 mg to the nearest 0.01 mg in a small weighing cup. Transfer to a 10-ml volumetric flask and dilute to volume with benzene. Prepare lower

concentrations by dilution. Make concentrations of approximately 2 $\mu\text{g}/\text{ml}$ to be used for tuning and 0.2 $\mu\text{g}/\text{ml}$ to be used as standard calibration.

(b) Inject 5 μl of the strong standard (2 $\mu\text{g}/\text{ml}$) in the GC-MS. As the TCDD elutes, fine tune the magnet and accelerating voltage alternator control to maximize the response at m/e 320, 322 and 324.

(c) Inject 3 μl of the weak standard (0.2 $\mu\text{g}/\text{ml}$ in the GC-MS and record the response. Measure the ion intensities at the chromatographic peak maximum using a tangent baseline.

9. Procedure A - Chlorinated Phenoxyacetic acids

(a) Preparation of Ion-exchange Resin, 21K Hydroxide form.

i) Place one liter of ion exchange resin 21K (Cl^- form) into a 2000 ml beaker and slurry with 500 ml of distilled water. Transfer the slurry to a 2 inch (I.D.) by 30 inch glass column having a glass wool plug above the stopcock made of Teflon plastic.

ii) Pass approximately 3000 ml of 5% sodium hydroxide through the resin at a flow rate of approximately 10 ml/min.

iii) Pass distilled water through the column until the eluent is no longer basic (as determined by testing with pHDrion paper).

iv) Pass approximately one gallon of methanol through the resin to remove the water.

v) Divide the resin into two equal portions; transfer each to a 1 liter amber bottle.

vi) Decant the excess methanol from the resin and fill the bottle with benzene. Shake vigorously. If the resin is not used immediately it should be refrigerated to retard decomposition.

(b) Transfer eighty ml of the 21K (OH^- form) resin to a 16 mm (I.D.) by 60 mm glass column having a glass wool plug above the stopcock made of Teflon plastic. Adjust the solvent level to slightly above the resin bed.

(c) Weigh 5.0 gram of sample into a 7-dram vial. Add 20 ml of 1:1 benzene/methanol (V/V) and shake until dissolved.

(d) Transfer the solution to the ion exchange column using a minimal amount of solvent for rinsing.

(e) Elute the dioxins from the column with 1:1 benzene/methanol. Discard the first 25 ml eluting from the column. Collect the next 125 ml eluting in a 150 ml beaker.

(f) Evaporate the solvent in the beaker to approximately 15-20 ml at ambient temperature using a stream of nitrogen. Transfer this (with adequate rinsing of the beaker) to a 7-dram vial.

(g) Evaporate the solvent in the vial to dryness at ambient temperature using a stream of dry nitrogen. Pipet 1.0 ml of benzene into the vial and cap tightly.

(h) Inject a 1.0 μ l portion into the GC-MS and monitor the three molecular ions at m/e 320, 322, 324 by either repetitively scanning over this region or by means of the multiple-ion detector assembly which will focus these three ions successively at 1/2 second intervals. Measure all three ion intensities at the chromatographic peak maximum using a tangent baseline.

10. Procedure B - Chlorinated Phenols

(a) Weigh 5.0 g of sample into a 400 ml beaker. Add 200 ml of methanol, 15 ml of 10% sodium hydroxide, and 200 ml of distilled water and stir until complete solution is obtained. Transfer to a 500 ml separatory funnel and extract with 50 ml hexane (or benzene). (Note 12b).

(b) Transfer the aqueous phase to the 400 ml beaker and the organic layer to a separate 125 ml separatory funnel. Repeat the extraction of the aqueous phase with fresh hexane (or benzene) and add the second organic portion of the first.

(c) Wash the combined organic phases with an equal volume of 1% sodium hydroxide followed by washing with an equal volume of water.

(d) Add the hexane (or benzene) in portions to a 5 dram (25 ml) vial and evaporate to near dryness at room temperature under a gentle stream of air. When the last portion has been evaporated, rinse the separatory funnel and evaporate just to dryness. CAUTION: Do not evaporate at elevated temperatures or allow the vial to be dry for more than several minutes.

(e) Add 0.5 ml benzene to the vial, cap securely, and rotate gently to wet all interior surfaces.

(f) Inject a 1.0 μ l portion into the GC-MS and monitor the three molecular ions at m/e 320, 322, 324 by either repetitively scanning over this region or by means of the multiple-ion detector accessory which will focus these three ions successively at 1/2 sec. intervals. Measure all three ion intensities at the chromatographic peak maximum using a tangent baseline.

11. Operating Conditions

Column: (glass) 1 m x 3 mm I.D..3% OV-3 on 80/100 Gas Chrom Z
Temperatures:

Column - 240°C.

Flash Heater - 250°C

Separator - 290°C

Ion Source - 270°C

Flow Rate: 40 cc/min. helium as read on rotameter

Trap Current: 60 μ A

Accelerating Voltage: 3.5 KV

Electron Energy: 70 ev

Multiplier: 4.9

Masses to be monitored. m/e 320, 322 and 324

Oscillograph chart speed: 1 cm/min.

12. Calculations

(a) The concentration of TCDD present is calculated from

$$C = \frac{a_n}{b_n} \times \frac{D_a}{D_b} \times \frac{1}{E} \times S$$

where C is the concentration of TCDD in the sample;

a_n = ion intensity as recorded in 10f. for m/e = 320 (sample)

b_n = ion intensity as recorded in 8b for m/e = 320 (standard)

D_a = Oscillograph attenuation range for largest on-scale ion intensity for sample

D_b = Oscillograph attenuation range for largest on-scale ion intensity for standard

E = ratio of sample weight in grams to final solution volume in ml. (usually 10)

S = concentration of the standard in μ g/ml

(b) The above calculation is repeated for m/e = 322 and for m/e = 324. The values should agree to $\pm 15\%$. If they do not, the value recorded is the lowest concentration calculated.

13. Recovery and Precision

The precision of this procedure for duplicate samples should be $\pm 20\%$ of the amount present. The lower limit of detection of this procedure is 0.01 to 0.02 ppm.

14. Notes

(a) For samples which are non-ionizable, such as esters, the TCDD can be separated from the matrix by substituting the following procedure for steps 10 a, b, and c.

1. Weigh a 5.0 gm sample and dissolve/slurry with 25 ml of 20% benzene in hexane. Pour (with rinsing) this sample onto 50 g of silica gel in a 16 mm. i.d. glass column previously equilibrated with 20% benzene/hexane. Allow 25 ml of solvent to elute. Collect the next 100 ml of effluent, adding 20% benzene/hexane to the column as necessary to maintain liquid above the silica gel bed. Continue as in 10d.

(b) The detection of other species present or suspected in the neutral residue can be accomplished by either scanning or focusing on the most characteristic ions formed from these species. Quantitation is achieved as described for TCDD.

The analytical procedures given herein have been adapted from literature sources or developed upon the basis of experimental data believed to be reliable. In the hands of a qualified analyst they are expected to yield results of sufficient accuracy for their intended purposes. Users are cautioned to confirm the suitability of the methods by appropriate tests. Anyone wishing to reproduce or publish the material in whole or in part should request written permission from The Dow Chemical Company.

METHOD 87596B

ISOOCTYL ESTER OF 2,4,5-T: THE TCDD METHOD HEREIN
DESCRIBED IS IDENTICAL TO THAT USED FOR ALL ESTERS OF
2,4,5-TRICHLOROPHENOXY ACETIC ACID AND 2,4,5-TRICHLORO-
PHENOXYPROPIONIC ACID



ANALYTICAL METHOD

February 12, 1976

Method No. 87596b

2,4,5-TRICHLOROPHENOXYACETIC ACID,
ISOOCTYL ESTERS

A. Scope

This method is applicable to the analysis of 2,4,5-trichlorophenoxyacetic acid, isooctyl esters. A procedure is given for the determination of the assay of 2,4,5-trichlorophenoxyacetic acid, (2,4,5-T) isooctyl esters, and free acid. The acid equivalent is calculated from the assay. A procedure is also given for the determination of 2,3,7,8-tetrachloro-dibenzo-p-dioxin. (TCDD).

B. Safety Precautions

The isooctyl esters of 2,4,5-T may cause mild eye and skin irritation but are not readily absorbed through the skin in toxic amounts. Avoid skin and eye contact. Handle with care.

Nitric acid is corrosive and a strong oxidizing agent. Wear gloves and goggles when handling nitric acid.

Sodium biphenyl and 30% hydrogen peroxide are strong oxidizers. Avoid eye and skin contact. Follow the safety precautions as recommended by the supplier.

Potassium cyanide (KCN) is TOXIC. Handle with care. To dispose of KCN solution flush sink drain with water for 15 minutes before and after KCN is poured down the sink.

Some of the other reagents used may also be hazardous to handle. Follow the recommendations given by the supplier for their safe handling.

1. Principle

The sample is decomposed using sodium biphenyl. Excess biphenyl reagent is precipitated and the soluble chloride is titrated potentiometrically with standard silver nitrate.

2. Apparatus

(a) Metrohm E - 436 Potentiograph, Brinkman Instruments, Cantiague Road, Westbury, N.J. 11590, or equivalent.



(b) Silver Billet electrode, Beckman #39261, or equivalent. Coat silver billet with silver chloride (see Section 4). The Beckman electrode is available from Beckman Lab Instruments 23511 Southfield Road, Southfield, MI 48075.

(c) Glass electrode, Sargent S3005-15C, or equivalent non-chloride containing reference electrode. The Sargent electrode is available from Sargent-Welch Scientific Co., 8560 West Chicago Avenue, Detroit, MI 48204.

(d) Hot plate.

3. Reagents

(a) Sodium biphenyl reagent, available from Southwestern Analytical Chemicals, Inc., P.O. Box 485, Austin, TX 48767. Reagent comes in 1/2 ounce bottles suitable for a single determination. Store in a refrigerator prior to use.

(b) 1,2-Dimethoxyethane (DME), available from Eastman Kodak Company, Rochester, NY 14650.

(c) Nitric acid, 1:1 solution. Carefully add 500 ml of concentrated nitric acid to 500 ml of distilled water.

(d) Silver nitrate, 0.1 N. Dissolve 17 g of reagent grade silver nitrate in 1 liter of distilled water. Standardize the silver nitrate solution with dry primary standard sodium chloride. Alternatively, an analytical concentrate "Dilut-it" from J. T. Baker 0.1 N-AgNO₃ available from Curtin Scientific, P.O. Box 1223, Midland, MI 48640 can be made to volume.

(e) Hydrogen Peroxide, 30%.

(f) Potassium chloride approximately 0.1 N. Dissolve 7.5 gm potassium chloride in 1 liter of distilled water.

(g) Potassium cyanide, 5%. Dissolve 5 g potassium cyanide in 100 ml of water. TOXIC. Handle with care. (Note-DO NOT add KCN to an acid solution).

4. Preparation of the Silver-Silver Chloride Electrode.

(a) Polish the silver portion with fine steel wool until it is shiny and clean. Chemically clean the electrode by briefly soaking it in a 5% solution of KCN and rinsing with water. The same KCN solution can be used repeatedly for cleaning the electrode.

(b) Coat the electrode with silver chloride by electro-deposition from an approximately 0.1 N solution of potassium chloride using a 3-volt dry cell, and a platinum wire electrode.

Connect the silver electrode to the positive pole of the battery and plate with chloride for 20 seconds. Then, by means of a switch, reverse the current for five seconds to partially remove the chloride. Repeat this operation twice and finally coat the silver electrode with chloride for 20 seconds. Coating must be uniform.

(c) In the presence of biphenyl, the silver chloride coating lasts for about 20 determinations. Silver billet should be coated with chloride daily.

5. Procedure

(a) Accurately weigh, to the nearest 0.1 mg, 100-200 mg of sample or a sufficient quantity to yield about 1.5 milliequivalents of chloride, into a dry 250-ml beaker.

(b) Add 10 ml of 1,2-dimethoxyethane (DME) to the sample. Swirl to dissolve. Run a blank on reagents used.

(c) Shake the contents of a sodium biphenyl reagent bottle and add in small increments with swirling. The mixture should remain green. If it is not, add more biphenyl reagent until green color persists indicating an excess of the reagent. Let it stand for 5 minutes.

(d) Carefully heat the mixture to boiling on the hot plate by swirling the beaker's contents to avoid any bumping. Cool to room temperature.

(e) Carefully add 50 ml of distilled water with swirling and 5 ml of 30% hydrogen peroxide. Add some glass beads and cover with a watch glass.

(f) Heat on the hot plate to incipient boiling and then transfer to a steam bath. Boil gently for 10 minutes.

(g) Remove from steam bath and let stand for 5 minutes. While stirring solution with a glass rod, add sufficient water to dilute to 150 ml. Let stand for 10 minutes.

(h) After standing, break up solidified biphenyl with a glass rod. (See Note 3b). Add 5 ml of 1:1 nitric acid.

(i) Place a magnetic stirring bar in the beaker. Titrate with a standard silver nitrate solution to past observed inflection point. The end point is taken as the mid-point of the inflection curve.

6. Calibration

$$\frac{(A - B) \times N \times 367.5}{C \times 30} = \text{2,4,5-trichlorophenoxyacetic acid, isooctyl esters.}$$

Where:

A = ml of silver nitrate used for the sample

B = ml of silver nitrate used for the blank

C = sample weight in grams

N = normality of silver nitrate solution

367.5 = molecular weight of 2,4,5-T isooctyl esters.

7. Precision

Data obtained by this procedure indicate a standard deviation of 0.05%. Any single determination may be expected to differ from the average by not more than $\pm 0.1\%$ (95% confidence limits).

8. Notes

(a) It is advisable to run an Analytical Standard of 2,4,5-T with each set of samples. This is available from the Sample Coordinator, Agricultural Products Department, 9003 Building, The Dow Chemical Company, Midland, MI 48640.

(b) Rapid cooling of solution will cause a thick crust of biphenyl precipitate to form which is difficult to break up. Also, possible occlusion of chloride in biphenyl crust can occur if solution is allowed to cool rapidly. Slow cooling causes a finer precipitate of biphenyl to form.

(c) Presence of bromide and chloride in some samples will be indicated by two breaks in the titration curve, with the bromide end-point occurring first.

D. Acid Equivalent

9. Principle

The acid equivalent is calculated from the assay as determined in Section 6.

10. Calculation

255.5 = molecular weight of 2,4,5-trichlorophenoxyacetic acid

367.5 = molecular weight of 2,4,5-trichlorophenoxyacetic acid, isooctyl esters.

$\frac{32,4,5\text{-trichlorophenoxy acid isooctyl esters (as obtained in Section 6)}}{367.5} \times 255.5 = 32,4,5\text{-T acid equivalent.}$

Or

$32,4,5\text{-trichlorophenoxyacetic acid isooctyl esters} \times 0.6952 = 32,4,5\text{-T acid equivalent}$

E. Free Acid

11. Principle

The free acid is determined by a potentiometric titration of an alcoholic solution of the ester with 0.1 N sodium hydroxide solution to an apparent pH of 7.0 (see Note 17a). The free acid is calculated as 2,4,5-trichlorophenoxyacetic acid.

12. Apparatus

- (a) pH Meter, any commercial instrument which is capable of giving reproducible results with a precision of ± 0.1 units.
- (b) Reference electrode, saturated calomel.
- (c) Glass electrode.

13. Reagents

- (a) Prepare 1 liter of 0.1 N NaOH by quantitatively transferring the contents of an analytical concentrate "Dilut-it" from J. T. Baker to a 1 liter volumetric flask. Dilute to exactly 1 liter with distilled water.
- (b) Ethanol, 95% Formula 2B or Formula 30 neutralized to an apparent pH of 7.0.
- (c) Buffer solution, equimolar phosphate, 0.05 M, pH 6.86.

Dissolve 3.533 g of ACS reagent grade disodium hydrogen phosphate (Na_2HPO_4) and 3.333 g of ACS reagent grade potassium dihydrogen phosphate (KH_2PO_4), each previously dried at 120°C for two hours, in sufficient distilled water to 1000 ml.

14. Procedure

(a) Standardize the electrode system at 25°C against the pH 6.85 aqueous buffer (13c).

(b) Accurately weigh, to the nearest 0.05 g, 10 g of sample into a 150 ml beaker.

(c) Add 100 ml of neutral alcohol and titrate the solution potentiometrically to an apparent pH of 7.0 with 0.1 N sodium hydroxide (see Note 17b).

15. Calculation

% Free acid as 2,4,5-trichlorophenoxyacetic acid =

$$\frac{\text{ml NaOH} \times N \times 0.2555 \times 100}{\text{grams of sample}}$$

16. Precision and Accuracy

The standard deviation for both the precision and the accuracy of this method has been determined by the analysis of three series of production and prepared standard samples to be 0.04% absolute. The expected limit of variation at the 95% confidence level is $\pm 0.08\%$ absolute for a range of free acid from 0 to 7%.

17. Notes

(a) The potentiometric endpoint for the titration of the phenoxyacetic acids is an apparant pH of 8.5. However, in the presence of the ester it is necessary to stop the titration at an apparent pH of 7.0 in order to avoid saponification. With a slight excess of alkali, the ester can be completely saponified at room temperature within a few minutes.

(b) Methyl red can be used as an indicator instead of the pH meter if the color change can be seen.

(c) Since the titration is stopped at pH 7 in order to avoid saponification of the ester, the free acid determination is always biased low.

F. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

18. Principle

The neutral components including any TCDD in the sample are separated from the matrix. This is accomplished by silica gel column chromatography. The residue is concentrated and a portion examined by gas chromatography-mass spectrometry for species which have ions of the same mass, number of chlorine atoms, and retention time as TCDD.

19. Interferences

No substances are encountered to an extent sufficient to cause interference down to approximately 10 ppb.

20. Specificity and Selectivity

The gas chromatography-mass spectrometry (GC-MS) technique is extremely sensitive and specific for the determination of TCDD. In order to assign a signal to TCDD three things must occur simultaneously: 1) the retention time must be correct (the peak maximum is reproducible to within 0.1 min.); 2) all three m/e 320, 322 and 324 must respond and; 3) they must respond in the proper ratio.

21. Safety Precautions

TCDD in amounts handled in the laboratory can produce chloracne if allowed to contact the skin. Extreme safety precautions should be exercised in the preparation and disposal of standard material and solutions. It is advisable to read "Summary of Safe Handling of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Laboratory," available from the Sample Coordinator, Agricultural Products Department, 9003 Building, The Dow Chemical Company, Midland, MI 48640.

Handle all solvents in a fume hood and avoid breathing vapors.

22. Apparatus

(a) Gas chromatograph-mass spectrometer: LKB-9000S (LKB Productor, Bromma, Sweden) or equivalent, with multiple ion detector.

(b) Gas chromatographic column for LKB: 1 m x 3 mm i.d. (glass) packed with 3% OV-3 on 80/100 Gas Chrom. Z.

(c) 30 cm x 11 mm i.d. glass chromatography columns with 125 ml reservoir.

23. Reagents

(a) Benzene: ACS reagent grade; available from Burdick & Jackson Laboratories, Inc., 1953 S. Harvey Street, Muskegon, MI 49442.

(b) Hexane: ACS reagent grade; available from Burdick & Jackson Laboratories, Inc., 1953 S. Harvey Street, Muskegon, MI 49442.

(c) Silica Gel: High purity 100-200 mesh, Davidson 923 Brand, available from Curtin Scientific, Midland, MI 48640.

(d) 20% benzene in hexane: Add 200 ml benzene to 800 ml hexane and mix well.

(e) Pure TCDD: available from the Sample Coordinator, Agricultural Products Department, 9008 Building, The Dow Chemical Company, Midland, MI 48640.

24. Calibration

(a) Standard TCDD: Weigh 1-2 mg to the nearest 0.01 mg in a small weighing cup. Transfer to a 10-ml volumetric flask and dilute to volume with benzene. Prepare lower concentrations by dilution. Accurately make concentrations of approximately 2 µg/ml to be used for tuning and 0.2 µg/ml to be used for standard calibration of the GC-MS.

(b) Inject 5 µl of the concentrated standard (2 µg/ml) in the GC-MS. As the TCDD elutes, fine tune the magnet and accelerating voltage alternator control to maximize the response at m/e 320, 322 and 324.

(c) Inject 3 µl of the diluted standard (0.2 µg/ml) in the GC-MS and record the response. Measure the ion intensities at the chromatographic peak maximum using a tangent baseline.

25. Operating Conditions

(a) Column: (glass) 1 m x 3 mm i.d. 3% OV-3 on 80/100 Gas Chrom. Z.

(b) Temperatures:

Column	240°C
Flash Heater	250°C
Separator	290°C
Ion Source	270°C

(c) Flow Rate: 40 cc/min. helium as read on rotameter.

(d) Trap Current: 60 µa

- (e) Accelerating Voltage: 3.5 KV
- (f) Electron Energy: 70 ev
- (g) Multiplier: 4.9
- (h) Masses to be monitored: m/e 320, 322 and 324.
- (i) Oscillograph chart speed: 1 cm/min.

26. Procedure

- (a) Weigh a 1.00 g sample of isooctyl ester of 2,4,5-T acid into a 5-dram vial and dissolve it with 5 ml of hexane.
- (b) Prepare a silica gel column in a glass tube (30 cm long x 11 mm i.d. with a 125 ml reservoir) with 14 g of silica gel equilibrated with 20% benzene-hexane.
- (c) Quantitatively transfer the sample onto the column with three 2-ml portions of 20% benzene-hexane. Add benzene-hexane to the column as necessary to maintain liquid above the silica bed, until 40 ml are collected.
- (d) Evaporate the column eluate until 1 to 2 ml remain in the beaker. Transfer to 1-dram vial; rinse the beaker twice with 1-ml portions of benzene-hexane, and add to the vial.
- (e) Evaporate just to dryness.
- (f) Add exactly 200 μ l of benzene to the vial, cap securely and rotate gently to wet all interior surfaces.
- (g) Inject a 3- μ l portion into the GC-MS and monitor the three molecular ions at m/e 320, 322 and 324 by means of the multiple-ion detector. Measure all three ion intensities at the chromatographic peak maximum using a tangent baseline.

27. Calculations

- (a) The concentration of TCDD present is calculated from

$$C = \frac{a_n}{b_n} \times \frac{D_a}{D_b} \times \frac{1}{E} \times S$$

where:

C is the concentration of TCDD in the sample (ppm)

a_n = ion intensity as recorded in Section 26g for m/e = 320 (sample)

b_n = ion intensity as recorded in Section 24c for m/e = 320 (standard)

D_a = Oscillograph attenuation range for largest on-scale ion intensity for sample

D_b = Oscillograph attenuation range for largest on-scale ion intensity for standard

S = concentration of the standard in ug/ml

28. Recovery and Precision

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METHOD 28855B

ESTERON* BRUSH KILLER: THE TCDD METHOD DESCRIBED
HEREIN IS IDENTICAL TO THAT USED FOR

ESTERON* 245
KURON*
VERTON* 2T
BRUSH KILLER LV-2-2
BRUSH KILLER TX
BRUSH KILLER X

*Trademark of The Dow Chemical Company.



THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48640

ANALYTICAL METHOD

December 2, 1976

Method No. 28855b

ESTERON* BRUSH KILLER

A. Scope

This method is applicable to the analysis of ESTERON Brush Killer. Procedures are given for the determination of total acid equivalent and specific gravity. The active ingredients which are the propylene glycol butyl ether esters of 2,4-dichlorophenoxyacetic (2,4-D) acid and 2,4,5-trichlorophenoxyacetic (2,4,5-T) acid, the inert ingredients and pounds of each acid equivalent (2,4-D acid and 2,4,5-T acid) per gallon (US) are calculated. A procedure is also given for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

The calculations are based on the assumption that equal weights of 2,4-D and 2,4,5-T acid equivalents have been mixed as required by the specification. If necessary, the actual ratio of the 2,4-D and 2,4,5-T acids present can be determined by other appropriate methods.

B. Safety Precautions

ESTERON Brush Killer may cause mild eye and skin irritation but is not readily absorbed through the skin in toxic amounts. Avoid skin and eye contact. Handle with care.

Nitric acid is corrosive and a strong oxidizing agent. Wear gloves and goggles when handling nitric acid.

Sodium biphenyl and 30% hydrogen peroxide are strong oxidizers. Avoid eye and skin contact.

Potassium cyanide (KCN) is TOXIC. Handle with care. To dispose of KCN solution flush sink drain with water for 15 minutes before and after KCN is poured down the sink.

Some of the other reagents used can also be hazardous. Follow the safe handling procedures recommended by the suppliers.

C. Assay

1. Principle

The sample is decomposed using sodium biphenyl. Excess biphenyl reagent is precipitated and the soluble chloride is titrated potentiometrically with standard silver nitrate.

2. Apparatus

(a) Metrohm E - 436 Potentiograph Brinkman, Instruments, Cantiague Road, Westbury, N.J. 11590, or equivalent.

(b) Silver Billet electrode, Beckman #39261, or equivalent. Coat silver billet with silver chloride (see Section 4). The Beckman electrode is available from Beckman Lab Instruments 25511 Southfield Road, Southfield, MI 48075.

(c) Glass electrode, Sargent S3005-15C, or equivalent non-chloride containing reference electrode. The Sargent electrode is available from Sargent-Welch Scientific Co., 8560 West Chicago Avenue, Detroit, MI 48204.

(d) Hot plate.

3. Reagents

(a) Sodium biphenyl reagent, available from Southwestern Analytical Chemicals, Inc., P.O. Box 485, Austin, TX 48767. Reagent comes in 1/2 ounce bottles suitable for a single determination. Store in a refrigerator prior to use.

(b) 1,2-Dimethoxyethane (DME), available from Eastman Kodak Company, Rochester, NY 14650.

(c) Nitric acid, 1:1 solution. Carefully add 500 ml of concentrated nitric acid to 500 ml of distilled water.

(d) Silver nitrate, 0.1 N. Dissolve 17 g of reagent grade silver nitrate in 1 liter of distilled water. Standardize the silver nitrate solution with dry primary standard sodium chloride. Alternatively, an analytical concentrate (J. T. Baker "Dilut-it" 0.1 N-AgNO₃, available from Curtin Scientific, P.O. Box 1228, Midland, MI 48640) can be made to volume.

(e) Hydrogen Peroxide, 30%.

(f) Potassium chloride approximately 0.1 N. Dissolve 7.5 gm potassium chloride in 1 liter of distilled water.

(g) Potassium cyanide, 5%. Dissolve 5 g potassium cyanide in 100 ml of water. TOXIC. Handle with care. (Note-DO NOT add KCN to an acid solution).

4. Preparation of the Silver-Silver Chloride Electrode.

(a) Polish the silver portion with fine steel wool until it is shiny and clean. Chemically clean the electrode by briefly soaking it in a 5% solution of KCN and rinsing with water. The same KCN solution can be used repeatedly for cleaning the electrode.

(b) Coat the electrode with silver chloride by electro-deposition from an approximately 0.1 N solution of potassium chloride using a 3-volt dry cell, and a platinum wire electrode.

Connect the silver electrode to the positive pole of the battery and plate with chloride for 20 seconds. Then, by means of a switch, reverse the current for five seconds to partially remove the chloride. Repeat this operation twice and finally coat the silver electrode with chloride for 20 seconds. Coating must be uniform.

(c) In the presence of biphenyl, the silver chloride coating lasts for about 20 determinations. Silver billet should be coated with chloride daily.

5. Procedure

(a) Accurately weigh, to the nearest 0.1 mg, 100-200 mg of sample or a sufficient quantity to yield about 1.5 milliequivalents of chloride, into a dry 250-ml beaker.

(b) Add 10 ml of 1,2-dimethoxyethane (DME) to the sample. Swirl to dissolve. Run a blank on reagents used.

(c) Shake the contents of a sodium biphenyl reagent bottle and add in small increments with swirling. The mixture should remain green. If it is not, add more biphenyl reagent until green color persists indicating an excess of the reagent. Let it stand for 5 minutes.

(d) Carefully heat the mixture to boiling on the hot plate by swirling the beaker's contents to avoid any bumping. Cool to room temperature.

(e) Carefully add 50 ml of distilled water with swirling and 5 ml of 30% hydrogen peroxide. Add some glass beads and cover with a watch glass.

(f) Heat on the hot plate to incipient boiling and then transfer to a steam bath. Boil gently for 10 minutes.

(g) Remove from steam bath and let stand for 5 minutes. While stirring solution with a glass rod, add sufficient water to dilute to 150 ml. Let stand for 10 minutes.

(h) After standing, break up solidified biphenyl with a glass rod. (See Note 8b). Add 5 ml of 1:1 nitric acid.

(i) Place a magnetic stirring bar in the beaker. Titrate with a standard silver nitrate solution to past observed inflection point. The end point is taken as the mid-point of the inflection curve.

6. Calculation

$$\frac{(A - B) \times N \times 374.9}{C \times 30} = \text{2,4,5-trichlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether esters.}$$

Where:

A = ml of silver nitrate used for the sample

B = ml of silver nitrate used for the blank

C = sample weight in grams

N = normality of silver nitrate solution

374.9 = weighted average molecular weight of 2,4,5-T and 2,4-D propylene glycol butyl ether esters.

7. Precision

Data obtained by this procedure indicate a standard deviation of 0.05%. Any single determination may be expected to differ from the average by not more than $\pm 0.1\%$ (95% confidence limits).

8. Notes

(a) It is advisable to run an Analytical Standard of 2,4,5-T and/or 2,4-D with each set of samples. These are available from the Sample Coordinator, Ag-Organic Department, 9001 Building, The Dow Chemical Company, Midland, MI 48640.

(b) Rapid cooling of solution will cause a thick crust of biphenyl precipitate to form which is difficult to break up. Also, possible occlusion of chloride in biphenyl crust can occur if solution is allowed to cool rapidly. Slow cooling causes a finer precipitate of biphenyl to form.

(c) Presence of bromide and chloride in some samples will be indicated by two breaks in the titration curve, with the bromide end-point occurring first.

D. Acid Equivalent

9. Principle

The acid equivalent is calculated from the assay as determined in Section 6.

10. Calculation

238.2 = weighted average molecular weight of 2,4,5-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid.

374.9 = weighted average molecular weight of 2,4,5-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid, propylene glycol butyl ether esters (as obtained in Section 6)
 $\times 238.2 = \%2,4,5\text{-T and } 2,4\text{-D acid equivalent.}$

374.9

Or

$\%2,4,5\text{-trichlorophenoxyacetic acid and } 2,4\text{-dichlorophenoxyacetic acid propylene glycol butyl ether esters} \times 0.6354 = \% \text{Total } (2,4,5\text{-T acid } 2,4\text{-D}), \text{ acid equivalent based on weighted averages.}$

E. Inert Ingredients

11. Calculation

$\% \text{Inert Ingredients} = 100 - \% \text{ active ingredient}$

F. Pounds 2,4,5-T Acid Equivalent per Gallon (US)

12. Principle

The specific gravity at 20/20°C (69/69°F) is determined and the pounds of 2,4,5-T and 2,4,-D acid equivalent per gallon is calculated.

13. Procedure

Determine the apparent specific gravity by weighing a known volume of sample in a pycnometer at a temperature $20.0 \pm 0.05^\circ\text{C}$ (68°F). The details of the method are given in "Method C" of the standard method of test for "Specific Gravity of Industrial Aromatic Hydrocarbons and Related Materials," ASTM Designation D 391-59, published by American Society for Testing and Materials, 1916 Race St., Philadelphia, Pennsylvania 19103.

14. Calculation

Total pounds of 2,4,5-T and 2,4-D acid equivalent/gallon (US)
= $\frac{\% \text{ total acid equivalent}}{100} \times 8.33 \times \text{sp. gr. } 20/20^{\circ}\text{C.}$

G. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

15. Principle

The neutral components including any TCDD in the sample are separated from the matrix. This is accomplished by silica gel column chromatography. The residue is concentrated and a portion examined by gas chromatography-mass spectrometry for species which have ions of the same mass, number of chlorine atoms, and retention time as TCDD.

16. Interferences

No substances are encountered to an extent sufficient to cause interference down to approximately 10 ppb.

17. Specificity and Selectivity

The gas chromatography-mass spectrometry (GC-MS) technique is extremely sensitive and specific for the determination of TCDD. In order to assign a signal to TCDD three things must occur simultaneously: 1) the retention time must be correct (the peak maximum is reproducible to within 0.1 min.); 2) all three m/e 320, 322 and 324 must respond and 3); they must respond in the proper ratio.

18. Safety Precautions

TCDD in amounts handled in the laboratory can produce chloracne if allowed to contact the skin. Extreme safety precautions should be exercised in the preparation and disposal of standard material and solutions. It is advisable to read "Summary of Safe Handling of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Laboratory," available from the Sample Coordinator, Ag-Organics Department, 9003 Building The Dow Chemical Company, Midland, MI 48640.

Handle all solvents in a fume hood and avoid breathing vapors.

19. Apparatus

(a) Gas chromatograph-mass spectrometer: LKB-9000S (LKB Productor, Bromma, Sweden) or equivalent, with multiple ion detector.

(b) Gas chromatographic column for LKB: 1 m x 3 mm i.d. (glass) packed with 3% OV-3 on 30/100 Gas Chrom. Z.

(c) 50 cm x 11 mm i.d. glass chromatography columns with 250 ml reservoir.

20. Reagents

(a) Benzene: ACS reagent grade; available from Burdick & Jackson Laboratories, Inc., 1953 S. Harvey Street, Muskegon, MI 49442.

(b) Hexane: ACS reagent grade; available from Burdick & Jackson Laboratories, Inc., 1953 S. Harvey Street, Muskegon, MI 49442.

(c) Silica Gel: High purity 100-200 mesh, Davidson 923 Brand, available from Curtin Scientific, Midland, MI 48640.

(d) 20% benzene in hexane: Add 200 ml benzene to 800 ml hexane and mix well.

(e) Pure TCDD: available from the Sample Coordinator, Ag-Organics Department, 9008 Building, The Dow Chemical Company, Midland, MI 48640.

21. Calibration

(a) Standard TCDD: Weigh 1-2 mg to the nearest 0.01 mg in a small weighing cup. Transfer to a 10-ml volumetric flask and dilute to volume with benzene. Prepare lower concentrations by dilution. Accurately make concentrations of approximately 2 µg/ml to be used for tuning and 0.2 µg/ml to be used for standard calibration of the GC-MS.

(b) Inject 5 µl of the concentrated standard (2 µg/ml) in the GC-MS. As the TCDD elutes, fine tune the magnet and accelerating voltage alternator control to maximize the response at m/e 320, 322 and 324.

(c) Inject 3 µl of the diluted standard (0.2 µg/ml) in the GC-MS and record the response. Measure the ion intensities at the chromatographic peak maximum using a tangent baseline.

22. Operating Conditions

(a) Column: (glass) 1 m x 3 mm i.d. 3% OV-3 on 80/100 Gas Chrom. Z.

(b) Temperatures:

Column	240°C
Flash Heater	250°C
Separator	290°C
Ion Source	270°C

(c) Flow Rate: 40 cc/min. helium as read on rotameter.

(d) Trap Current: 60 µa

(e) Accelerating Voltage: 3.5 KV

(f) Electron Energy: 70 ev.

(g) Multiplier: 4.9

(h) Masses to be monitored: m/e 320, 322 and 324.

(i) Oscillograph chart speed: 1 cm/min.

23. Procedure

(a) Weigh a 5.00 g sample of ESTERON Brush Killer into a 30-ml beaker and dissolve it with 15 ml of hexane.

(b) Prepare a silica gel column in a glass tube (50 cm long x 16 mm i.d. with a 250 ml reservoir) with 50 g of silica gel equilibrated with hexane.

(c) Quantitatively transfer the sample onto the column with three 5-ml portions of hexane. Add hexane to the column as necessary to maintain liquid above the silica bed, until 120 ml are collected. This eluate is discarded.

(d) Add 120 ml of 20% benzene in hexane to the column and collect the eluate in a 150-ml beaker.

(e) Add the column eluate in portions to a 5-dram vial (25 ml) and evaporate until all volatile solvents are gone. (Some heavy oily material may remain.)

(f) Add exactly 0.5 ml of benzene to the vial, cap securely with a Polyseal cap and rotate gently to wet all interior surfaces.

(g) Inject a 3 µl portion into the GC-MS and monitor the three molecular ions at m/e 320, 322 and 324 by means of the multiple-ion detector. Measure all three ion intensities at the chromatographic peak maximum using a tangent baseline.

(h) With 1-ml syringe measure the volume of liquid in the vial.

24. Calculations

(a) The concentration of TCDD present is calculated from

$$C = \frac{a_n}{b_n} \times \frac{D_a}{D_b} \times \frac{1}{E} \times S$$

where:

C is the concentration of TCDD in the sample (ppm)

a_n = ion intensity as recorded in Section 23g for m/e = 320 (sample)

b_n = ion intensity as recorded in Section 21c for m/e = 320 (standard)

D_a = Oscillograph attenuation range for largest on-scale ion intensity for sample

D_b = Oscillograph attenuation range for largest ion intensity for standard

E = ratio of sample weight in grams to final solution volume (usually less than 10 ml)

S = concentration of the standard in ug/ml

(b) The above calculation is repeated for $m/e = 322$ and for $m/e = 324$. The values should agree to $\pm 15\%$. If they do not, the value recorded is the lowest concentration calculated.

25. Recovery and Precision

The precision of this procedure is within 20% of the amount present. This is the acceptable range at this level.

The detection limit is 0.01 ppm, therefore, it meets the dioxin specification of less than 0.1 ppm based on the total acid equivalent.

* * * * *

The analytical procedures given herein have been adapted from literature sources or developed upon the basis of experimental data believed to be reliable. In the hands of a qualified analyst, they are expected to yield results of sufficient accuracy for their intended purposes, but recipients are cautioned to confirm the suitability of the methods by appropriate tests. Recipients are also cautioned that The Dow Chemical Company makes no representation or warranty that the practice of the method described herein does not infringe third party patents. Anyone wishing to reproduce or publish the materials in whole or in part should request written permission from The Dow Chemical Company.

APR 2 1980

Exhibit F

Exhibit F has been filed with the second portion of Mr. Thomas' testimony which is filed Under Seal for use in camera.

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MAR 2 1980

CURRICULUM VITAE

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MAR 2 1986

Curriculum Vitae

RONALD F. THOMAS

Date of Birth: February 14, 1934

Place of Birth: Baltimore, Md.

Education: B.S., Washington College
Chestertown, Md. 1956

Former Employment: Federal Food and Drug Administration
1956 - 1961

U.S. Department of Agriculture
1961 - 1970

U.S. Environmental Protection Agency
1970 to Present

Present Position: Supervisor, Residue Support Unit
CBIB, BFSD, OPP, EPA

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Attachment

Publications List

1. "Microcoulometric Determination of S,S,S-Tributyl Phosphorotriithioate in Cottonseed." R.F. Thomas, T.H. Harris. JAFC Vol. 13, No. 6, 1965.
2. "Residues in Fat of Steers Sprayed with a Dieldrin Contaminated Fly Control Product." Medley, Younger, and Thomas. Bulletin of Env. Cont. & Tox., Vol. 12, No. 5, 1974.
3. "Residues of Lindane on Food and Utensils from a Thermal Lindane Vaporizer." R.F. Thomas. JAOAC Vol. 56, No. 2, 1973.
4. "Effects on Eggs of Applications to Poultry of Pesticide Formulations Contaminated with Chlorinated Hydrocarbons." R.F. Thomas, J.G. Medley, JAOAC Vol. 54, No. 3, May 1971.
5. "Survey of Polychlorodibenzo-p-dioxin Content in Selected Pesticides." E. A. Woolson, R.F. Thomas, P.D.J. Ensor. JAFC Vol. 20, No. 2, 1972.
6. "Polychlorinated Biphenyl (PCB) Degrading Bacteria in Estuarine and Marine Environment." Sayler, Thomas, Colwell. Submitted to Estuarine & Coastal Marine Science.
7. "Analytical Properties of Polychlorodibenzo-p-dioxins." Woolson & Thomas. "Methods in Residue Analysis." Proceedings of the Second International IUPAC Congress on Pesticide Chemistry.
8. "Disposable Inner Liners - Tests for Permeation." Presented at the National Conference on Pesticide Containers, 1972.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

BEFORE THE ADMINISTRATOR

In re:

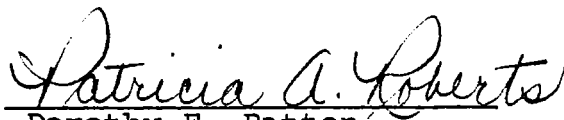
The Dow Chemical Company, et al.

) FIFRA Docket Nos. 415, et al.
)
)
)

PROPOSED CORRECTIONS TO TRANSCRIPT
OF TESTIMONY OF MR. RONALD THOMAS

Respondent hereby submits its proposed corrections to the transcript of the testimony of Mr. Ronald Thomas. The proposed corrections are listed in the attached Appendices. Appendix A contains the corrections for the public session of Mr. Thomas' testimony; Appendix B contains the corrections for the in camera session. Rule 13 of the Rules of Procedure, December 18, 1979, provide that each party shall submit lists of proposed corrections to the transcript of testimony within four weeks of receipt of each volume of transcripts. We respectfully request that these corrections be accepted notwithstanding the fact that they are being submitted beyond the specified time period.

Respectfully submitted,



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July 16, 1980

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APPENDIX B

Mr. Ronald Thomas
(in camera session)

Page	Line	Correction
6	14	"trade" should read "trace".
7	8	"introduction" should read "production".
15	1	"base" should read "Bates".
19	24	The line should read "It would <u>not</u> lead".
26	6	The line should read "supplied to Dow by".
28	21	Delete "no".