APPENDIX H. CHEMICAL CHARACTERIZATION

I. Identity and Purity Determinations of Decabromodiphenyl Oxide Performed by the Analytical Chemistry Laboratory

	Determined	Literature Values
A. Lot No. 08287-2		
1. Physical properties		
a. Appearance:	Fine, off-white, microcrystalline powder	
b. Melting point:	298.4°-302.5° C 299.0°-302.0° C (Dupont 900 DTA)	290°-306° C (Norris et al., 1973; Kociba et al., 1975; AIHA, 1981)
2. Spectral data		
a. Infrared		
Instrument:	Beckman IR-12	
Phase:	1% potassium bromide pellet	
Results:	See Figure 5	No literature reference found. Consistent with structure.
b. Ultraviolet/visible		
Instrument:	Cary 118	
Solvent:	*p*-Dioxane	
Results:	There was no absorbance between 800-350 nm.	No literature reference found. Consistent with structure.

λ_{max} (nm)	$\varepsilon \times 10^{-3}$
306 (shoulder)	2.480 ± 0.007
277	5.593 ± 0.033

3. Water analysis (Karl Fischer): 0.04% ± 0.01 (δ)%

4. Elemental analysis

Element	C	Br
Theory (T)	15.02	83.31
Determined (D)	15.02 15.20	83.39 83.26
Percent D/T	100.60	100.02

FIGURE 5. INFRARED ABSORPTION SPECTRUM OF DECABROMODIPHENYL OXIDE
(LOT NO. 08287-2)

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5. **Chromatographic analyses**

   a. **Thin-layer chromatography**

   Plates: System I--Whatman KC_{18} reversed-phase with fluorescent indicator
   System II--Silica Gel 60 F-254
   Reference standard: 2,4,6-Tribromophenol, 40 µg (10 µg/µl in toluene)
   Amount spotted: 100 and 300 µg (10 µg/µl in toluene)
   Visualization: Ultraviolet, 254 nm

   System 1: Methanol (100%)

   R_f: 0.47 (major)
   R_{st}: 0.56

   System 2: Hexane (100%)

   R_f: 0.68 (major)
   R_{st}: 6.48

   b. **High-performance liquid chromatography**

   Instrument: Waters Programmable Component System
   Column: µBondapak C_{18}, 300 mm × 4 mm, ID
   Detector: Ultraviolet, 254 nm
   Flow rate: 1 ml/min
   Sample injected: 10 µl of 0.5 mg/ml in tetrahydrofuran
   Solvent program: Methanol (Fischer HPLC):water (95:5)
   Results: Major peak, one minor peak, and one shoulder on the minor peak

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	9.1	0.76 }	1.31
2	9.8	0.82 }	
3	12.0	1.00	100

Note: Reducing the solvent ratio from 95% methanol to 70% methanol increased the retention time of the major peak to 18 minutes. No additional impurity peaks were detected.

   c. **Gas chromatography:** Gas chromatography was attempted with an SP-2100 column. The compound would not elute even at high isothermal temperatures.

6. **Conclusions:** The results of elemental analysis for carbon and bromine were consistent with the theoretical values. Thin-layer chromatography by two systems indicated a major spot only. High-performance liquid chromatography indicated two impurities before the major peak. The smaller of these impurities was detected as a shoulder on the larger impurity peak. The combined area of the two impurities was 1.3% of the major peak area. The infrared and the ultraviolet/visible spectra were consistent with the structure.

	Determined	Literature values
B. Lot no. D12478		
1. Physical properties		
Appearance:	White, microcrystalline powder	
2. Spectral data		
a. Infrared		
Instrument:	Beckman IR-12	
Phase:	1% in potassium bromide pellet	
Results:	See Figure 6	No literature reference found. Consistent with structure.
b. Ultraviolet/visible		
Instrument:	Cary 118	
Solvent:	1,4-dioxane	
Results:	No maximum from 800 to 350 nm, but a gradual increase in absorbance toward 350 nm.	No literature reference found. Consistent with structure.

λ_{max} (nm)	$\varepsilon \times 10^{-3}$
306	2.314 ± 0.02(δ)
277	5.313 ± 0.09(δ)

3. Water analysis (Karl Fischer): <0.05%

4. Elemental analysis

Element	C	Br
Theory (T)	15.02	83.31
Determined (D)	14.79 14.75	83.55 83.74
Percent D/T	98.34	100.40

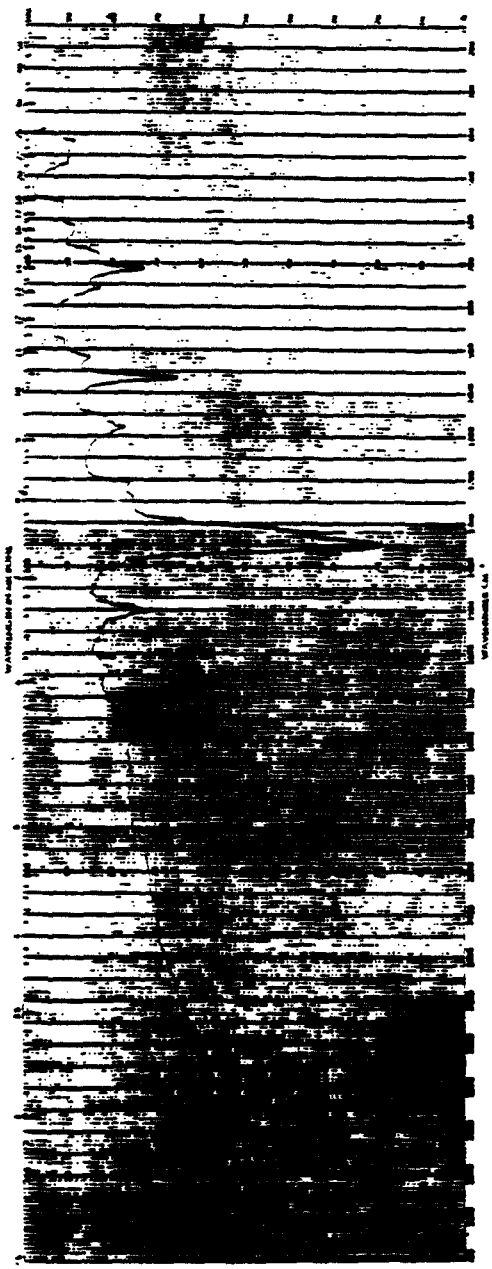

FIGURE 6. INFRARED ABSORPTION SPECTRUM OF DECABROMODIPHENYL OXIDE
(LOT NO. D12478)

5. **Chromatographic analysis**

   a. **Thin-layer chromatography**

   Plates: Silica Gel 60 F-254, 0.25 mm layer
   Reference standard: 2,4,6-Tribromophenol (10 mg/ml in toluene)
   Amount spotted: 10 and 30 µg (1 mg/ml in toluene)
   Visualization: Ultraviolet (254 nm)
   Solvent system: Hexane (100%)

   Results

   R_f: 0.35 (major)
   R_{st}: 9.6

   b. **High-performance liquid chromatography (HPLC)**

   Instrument system
   Pump(s): Waters 6000A
   Programmer: Waters 660
   Detector: Waters 440
   Injector: Waters U6K
   Column: µBondapak C_{18}, 300 mm × 3.9 mm, ID
   Detection: Ultraviolet, 254 nm
   Guard column: CO:PELL ODS, 72 mm × 2.3 mm, ID
   Solvent system: Acetonitrile:water (90:10)
   Flow rate: 1 ml/min
   Samples injected: 10 µl solution of 0.4 mg decabromodiphenyl oxide per 1 ml tetrahydrofuran

   Results: Major peak and two impurities before the major peak with relative areas of 1.5% and 1.3%. A system using 100% acetonitrile, isocratic, revealed no additional impurities, and the retention time of the major peak was 7.5 minutes.

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	13.5	0.81	1.5
2	14.5	0.87	1.3
3	16.75	1.00	100

6. **Conclusions:** The result of elemental analysis for bromine was in agreement with the theoretical value, whereas that for carbon was slightly low. Thin-layer chromatography indicated a major spot only. High-performance liquid chromatography indicated two impurities before the major peak with areas totaling 2.8% of the area of the major peak. The two impurities had areas 1.5% and 1.3% of the major peak area. This HPLC analysis compares with two impurities before the major peak with a combined area of 1.3% of the major peak for lot no. 08287-2. The infrared and ultraviolet/visible spectra were consistent with the structure of decabromodiphenyl oxide and with the spectra for lot no. 08287-2.

	Determined	Literature Values
C. Lot no. MM04080-1		
1. Appearance:	White, microcrystalline powder	
2. Spectral data		
a. Infrared		
Instrument:	Beckman IR-12	
Phase:	1% potassium bromide pellet	
Results:	See Figure 7	No literature reference found. Consistent with structure.
b. Ultraviolet/visible		
Instrument:	Cary 118	
Solvent:	*p*-Dioxane	
Results:	There was no absorbance between 800-350 nm at a concentration of 0.13 mg/ml	No literature reference found. Consistent with structure.

λ_{max} (nm)	$\varepsilon \times 10^{-3}$
306	2.46 ± 0.03
(a) 296	2.77 ± 0.02
276	5.57 ± 0.08

(a) Observed in spectrum of lot no. D12478 but not calculated or reported

3. Water analysis (Karl Fischer): <0.1%

4. Elemental analysis

Element	C	Br
Theory (T)	15.02	83.31
Determined (D)	14.52 14.34	82.73 82.79
Percent D/T	96.07	99.34

FIGURE 7. INFRARED ABSORPTION SPECTRUM OF DECABROMODIPHENYL OXIDE
(LOT NO. MM04080-1)

5. **Chromatographic analyses**

a. **Thin-layer chromatography**

Plates: System I--Silica Gel 60 F-254, 0.25 mm layer; System II--Whatman KC_{18} reversed-phase with fluorescent indicator, 0.25 mm layer
Reference standard: 2,4,6-Tribromophenol, 10 µg (1 µl of a 10 µg/µl solution in toluene)
Amount spotted: 1, 10, and 30 µg (1, 10, and 30 µl of a 1 µg/µl solution in toluene)
Visualization: Ultraviolet, 254 nm

System 1: Hexane (100%)
System 2: Methanol (100%)

Results

Spot Intensity	R_f	R_{st}
System 1		
Major	0.52	5.2
Trace	origin	--
Reference	0.16	--
System 2		
Major	0.21	0.29
Trace	origin	--
Reference	0.72	--

b. **High-performance liquid chromatography**

Instrument system
Pump: Varian 5020 liquid chromatograph
Detector: Waters 440
Injector: Waters U6K
Column: µBondapak C_{18}, 300 mm × 3.9 mm, ID
Guard column: Whatman CO:PELL ODS, 72 × 2.3 mm, ID
Detector: Ultraviolet, 254 nm
Solvent system: Water:acetonitrile (23:77), isocratic
Flow rate: 1 ml/min
Sample injected: 10 µl of 0.5 mg/ml in tetrahydrofuran, filtered

Results: Major peak and three impurities before the major peak with relative areas of 0.23%, 2.0%, and 2.3% that of the major peak. Another impurity before the major peak had a relative area of less than 0.1% that of the major peak. No additional impurities were observed when the sample solution was injected at 100%, 90%, and 80% acetonitrile. A visual comparison of profiles between lot no. D12478 and lot no. MM04080-1 indicated the same impurities in both samples but at lower levels in lot no. D12478.

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	13.1	0.68	0.23
2	14.8	0.76	2.0
3	16.0	0.82	2.3
4	19.4	1.00	100

6. **Conclusions:** The results of the elemental analysis for carbon and bromine were low when compared with the theoretical values. Water content was found to be less than 0.1% by Karl Fischer analysis, compared with less than 0.05% for lot no. D12478. Thin-layer chromatography indicated a major spot and a trace impurity on each of two systems. High-performance liquid chromatography indicated three impurities before the major peak with relative areas that were 0.23%, 2.0%, and 2.3%. One additional impurity before the major peak had a relative area of less than 0.1% that of the major peak. Major peak comparison of lot nos. D12478 and MM04080-1 indicated that lot no. MM04080-1 was 95.2% ± 1.0(δ)% when normalized to lot no. D12478. The infrared and ultraviolet/visible spectra were consistent with the structure of decabromodiphenyl oxide and with the spectra for lot no. D12478.

	Determined	Literature Values
D. Lot no. MM811102-3-1		
1. Appearance:	White, crystalline powder	
2. Spectral data		
a. Infrared		
Instrument:	Perkin-Elmer 283	
Phase:	2% in potassium bromide pellet	
Results:	See Figure 8	No literature reference found. Consistent with structure.
b. Ultraviolet/visible		
Instrument:	Cary 219	
Solvent:	*p*-Dioxane	
Results:	No absorbance observed from 800 to 350 nm at a concentration of 0.12 mg/ml	No literature reference found; spectra consistent with structure.

λ_{max} (nm)	$\varepsilon \times 10^{-3}$
306	2.46 ± 0.01(δ)
(a) 296 (shoulder)	2.74 ± 0.01(δ)
276 (shoulder)	5.55 ± 0.02(δ)

(a) Observed in spectrum of lot no. D12478 but not calculated or reported

3. Water analysis (Karl Fischer): 0.010% ± 0.001(δ)%

4. Elemental analysis

Element	C	Br
Theory (T)	15.02	83.31
Determined (D)	14.87 14.83	83.30 83.42
Percent D/T	98.87	100.06

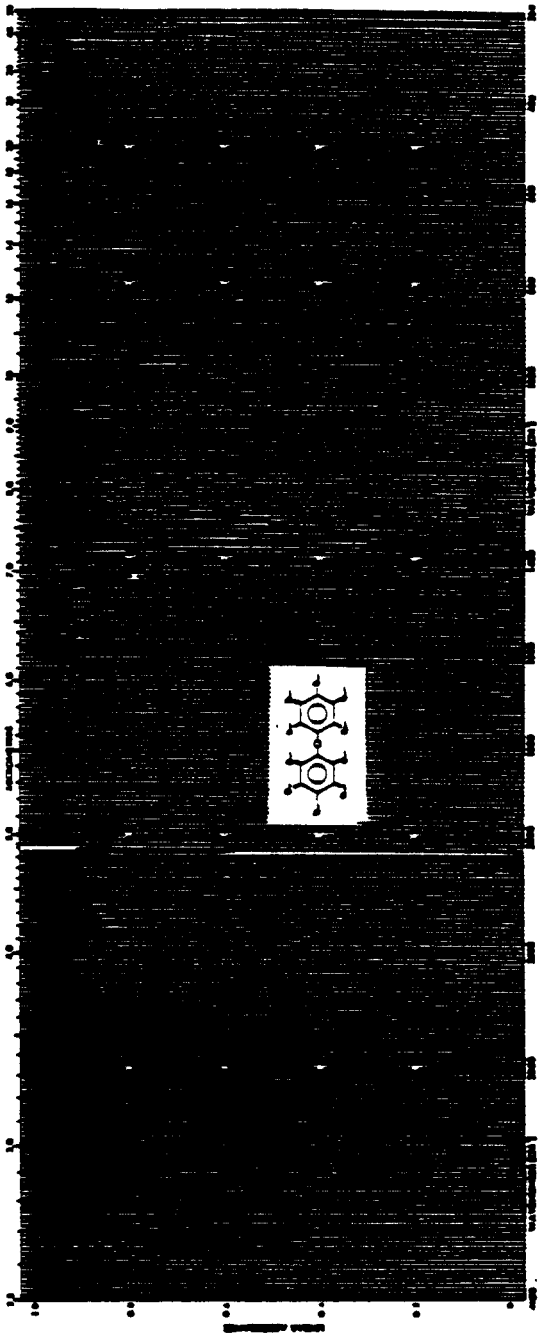

FIGURE 8. INFRARED ABSORPTION SPECTRUM OF DECABROMODIPHENYL OXIDE
(LOT NO. MM811102-3-1)

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5. **Chromatographic analysis**

a. **Thin-layer chromatography**

Plates: System 1--Silica Gel 60 F-254, 0.25 mm layer; System 2--Whatman KC_{18} reversed-phase with fluorescent indicator, 0.20 mm layer
Reference standard: 2,4,6-Tribromophenol, 10 µg (1 µl of a 10 µg/µl solution in toluene)
Amount spotted: 1, 10, and 30 µg (1, 10, and 30 µl of a 1 µg/µl solution in toluene)
Visualization: Ultraviolet (254 nm)

System 1: Hexanes (100%)
System 2: Methanol (100%)

Results

Spot Intensity	R_f	R_{st}
System 1		
Major	0.39	6.5
Reference	0.06	--
System 2		
Major	0.23	0.32
Minor	origin	--
Reference	0.72	--

b. **High-performance liquid chromatography**

(1) **Impurity profile**

Instrument system
Pump(s): Waters 6000A
Programmer: Waters 660
Detector: Waters 440
Injector: Rheodyne 7125 with 10 µl loop
Column: µBondapak C_{18}, 300 mm × 3.9 mm, ID
Detection: Ultraviolet, 254nm
Guard column: Whatman CO:PELL ODS, 72 mm × 2.3 mm, ID
Solvent system: Water:acetonitrile (12:88), isocratic
Flow rate: 1 ml/min
Samples injected: 0.45 mg/ml decabromodiphenyl oxide in tetrahydrofuran, filtered
Volume injected: 10 µl

Results: Major peak and three impurities before the major peak with relative areas greater than or equal to 0.1% of the major peak area. Two of the impurities had areas 1.3% that of the major peak. A system using 100% and 90% acetonitrile showed no additional peaks.

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (a) (percent of major peak)
1	15.2	0.70	0.1
2	17.0	0.78	1.3
3	18.4	0.85	1.3
4	21.7	1.00	100

(a) Detector response is very dependent on the absorbance of a substance at the detection wavelength used. The values reported are absolute areas expressed as percentages of the area of the major peak and do not take into account the different molar absorptivity values of the compound and its impurities. Therefore, the areas reported do not necessarily reflect the actual weight percentages of the impurities in the sample.

(2) **Major peak lot comparison:** Lot no. MM811102-3-1 and lot no. D12478 were analyzed for content of decabromodiphenyl oxide by high-performance liquid chromatography. Major peak areas were compared with internal standard peak areas, and the percent of decabromodiphenyl oxide in lot MM811102-3-1 was calculated relative to lot no. D12478. The instrument system described in I.D.5.b.(1) was used with the following changes.

Integrator: Varian CDS111L
Guard column: None
Solvent system: Water:acetonitrile (6:94), isocratic
Samples injected: Accurately weighed solutions containing approximately 0.24 mg/ml of decabromodiphenyl oxide and 8.86×10^{-3} mg/ml of the internal standard, anthracene, in tetrahydrofuran. The solutions were filtered into amber septum vials.
Retention times: Anthracene--4.0 min; decabromodiphenyl oxide--10.5 min

Results

Sample	Percent Decabromodiphenyl Oxide Compared with Lot No. D12478
Lot no. D12478	100.0 ± 0.1(δ)
Lot no. MM811102-3-1	100.5 ± 0.2(δ)

6. **Conclusions:** The results of the elemental analysis for carbon and bromine were consistent with the theoretical values. Water content (Karl Fischer titration) was 0.010% ± 0.001(δ)% compared with less than 0.05% for lot no. D12478. Thin-layer chromatography indicated a major spot and a minor impurity by one system and a major spot only by a second system. High-performance liquid chromatography indicated three impurities with areas totaling 2.7% that of the major peak. The three impurities before the major peak had relative areas of 0.1%, 1.3%, and 1.3% that of the major peak. A similar impurity profile was detected for lot no. D12478: three impurities before the major peak with relative areas of 0.1%, 1.6%, and 1.3% that of the major peak. Major peak comparison of lot no. D12478 and lot no. MM811102-3-1 indicated that lot no. MM811102-3-1 was 100.5% ± 2.0(δ)% pure when normalized to lot no. D12478. The infrared and ultraviolet/visible spectra were consistent with the spectra for lot no. D12478 and with the structure of decabromodiphenyl oxide.

II. Chemical Stability Study Performed by the Analytical Chemistry Laboratory

A. Sample storage: The decabromodiphenyl oxide samples were stored at −20°, 5°, 25°, and 60° C for 2 weeks in glass tubes with Teflon®-lined lids.

B. Analytical method: The high-performance liquid chromatographic system used is described below. The major peak areas of the 5°, 25°, and 60° C samples were compared with the average of the major peak areas for the −20° C sample injections, which served as the standard. Each area was adjusted for the weight of the sample.

Instrument: Waters Programmable Component System
Column: μBondapak C_{18}, 300 × 4 mm, ID
Detector: Ultraviolet, 254 nm
Solvent: Acetonitrile (100%), 1 ml/min
Retention time: 5.9 min

C. Results

Storage Temperature	Percent Purity
−20° C	100.0 ± 0.9
5° C	99.2 ± 0.9
25° C	100.3 ± 0.9
60° C	99.6 ± 0.9

D. Conclusion: Decabromodiphenyl oxide is stable as the bulk chemical for 2 weeks at temperatures up to 60° C.

III. Chemical Stability Study at the Study Laboratory

A. Identity determination by infrared spectroscopy

Instrument: Perkin-Elmer 597
Phase: 1% Potassium bromide pellet

B. Purity determination

1. **Thin-layer chromatography:** Solutions of decabromodiphenyl oxide were prepared and processed simultaneously with an internal standard solution of 2,4,6-tribromophenol.

   Plates: Silica gel 60, F-254 nm, 0.25-mm layer
   Solvent system: 100% Hexane at ambient temperature
   Visualization: Ultraviolet lamp at 254 nm
   Reference standard: 2,4,6-tribromophenol (10 mg/ml in toluene)
   Sample solutions: Decabromodiphenyl oxide (1 mg/ml in toluene)
   Amount spotted: 20 µl of each

2. **High-performance liquid chromatography**

   Instrument: Waters HPLC model 440 with ultraviolet detector at 254 nm
   Column: µBondapak C_{18} (3.9 mm × 300 mm) with CO:PELL ODS guard column
   Mobile phase: Water:acetonitrile (10:90), isocratic, 1.0 ml/min
   Chart speed: 0.5 in/min
   Attenuation: 0.1
   Standard: Solutions of 0.4 mg/ml decabromodiphenyl oxide in tetrahydrofuran
   Injection volume: 10 µl

C. Results

1. **Thin-layer chromatography**

Date of Analysis	Lot No.	Reference R_f	Reference R_{st}	Bulk R_f	Bulk R_{st}
04/02/79	08287-2	0.69	5.31	0.66	6.25
04/13/79	08287-2	0.58	5.80	0.57	5.70
08/13/79	D12478	0.70	7.00	0.70	7.00
12/09/79	D12478	0.60	5.00	0.60	5.00

2. **High-performance liquid chromatography**

Date of Analysis	Lot No.	Purity (percent) Reference	Bulk
02/11/80	08287-2	97.24	97.09
02/11/80	D12478	97.24	97.28
05/09/80	MM04080-1	97.75	96.67
09/24/80	MM04080-1	96.47	96.47
01/07/81	MM04080-1	95.36	95.34
05/12/81	MM04080-1	95.56	95.65
09/25/81	MM04080-1	95.99	95.56
01/27/82	MM04080-1	96.22	95.60
03/18/82	MM811102-3-1	95.50	97.30
10/18/82	MM811102-3-1	97.75	97.70

High-performance liquid chromatography replaced thin-layer chromatography as the purity analytical method because the purity results obtained from the thin-layer chromatography analyses were not consistent.

D. **Conclusion:** No notable degradation of the test material occurred during the studies.

IV. Isolation and Identification of Impurities in Decabromodiphenyl Oxide

A. Introduction: The purpose of this analysis was to isolate and identify two impurities previously observed in the lot no. MM811102-3-1 of decabromodiphenyl oxide by high-performance liquid chromatography (HPLC). The two impurity peak areas were 1.3% and 1.6% relative to the major peak in the previous HPLC analysis.

The HPLC method developed by the Dow Chemical USA was used without modification for the impurity profile analysis and subsequent isolation of the major impurities in this lot of decabromodiphenyl oxide. These HPLC fractions were then analyzed by direct inlet mass spectrometry to identify the two impurities.

B. High-performance liquid chromatography

1. **Sample preparation:** A solution (approximately 0.5 mg/ml) of decabromodiphenyl oxide was prepared in tetrahydrofuran and filtered for HPLC analysis.

2. **Instrumental system**

Solvent delivery system: Varian 5020 HPLC
Detector: Tracor 970A
Injector: Waters WISP 710B
Electronic integration: Nelson 4400 Data System
Detection: Ultraviolet, 220 nm (254 nm was used in the previous analysis)
Column: Dupont Zorbax ODS, 250 × 4.6 mm ID
Guard column: Whatman CO:PELL ODS, 23 × 3.9 mm ID
Mobile phase: 100% Acetonitrile, 1.2 ml/min
Volume injected: 10 µl
Column temperature: 40° C

3. **Results:** A major peak and three impurity peaks, with areas greater than 0.1% relative to the major peak (Figure 9), were observed. All the impurity peaks eluted before the major peak.

Peak No.	Retention Time (min)	Retention Time (relative to major peak)	Area (a) (percent of major peak)
1	7.7	0.73	0.3
2	8.2	0.78	3.7
3	9.3	0.89	1.7
4	10.5	1.00	100

(a) Detector response is very dependent on the absorbance of a substance at the detection wavelength used. The values reported are absolute areas expressed as percentages of the area of the major peak and do not take into account the different ε values of the compound and its impurities. Therefore, the areas reported do not necessarily reflect the actual weight percentages of the impurities in the sample.

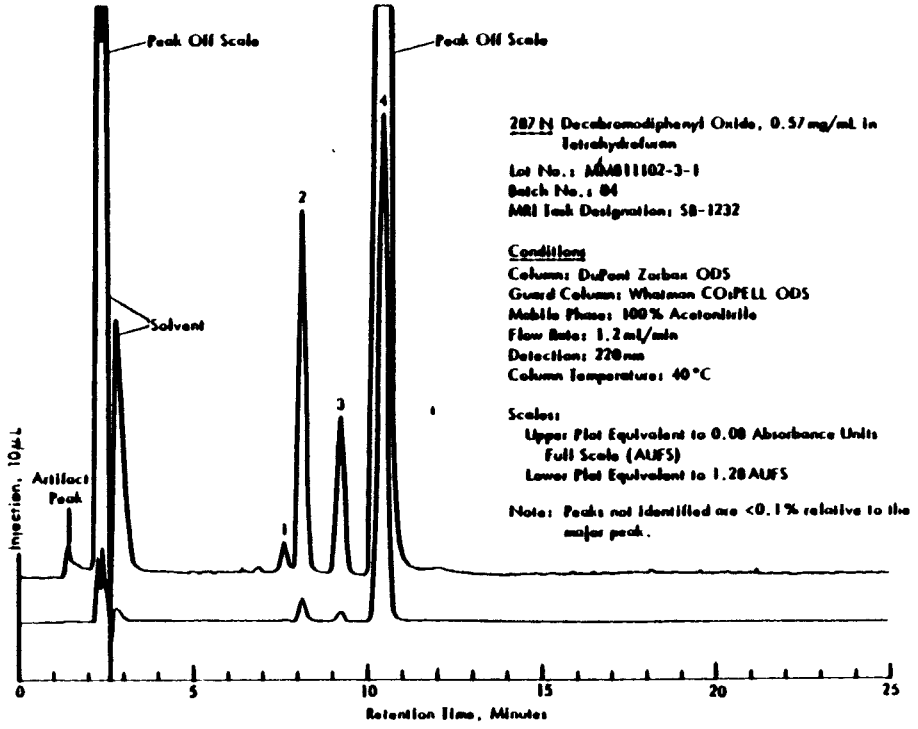

FIGURE 9. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PROFILE OF DECABROMODIPHENYL OXIDE (LOT NO. MM811102-3-1)

C. **Ultraviolet/visible spectra of the major component and impurities**

1. **Sample preparation:** A 2.3 mg/ml solution of decabromodiphenyl oxide was prepared volumetrically in tetrahydrofuran. The solution was filtered for HPLC analysis.

2. **Instrument system:** Ultraviolet/visible spectra of the major peak and the impurity peaks were obtained with a Hewlett-Packard 1040A high-speed spectrophotometric HPLC detector. The HPLC conditions described above were used, with the following exceptions:

   Injector: Rheodyne 7125
   Detector: Hewlett-Packard 1040A
   Monitoring wavelength: 220 nm
   Lamp current: Low
   Scanning range: 190-600 nm
   Scanning step: 2 nm

3. **Results:** The spectra of decabromodiphenyl oxide and its major impurities are presented in Figure 10. The spectra are very similar, indicating that the two impurities are probably compounds that have structures closely related to the major component. The absorbance maxima of the three peaks are also quite near the detection wavelength used in the impurity profile analysis. The relative area percent values reported for the impurities in the impurity profile should therefore closely approximate their actual concentrations in the sample.

D. **Isolation of the major component and two impurities**

1. **Procedure:** A concentrated solution of decabromodiphenyl oxide was prepared in tetrahydrofuran and repeatedly injected into an HPLC system similar to that used for the impurity profile. The fractions containing the two largest impurities and the major peak were collected as they eluted from the analytical column. The fractions were immersed in a 50° C water bath and evaporated to dryness under a stream of purified nitrogen. The samples were then stored at −20° C before analysis by direct inlet mass spectrometry (Section IV.E.).

2. **Instrument system:** The instrumental parameters described in IV.B.2. were used with the following exceptions:

   Injector: Rheodyne 7125
   Samples injected: Solutions of 6.0 mg/ml decabromodiphenyl oxide in tetrahydrofuran, filtered

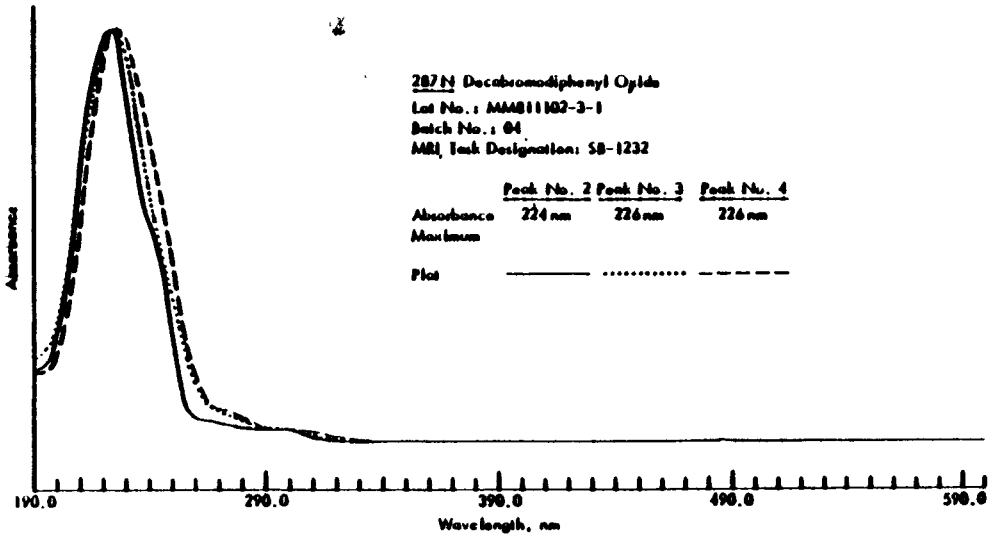

FIGURE 10. ULTRAVIOLET/VISIBLE SPECTRUM OF DECABROMODIPHENYL OXIDE AND TWO MAJOR IMPURITIES

E. Identification of impurities by mass spectrometry

1. **Sample preparation:** The three HPLC fractions were reconstituted in 50 µl of acetonitrile. Aliquots (1-5 µl) of the reconstituted samples were evaporated in a gold cup for direct inlet mass spectrophotometric analysis.

2. **Instrument system**

Instrument: Finnigan MAT 311-A mass spectrometer
Data processor: Incos 2400 Data System
Electron energy: 70 eV
Scan range: 50-1,075 amu
Scan rate: 7.00 sec/scan
Scan times: Up: 5.70 Top: 0.30
Down: 0.00 Bottom: 1.00
Electron multiplier voltage: −1800 V
Emission current: 1 mA
Resolution: 1,000
Accelerator voltage: 3000 V
Sample introduction: Direct inlet probe (gold cup)
Probe temperature program: 30°-450° C in 1,000 sec
Probe temperature at sampling point: Approximately 250° C

3. **Results**

Peak no. 4 (major component of decabromodiphenyl oxide): The mass list is presented in Table H1. The spectrum was found to be consistent with a literature reference mass spectrum of decabromodiphenyl oxide (EPA/NIH, 1980). An abundant molecular ion cluster (m/z 950-m/z 958) was observed with an isotopic ratio consistent with that for a molecule containing 10 bromine atoms. The fragmentation observed indicated several losses of Br and Br_2 from the molecular ion. A mass spectrum of the major component is presented in Figure 11.

Peak no. 2: The mass list obtained from peak no. 2 is presented in Table H2. This impurity was identified from the mass spectrum as an isomer of nonabromodiphenyl oxide ($C_{12}HOBr_9$). A molecular ion was observed at m/z 871 (nominal mass for ^{79}Br, 78.9183, was used for molecular weight calculations). The isotopic ratio for the molecular ion cluster was consistent with the theoretical isotopic pattern for a molecule containing nine bromine atoms. An initial loss of 80 amu (HBr) from the molecular ion was observed. Subsequent losses of 79 (Br) and 159 amu (Br_2) were repeatedly observed, yielding a fragmentation pathway analogous to that observed for the major component. The loss of 28 amu from the m/z 640 ion was observed at m/z 612, indicating the loss of CO which is characteristic of aromatic diphenylethers. A specific isomer of nonabromodiphenyl oxide was not identified. A mass spectrum of the impurity is presented in Figure 12.

TABLE H1. TABULATED MASS SPECTRUM FOR DECABROMODIPHENYL OXIDE (PEAK NO. 4)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
35.32	1.08	302.75	5.23
43.98	2.45	303.78	1.55
60.03	1.53	304.78	2.91
66.02	1.27	305.78	4.09
72.02	1.77	306.75	3.04
78.96	3.05	307.75	1.13
79.97	3.40	308.78	2.87
80.95	3.14	310.78	8.08
81.97	3.27	312.75	8.10
106.97	0.27	314.75	2.51
132.00	5.50	317.75	3.73
138.92	7.49	318.75	8.68
140.92	7.50	319.25	1.25
144.92	1.88	319.75	12.06
145.92	3.34	320.25	1.59
146.94	2.04	320.75	8.71
150.92	4.94	321.75	1.26
152.92	5.00	321.75	3.51
166.92	1.31	343.25	1.04
168.95	2.46	344.25	3.06
186.42	1.53	345.25	5.11
210.94	1.78	346.25	5.12
217.84	6.66	347.25	2.90
219.84	13.29	358.25	1.64
220.86	1.22	358.75	2.04
221.84	6.30	359.22	2.86
224.84	1.24	359.75	3.38
225.84	1.74	360.22	2.98
226.84	1.19	360.75	3.24
229.84	10.14	361.22	1.71
231.84	20.14	361.75	1.89
232.84	1.67	368.78	1.56
233.84	9.87	370.78	4.26
234.86	1.07	372.78	4.28
238.87	1.22	374.75	1.48
239.84	1.22	377.69	1.79
240.87	1.10	379.69	2.68
247.84	1.31	381.69	1.68
265.31	5.94	387.69	1.67
265.81	1.46	389.69	6.24
266.31	6.20	391.69	9.43
267.28	3.33	393.69	6.13
274.75	1.12	395.69	2.54
276.75	1.07	396.69	7.65
289.84	1.74	397.19	1.07
291.84	3.42	397.69	24.93
293.84	1.78	398.19	3.34
296.75	5.67	398.69	49.90
298.78	15.79	399.19	6.66
300.75	15.76	399.69	60.61

TABLE H1. TABULATED MASS SPECTRUM FOR DECABROMODIPHENYL OXIDE (PEAK NO. 4)
(Continued)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
400.19	8.10	715.44	1.14
400.69	47.96	716.44	2.53
401.19	6.41	717.44	3.49
401.69	23.00	718.44	4.64
402.19	3.27	719.44	5.62
402.69	6.57	720.44	4.62
403.69	1.93	721.44	5.63
405.69	4.03	722.44	3.02
407.69	5.61	723.44	3.22
409.69	3.65	724.44	1.15
438.16	1.30	725.44	1.10
439.16	2.09	791.37	1.62
440.16	2.15	793.37	12.50
441.16	1.29	794.37	1.63
454.62	1.59	795.37	41.89
456.62	6.29	796.37	5.64
458.62	11.77	797.37	82.89
460.62	11.47	798.37	11.11
462.62	5.48	799.37	100.00
464.62	1.10	800.37	13.39
470.62	1.20	801.37	78.40
472.62	1.15	802.37	10.69
477.69	1.80	803.37	38.71
479.69	2.64	804.37	4.96
481.69	1.67	805.37	11.29
486.62	1.21	806.37	1.48
488.62	1.38	807.37	1.26
528.62	3.88	875.31	1.02
530.62	8.00	876.31	1.15
531.62	1.02	877.31	2.38
532.62	7.75	878.31	1.89
534.62	3.79	879.31	3.38
558.62	1.61	880.31	1.86
560.62	1.42	881.31	3.55
609.56	1.51	882.31	1.43
611.56	1.71	883.31	2.09
613.56	1.57	951.19	2.54
635.56	4.23	953.25	11.58
637.56	10.68	955.19	31.23
638.56	1.49	957.12	55.13
639.50	14.13	958.25	6.76
640.50	2.14	959.19	60.51
641.50	10.51	960.25	7.80
642.56	1.42	961.25	48.97
643.50	4.22	962.25	6.43
686.44	1.11	963.25	27.66
688.44	3.28	964.25	3.89
690.50	5.03	965.25	10.28
692.50	4.88	966.87	3.65
694.50	3.09		

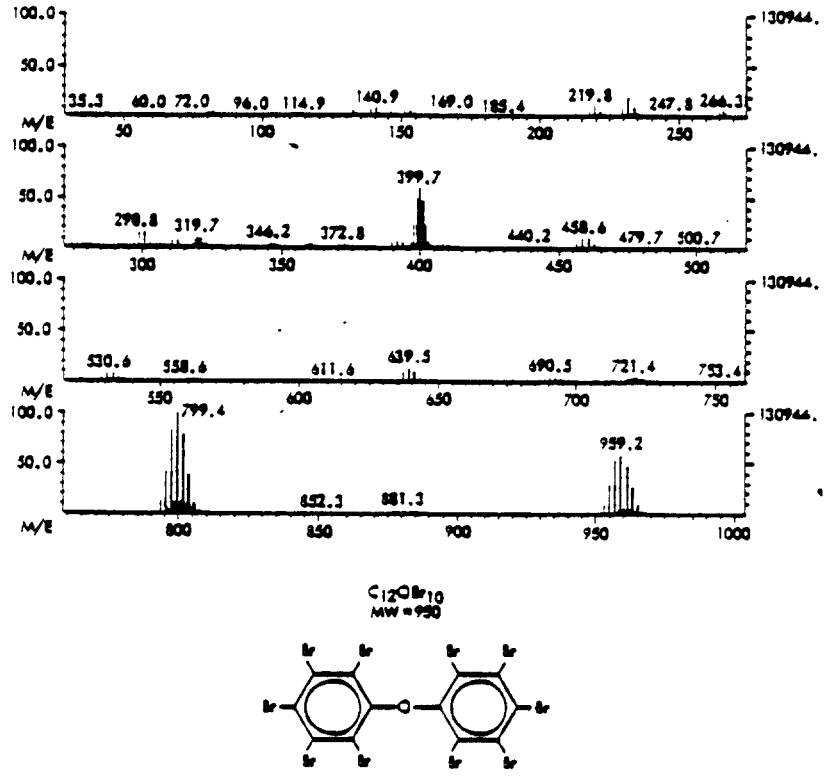

FIGURE 11. MASS SPECTRUM OF DECABROMODIPHENYL OXIDE (PEAK NO. 4)

TABLE H2. TABULATED MASS SPECTRUM FOR NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 2)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
35.26	1.94	87.09	3.42
35.31	4.05	88.09	1.77
35.36	2.54	89.07	1.86
35.40	6.84	90.96	1.46
35.45	9.07	91.10	1.52
36.88	2.27	92.95	1.52
37.93	7.61	93.11	1.25
38.98	13.20	95.12	3.81
40.00	28.07	96.03	13.34
41.02	61.34	96.12	1.63
42.02	3.84	97.02	15.24
43.01	4.94	97.12	4.43
43.05	4.40	98.09	2.45
43.99	100.00	99.11	1.63
45.00	3.66	100.01	3.10
48.01	4.20	101.05	1.83
49.02	1.62	105.09	1.64
50.02	1.44	105.48	4.04
53.06	1.01	105.99	42.06
54.06	1.54	106.49	9.11
55.07	9.99	109.99	41.59
56.09	3.87	107.10	1.18
57.10	8.28	107.49	4.56
60.04	30.67	108.03	5.88
61.04	20.58	109.02	7.18
62.04	1.34	109.12	1.48
66.02	56.50	109.95	3.30
66.52	34.13	110.11	1.94
67.02	6.41	110.94	1.51
67.07	3.10	111.14	2.33
68.07	1.45	112.13	1.05
69.01	24.22	113.12	1.44
69.08	7.06	114.94	4.06
70.09	3.95	115.09	1.05
71.10	7.13	115.94	1.57
72.02	14.74	116.94	2.87
73.04	21.93	119.02	10.25
74.05	2.63	120.01	6.94
75.05	4.18	121.01	3.50
76.05	1.07	123.13	1.28
77.06	2.83	125.14	1.33
78.97	11.80	127.13	1.35
79.09	1.83	129.09	1.13
79.98	13.51	131.00	3.83
80.55	2.46	132.02	46.42
80.96	11.25	133.03	57.10
81.10	4.59	134.02	8.74
81.98	13.59	135.00	3.40
82.12	2.42	138.94	12.89
83.13	6.00	139.94	11.83
84.05	10.23	140.94	13.45
84.14	1.98	141.94	10.07
85.05	6.39	143.00	1.16
85.15	4.16	144.94	2.90
86.06	1.03	145.44	8.13

TABLE H2. TABULATED MASS SPECTRUM FOR NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 2) (Continued)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
145.94	5.91	233.86	8.19
146.44	13.60	234.87	6.35
146.98	21.75	235.02	4.17
147.44	6.43	239.87	1.60
147.95	1.80	240.41	1.45
149.05	1.24	241.91	1.13
150.02	4.56	263.03	1.24
150.94	7.64	264.84	2.17
151.95	20.20	265.84	4.29
152.94	8.50	266.34	1.72
153.95	20.58	266.81	4.25
154.95	2.04	267.81	2.63
161.08	1.28	274.78	1.14
162.94	1.21	276.78	1.21
164.94	1.31	277.84	1.33
166.95	1.19	278.81	5.96
169.00	37.77	279.84	11.41
169.97	1.78	280.34	1.26
178.02	1.92	280.84	12.26
184.98	4.63	281.34	1.60
185.44	1.33	281.81	5.36
185.94	9.19	282.81	1.07
186.44	2.30	285.00	4.63
186.95	9.23	290.87	10.90
187.45	1.05	291.87	2.34
187.95	3.94	292.87	20.73
188.95	1.03	293.87	2.73
189.95	1.39	294.87	9.61
193.91	1.13	295.87	1.03
195.89	1.61	296.78	3.65
198.94	1.57	298.78	11.50
199.94	3.80	299.78	2.93
200.94	3.28	300.78	10.95
210.95	1.51	301.78	3.04
211.95	11.74	302.78	3.41
212.95	5.10	303.78	1.28
213.95	11.74	304.28	1.95
214.95	2.58	305.28	4.25
217.86	6.24	306.28	5.37
218.89	8.93	307.28	3.66
219.86	12.59	308.28	1.69
220.87	15.81	308.78	1.13
221.87	6.39	309.81	2.16
222.87	7.03	310.78	3.46
224.39	1.98	311.78	6.48
225.36	10.68	312.78	4.43
225.87	1.96	313.03	1.15
226.36	14.83	313.78	5.96
226.87	2.39	314.78	1.51
227.36	10.77	315.78	1.89
227.87	1.31	319.28	1.46
228.36	2.02	319.78	1.63
229.86	6.45	320.28	2.51
230.87	5.78	320.81	2.82
231.86	13.81	321.28	2.10
232.87	11.08	321.78	1.12

TABLE H2. TABULATED MASS SPECTRUM FOR NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 2) (Continued)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
325.78	1.03	532.62	1.05
327.78	4.11	533.69	2.43
329.81	3.59	535.69	1.24
331.78	1.00	557.62	6.59
335.00	5.33	558.62	1.14
357.75	4.66	559.62	13.17
358.75	14.23	560.69	1.55
359.25	1.54	561.62	13.04
359.75	22.37	562.62	1.98
360.25	3.18	563.62	6.09
360.75	22.40	565.62	1.20
361.25	3.51	608.56	2.57
361.75	12.90	610.56	5.91
362.25	1.60	612.56	7.03
362.75	3.91	614.56	5.02
369.84	1.10	616.56	1.80
371.78	4.18	636.56	1.40
372.81	1.83	637.62	1.07
373.78	4.31	638.56	3.98
374.81	1.10	639.56	2.71
375.78	1.54	640.56	5.38
376.75	1.13	641.56	2.55
377.69	1.16	642.56	3.57
378.72	2.80	643.56	2.33
379.72	2.36	644.56	1.39
380.72	4.47	645.56	1.10
381.72	1.19	713.56	1.29
382.72	2.34	715.50	11.56
389.72	2.66	716.44	1.31
391.72	3.67	717.50	33.29
393.72	2.46	718.50	5.10
397.81	1.57	719.50	54.53
399.78	4.23	720.50	7.17
400.78	1.50	721.50	52.80
401.78	4.09	722.50	6.70
403.78	1.75	723.50	29.65
405.72	1.91	724.50	4.06
407.72	2.62	725.50	9.77
409.72	1.69	726.50	1.04
448.72	2.71	727.50	1.31
450.72	11.26	799.44	1.28
451.72	1.56	801.37	1.09
452.72	16.35	803.37	1.00
453.72	2.05	873.37	1.66
454.72	11.63	875.31	6.90
455.72	1.01	876.37	1.00
456.69	4.60	877.37	15.93
458.66	4.82	878.31	1.64
460.62	5.47	879.37	22.61
462.66	2.09	880.37	2.76
464.62	0.56	881.31	21.45
478.75	3.16	882.31	2.96
480.72	5.36	883.31	14.42
481.75	1.13	884.31	1.93
482.72	3.34	885.31	5.76
531.62	2.32	887.37	1.61

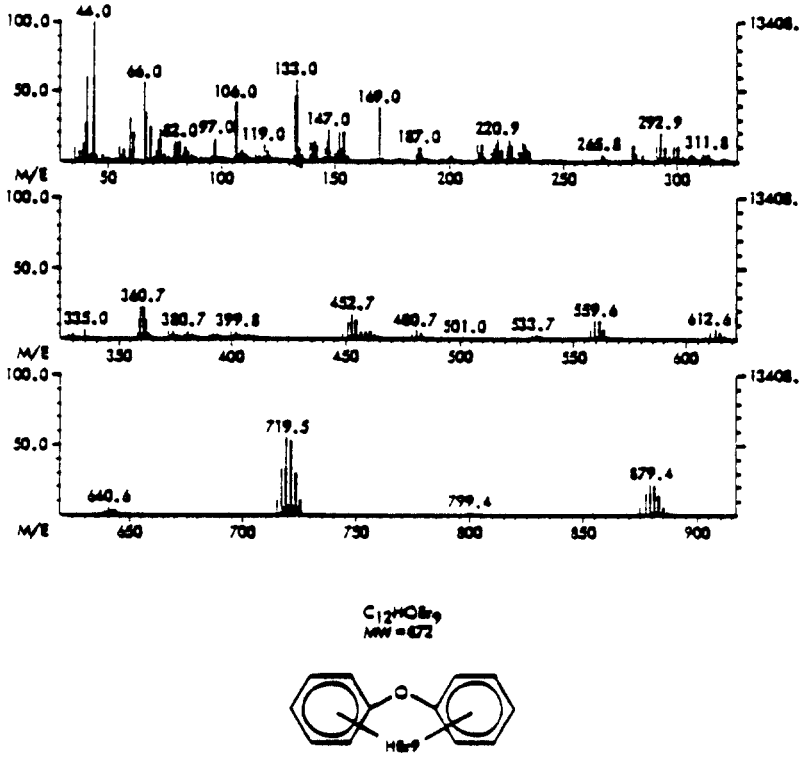

FIGURE 12. MASS SPECTRUM OF NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 2)

Peak no. 3: The mass list obtained from peak no. 3 is presented in Table H3. This impurity was identified as a second isomer of nonabromodiphenyl oxide ($C_{12}HOBr_9$). The mass spectrum obtained from peak no. 3 resembled the mass spectrum obtained from peak no. 2. A specific isomer on nonabromodiphenyl oxide was not identified. A mass spectrum of this impurity is presented in Figure 13.

4. **Conclusions:** High-performance liquid chromatographic analysis detected two impurities that were estimated at 3.7% and 1.7% relative to the major component by peak area comparison at 220 nm. HPLC analysis with a spectrophotometric detector revealed similar ultraviolet/visible spectra for the major component and the two impurities. These two impurities were isolated by HPLC and identified by direct inlet mass spectrometry as two isomers of nonabromodiphenyl oxide.

TABLE H3. TABULATED MASS SPECTRUM FOR NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 4)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
35.36	1.09	111.14	1.68
35.41	1.68	113.12	1.13
35.46	1.72	114.95	2.77
38.98	1.44	115.95	2.05
40.00	2.94	116.95	2.28
41.02	7.81	119.04	2.56
43.06	1.54	120.00	4.07
43.99	17.43	120.99	3.67
45.04	1.44	123.95	1.23
55.08	3.15	124.61	1.10
56.10	1.44	125.14	1.29
57.10	4.19	130.95	1.10
60.04	7.19	132.02	18.69
61.04	8.87	133.03	35.43
66.02	25.17	133.95	2.40
66.52	19.82	134.05	4.23
67.03	3.82	134.94	1.46
69.01	3.51	135.08	3.34
69.09	2.73	138.94	8.80
70.09	1.28	139.95	9.87
71.10	3.07	140.94	10.12
72.03	4.53	141.95	8.95
73.04	8.46	144.94	3.43
75.06	2.18	145.44	12.08
77.07	1.28	145.94	7.69
78.97	4.21	146.44	22.65
79.98	7.24	146.97	10.68
80.55	2.99	147.44	10.90
80.97	4.23	147.95	1.95
81.11	2.20	149.05	5.21
81.98	7.23	149.91	1.20
82.12	1.09	150.05	1.29
83.13	2.80	150.25	1.69
84.05	2.93	150.94	8.97
84.14	1.24	151.59	1.62
85.06	2.21	151.95	14.28
85.16	2.06	152.95	6.92
87.09	1.41	153.95	13.58
89.09	2.74	154.95	1.19
95.12	2.05	161.00	1.16
76.04	4.30	164.94	1.22
96.12	1.11	166.97	1.36
97.02	4.52	169.00	9.28
97.13	2.66	177.17	2.30
98.10	1.16	177.95	1.83
99.12	1.04	184.95	6.69
105.09	1.30	185.45	2.08
105.49	4.23	185.95	17.01
106.00	45.74	186.44	4.62
106.49	8.91	186.61	1.25
106.99	45.52	186.95	20.04
107.50	5.84	187.27	1.38
108.03	2.39	187.45	2.89
108.95	1.68	187.95	7.24
109.06	3.50	188.97	1.37
109.95	3.46	189.95	1.37
110.95	1.45	193.91	1.45

TABLE H3. TABULATED MASS SPECTRUM FOR NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 4) (Continued)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
195.91	1.62	281.34	4.18
196.91	1.75	281.84	15.55
198.94	2.70	282.34	1.82
199.95	5.62	282.84	3.17
200.94	5.93	285.00	1.32
201.95	2.35	290.87	10.97
210.94	1.09	291.87	2.31
211.95	9.87	292.87	22.20
212.95	3.39	293.87	3.20
213.94	10.01	294.87	11.22
214.95	1.75	295.87	1.37
217.86	5.98	296.81	4.97
218.87	9.95	297.81	1.58
219.86	13.38	298.78	13.34
220.87	16.17	299.81	4.54
221.86	7.41	300.78	13.61
222.87	7.51	301.81	4.34
224.37	5.27	302.78	4.32
224.86	1.33	303.78	1.55
225.37	23.01	304.31	6.05
225.87	4.01	304.81	1.02
226.37	34.19	305.28	13.47
226.87	4.89	305.78	1.62
227.37	22.28	306.28	16.96
227.87	3.07	306.78	1.19
228.37	5.23	307.28	12.57
229.86	8.48	307.78	1.30
230.87	4.58	308.28	4.76
231.86	18.16	308.78	2.77
232.87	9.28	309.81	2.22
233.86	9.30	310.78	6.61
234.91	5.91	311.81	6.72
239.28	3.29	312.81	7.60
239.86	4.37	318.81	6.59
240.34	2.73	314.78	2.50
240.52	2.34	315.81	2.16
240.87	1.65	318.31	1.98
241.19	2.02	318.84	1.42
241.39	1.48	319.28	4.33
241.87	2.05	319.78	2.15
263.84	1.07	320.28	5.96
264.84	5.57	320.81	3.87
265.34	1.59	321.28	4.15
265.84	11.98	321.81	2.05
266.34	2.62	322.28	1.70
266.84	11.83	322.81	1.17
267.31	2.29	325.81	1.30
267.84	6.47	327.81	3.56
268.31	1.10	329.81	3.34
268.87	1.62	331.78	1.01
274.78	2.20	335.03	1.45
276.78	1.93	356.78	2.00
277.84	2.97	357.78	15.58
278.84	15.70	358.28	2.12
279.34	2.08	358.78	44.51
279.84	29.26	359.28	5.86
280.34	3.83	359.78	72.53
280.84	28.42	360.28	9.16

TABLE H3. TABULATED MASS SPECTRUM FOR NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 4) (Continued)

Mass	Relative Abundance (percent of base peak)	Mass	Relative Abundance (percent of base peak)
360.78	69.06	533.69	3.71
361.25	9.09	535.69	2.05
361.78	41.37	555.69	1.59
362.28	5.40	557.69	8.03
362.75	14.00	558.69	1.14
363.28	1.73	559.69	15.53
363.78	1.85	560.69	2.40
369.81	1.51	561.69	15.47
371.81	4.27	562.62	2.10
372.81	1.57	563.69	7.79
373.81	4.60	564.69	1.08
374.81	1.31	565.69	1.62
375.78	2.24	608.62	4.18
376.72	2.21	610.62	9.75
377.72	2.01	611.62	1.44
378.72	7.60	612.62	12.99
379.72	3.21	613.62	1.54
380.72	11.59	614.56	9.71
381.72	2.12	616.56	3.90
382.72	7.32	636.62	2.11
384.72	1.88	637.62	1.21
387.37	1.16	638.62	4.74
389.72	4.49	639.62	2.19
391.72	6.68	640.56	6.88
392.72	1.28	641.62	3.24
393.72	3.82	642.56	5.17
397.78	1.50	643.56	2.61
399.25	1.81	644.62	2.10
399.81	4.78	613.56	3.15
400.22	1.44	715.56	20.74
400.78	1.39	716.50	3.07
401.22	1.31	717.50	60.31
401.81	4.72	718.50	8.17
403.78	2.04	719.50	100.00
405.72	2.67	720.50	12.67
406.75	1.56	721.50	98.65
407.75	3.58	722.50	13.33
408.75	2.01	723.50	57.17
409.72	2.52	724.50	7.65
410.75	1.30	725.50	18.61
448.75	4.07	726.50	2.60
450.75	14.28	727.50	2.11
451.75	1.94	799.44	1.44
452.75	21.38	801.44	1.33
453.75	2.80	873.37	2.62
454.72	14.57	875.37	10.66
455.75	1.95	876.37	1.42
456.69	6.95	877.37	25.62
458.66	6.94	878.37	3.23
460.66	6.57	879.37	36.94
462.62	2.90	880.37	5.11
478.75	3.93	881.37	35.82
480.75	4.83	882.37	4.59
481.75	1.10	883.37	24.10
482.72	3.66	884.37	3.32
484.75	1.08	885.37	9.82
531.69	4.17	886.37	1.44
532.69	1.12	887.37	2.53

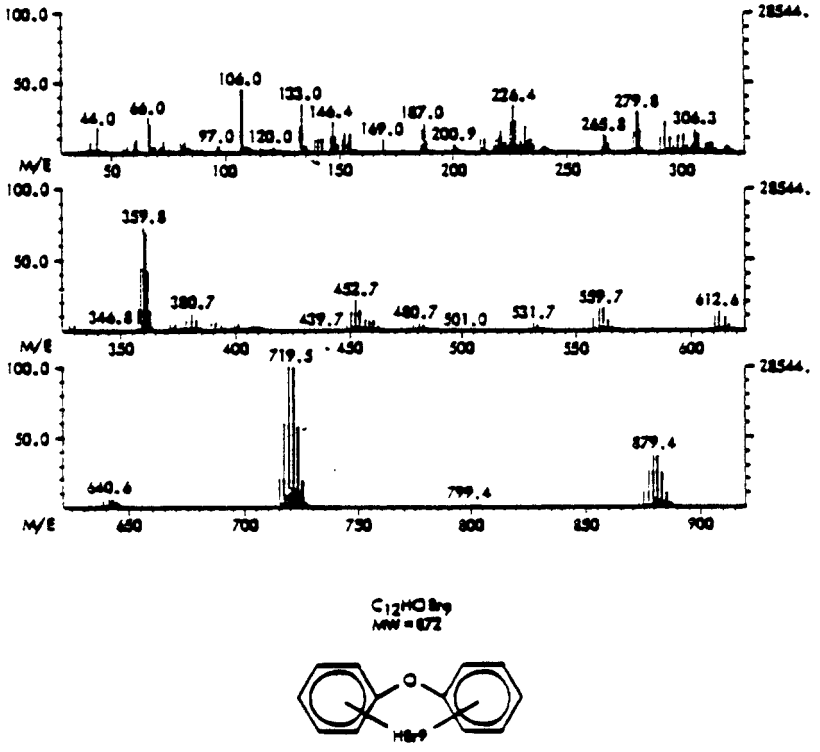

FIGURE 13. MASS SPECTRUM OF NONABROMODIPHENYL OXIDE ISOMER (PEAK NO. 3)

APPENDIX I

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

APPENDIX I. PREPARATION AND CHARACTERIZATION

I. Studies Conducted at the Analytical Chemistry Laboratory

A. Preparation procedure

1. **Premix:** Decabromodiphenyl oxide (309.9 ± 0.1 mg) was added directly to 200 g of Wayne Lab Blox® rodent feed. This premix was homogenized by rotation in a 1-quart, large-mouth, glass jar for 15 minutes on a ball-mill-type tumbler apparatus.

2. **Bulk mixing:** The above premix and 1,300 g more feed were mixed in a Patterson-Kelly® twin-shell blender with pin-type intensifier bar for a total of 15 minutes. The blender was loaded from the top of the shells as follows: 650 g of feed was poured in and allowed to settle and level at the bottom (vertex of the "V"); then the dry premix was poured in on top of the feed from each side; this layer was covered with the remaining 650 g of feed poured in from each side. After elapsed mixing times of 10 and 15 minutes, duplicate 5-g samples were removed from the top of each shell and the bottom trap of the blender for subsequent analysis.

3. **Extraction and analysis:** Each sample was placed in a 200-ml centrifuge bottle and triturated with 50 ml of nonstabilized tetrahydrofuran (high-performance liquid chromatography grade) for 30 seconds in a Brinkmann Polytron® high-speed blender. The mixture was placed in an ultrasonic vibratory bath for 2 minutes and centrifuged for 15 minutes. The supernatant solution was pipetted into a 100-ml volumetric flask. The feed residue was mixed with an additional 50 ml of tetrahydrofuran and treated again as described above. The combined supernatant solutions were brought to volume (100 ml) with additional tetrahydrofuran. After filtration through Millipore® (0.5 µ) filters, the sample extract solutions were analyzed by the high-performance liquid chromatographic system described below.

   Instrument: Waters Programmable Component System
   Column: µBondapak C_{18}, 300 mm × 4 mm, ID
   Column temperature: Ambient
   Solvent: 100% Methanol, 1 ml/min
   Detection: Ultraviolet, 254 nm
   Retention time: 6.5 min

4. **Quality control:** Two blank (undosed) feed samples and three individually spiked mixtures (at the 200-ppm concentration) were extracted and prepared for analysis in the manner described above for the test samples. No interference from feed was found at the retention time of decabromodiphenyl oxide in the chromatograms. Chromatographic detector linearity was determined with standard solutions of the study chemical in tetrahydrofuran. A standard curve, from which the decabromodiphenyl oxide content of the test sample extracts was determined, was also constructed from these standard solutions. The stability of the test solutions and the chromatographic system was monitored throughout the analysis by periodic injections of the 11 µg/ml standard solution.

B. Homogeneity

1. Results

Sample Location	Sampling Time (min)	Average Concentration (ppm) Found in Formulated Diet (a,b)
Right	10	229 ± 12
Left	10	245 ± 14
Bottom	10	209 ± 11
Right	15	198 ± 11
Left	15	225 ± 12
Bottom	15	205 ± 11

(a) Corrected for a spiked recovery yield of 88% ± 3(δ)%. The target concentration of chemical in feed was 206.6 ± 0.6 ppm.
(b) Error values are average deviations obtained in the instrumental measurements of the test solutions.

2. **Conclusion:** Decabromodiphenyl oxide mixed with stock rodent feed at the 200-ppm concentration was found more homogenous when mixed for 15 minutes (rather than 10 minutes) in a Patterson-Kelly®, 4-quart, twin-shell blender with a pin-type intensifier bar.

C. Stability

1. **Sample mixing and storage:** A stock solution of decabromodiphenyl oxide in nonstabilized high-performance liquid chromatography grade tetrahydrofuran (0.2418 mg/ml) was prepared, and 5 ml of this solution was added to individual 5-g samples of Wayne Lab-Blox® rodent feed. The tetrahydrofuran was then removed from the samples on a rotary evaporator (30 minutes, 25° C water bath temperature). The dried samples were stored, in duplicate, at −20°, 5°, 25°, and 45° C for 2 weeks.

2. **Extraction and analysis:** Each stability sample was quantitatively transferred to a 200-ml centrifuge bottle and extracted according to the procedure described in section I.A.3. An aliquot (5 ml) of each extract solution was filtered through a 0.5-μ Millipore® filter and then analyzed by the same high-performance liquid chromatographic system described in section I.A.3.

3. **Quality control:** Undosed feed samples and individual samples (at the 200-ppm concentration) were extracted and prepared for analysis in the manner described for the test samples. The blank showed no feed interference.

4. **Results**

Storage Temperature	Average Concentration (ppm) Chemical Found in Formulated Diet (a,b)
−20° C	249 ± 14
5° C	244 ± 14
25° C	242 ± 13
45° C	221 ± 12

(a) Corrected for a spiked recovery yield of 88% ± 3(δ)%. The target concentration of chemical in feed was 242 ± 5 ppm.
(b) Error values are average deviations obtained in the instrumental measurements of the test solutions

5. **Conclusion:** Decabromodiphenyl oxide mixed with stock rodent feed at 240 ppm was found to be stable over a 2-week storage period at temperatures of 25° C and below. Samples stored for 2 weeks at 45° C showed slight but significant loss of major component.

II. Studies Conducted at the Study Laboratory

A. Preparation: Decabromodiphenyl oxide was weighed and mixed with a small amount of feed for 2 minutes. The premix was transferred to a Hobart® mixer with 5 kg of NIH 07 Rat and Mouse Ration and mixed for 1 minute/kg of feed. This mixture was transferred to a Patterson-Kelley® twin-shell blender with the required amount of feed and mixed for 1 min/kg of feed.

B. Homogeneity

A 5-g sample in a 50-ml test tube was extracted with 40 ml of tetrahydrofuran for 10 minutes on a horizontal shaker. The sample was centrifuged at 2,500 rpm for 15 minutes, and the supernatant was transferred to a 125-ml Erlenmeyer flask. The feed residue was extracted again with 40 ml of tetrahydrofuran. The combined extracts were filtered through Whatman #1 filter paper into a 100-ml volumetric flask. The solutions were brought to volume with tetrahydrofuran. Dilutions from 1:2 to 1:10 were made in order to inject 10-µl aliquots into the high-performance liquid chromatograph under the following conditions:

Instrument: Waters Model 6000A high-performance liquid chromatograph with U6K injector linked to a Waters Data Module System
Column: µBondapak C_{18}, 300 mm × 25 mm
Solvent: Water:acetonitrile (90:10), 1 ml/minute
Detection: Waters 440 model, ultraviolet, 254 nm
Retention time: 15.29 min for major peak

All feed samples were analyzed in duplicate, including control feed. Samples were quantitated against a standard of decabromodiphenyl oxide by the Data Module Integration System.

2. **Results**

Sample Location	Target Concentration (ppm)	Determined Concentration (ppm)	Determined Concentration as Percent of Target Concentration (wt/wt)
Top left	25,000	24,600	98.4
Top right	25,000	24,500	98.0
Bottom	25,000	23,800	95.2
Top left	50,000	47,900	95.8
Top right	50,000	51,300	102.6
Bottom	50,000	48,700	97.4

C. **Conclusion:** The homogeneity of both mixes was excellent. All results were within specifications (±10%).

APPENDIX J

METHODS OF ANALYSIS OF FORMULATED DIETS

APPENDIX J. METHODS OF ANALYSIS

I. Study Laboratory

A. Preparation and analysis of dosed feed samples:

A 5-g sample of feed was weighed in duplicate and transferred into 50-ml test tubes containing 40 ml of tetrahydrofuran. The test tubes were shaken on a horizontal shaker for 10 minutes or ultrasonicated for 2 minutes. The samples were centrifuged at 2,500 rpm for 15 minutes, and the supernatant was transferred to a 125-ml Erlenmeyer flask. The feed was reextracted with 40 ml of tetrahydrofuran, shaken, and centrifuged as above. The extracts were combined and filtered through Whatman #1 filter paper into a 100-ml volumetric flask. The samples were diluted to 1:50 or 1:100 for injection.

Instrument parameters

Instrument: Waters Data Module System, equipped with 6000A pump and U6K injector
Detector: Waters Model 440, ultraviolet, 254 nm
Column: Waters μBondapak C_{18}, 300 mm × 3.9 mm
Solvent: 10% water:90% acetonitrile, isocratic, 1 ml/min
Retention time: 14 min for major peak

B. Preparation and analysis of spiked feed samples: Appropriate amounts of decabromodiphenyl oxide were weighed into 5-g aliquots of feed to obtain final concentrations similar to the levels to be analyzed. The spiked feed samples were processed simultaneously with the dosed feed samples.

II. Analytical Chemistry Laboratory

A. Preparation of spiked feed standards: Two standard solutions of decabromodiphenyl oxide were prepared independently in high-performance liquid chromatography (HPLC) grade tetrahydrofuran. These solutions were diluted with tetrahydrofuran to make six standards. Aliquots (100 ml) of the six standard solutions were pipetted into individual 200-ml centrifuge bottles containing 10 g of undosed feed to make spiked feed standards bracketing the specified concentration range of the referee sample. One 200-ml centrifuge bottle containing 10 g of undosed feed was treated with 100 ml of tetrahydrofuran for use as a blank. The spiked feeds and the feed blank were sealed and allowed to stand overnight at room temperature before analysis.

B. Preparation of the referee sample: Triplicate weights of the referee feed sample (~10 g weighed to the nearest 0.01 g) were transferred to individual 200-ml centrifuge bottles. HPLC-grade tetrahydrofuran (100 ml) was pipetted into each sample; then the bottles were sealed and allowed to stand overnight at room temperature before analysis.

C. Analysis: Feed samples (10 g treated with 100 ml of tetrahydrofuran in 200-ml centrifuge bottles) were placed on a Burrell Model 75 Wrist-Action® shaker and were shaken at maximum stroke for 20 minutes. The extraction mixtures were centrifuged for 10 minutes; then 3-ml aliquots of the supernatant solutions were diluted to 50 ml with tetrahydrofuran and thoroughly mixed. The solutions were filtered through a 0.5-μ Millipore® filter, and the decabromodiphenyl oxide content of the filtrate was determined by the high-performance liquid chromatography analysis described below.

Instrument parameters

Instrument: Waters Data Module System, equipped with 6000A pump and U6K injector
Detector: Waters Model 440, ultraviolet, 254 nm, 0.5 AUFS
Column: Waters μBondapak C_{18} (3.9 mm × 300 mm, ID)
Solvent: 100% methanol, 1 ml/min
Volume injected: 15 μl
Retention time: 3.6 min

The amount of decabromodiphenyl oxide in the referee sample was determined from the linear regression equation computed for the standard data, using peak area measurements and the amount of decabromodiphenyl oxide added to the spiked feed standards.

D. **Quality Assurance:** The referee feed sample was analyzed in triplicate, and the undosed feed sample was analyzed once. For calibration, six spiked feed standards bracketing the specified concentration range of the referee sample were made from two independently prepared standard solutions. Triplicate injections of each standard and sample solution were made into the liquid chromatograph in a randomized order.

APPENDIX K

RESULTS OF ANALYSIS OF FORMULATED DIETS

TABLE K1. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF DECABROMODIPHENYL OXIDE (a)

Date Mixed	Concentration of Decabromodiphenyl Oxide in Feed (ppm)	
	Target	Determined
02/06/79	3,100	2,850
	3,100	2,850
	3,100	3,140
	6,300	(b) 5,440
	12,500	(b) 14,180
	25,000	(c) 27,590
	50,000	59,800
	50,000	52,490
	50,000	52,000
02/27/79	6,300	(d) 6,890
	6,300	(d) 6,450
	12,500	(d) 12,500
	12,500	(d) 12,500

(a) Results of duplicate analysis
(b) Sample out of specification; remixed.
(c) Sample out of specification; not remixed.
(d) Remix

TABLE K2. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF DECABROMODIPHENYL OXIDE (a)

Date Mixed	Determined Concentration for Target Concentration of	
	25,000 ppm	50,000 ppm
07/08/80	24,300	50,000
07/24/80	24,655	51,600
07/31/80		46,050
08/21/80	22,550	
11/06/80	25,000	49,200
12/31/80	23,300	46,800
01/29/81	22,270	46,500
04/02/81	22,750	48,050
10/08/81	26,050	48,050
12/10/81	24,600	50,950
01/28/82	23,150	48,250
03/18/82	24,350	51,300
05/06/82	26,450	53,600
06/24/82	26,000	50,600
08/19/82	22,650	47,950
Experimental mean	24,148	49,207
Standard deviation	1,403	2,206
Coefficent of variation (percent)	5.8	4.5
Range	22,270-26,450	46,050-53,600
Number of samples	14	14

(a) Results of duplicate analysis

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TABLE K3. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF DECABROMODIPHENYL OXIDE

Date Mixed	Target Concentration (ppm)	Determined Concentration	
		Study Laboratory	Analytical Laboratory
07/24/80	50,000	51,600	49,660
04/02/81	25,000	22,750	25,200
12/10/81	50,000	50,950	51,900
06/24/82	50,000	50,600	50,200
08/19/82	25,000	22,650	25,800

APPENDIX L

SENTINEL ANIMAL PROGRAM

APPENDIX L. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen $B6C3F_1$ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests are performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai (6, 12, 18 mo)	M.Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) MHV (6,12 mo) Sendai (24 mo)	MHV (mouse hepatitis virus) (18, 24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (6, 12, 18 mo)	RCV (rat coronavirus) Sendai (24 mo)	

II. Results

Results are presented in Table L1.

TABLE L1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF DECABROMODIPHENYL OXIDE (a)

	Interval (months)	No. of Animals	Positive Serologic Reaction for
Rats	6	--	None positive
	12	9/10	RCV
	18	2/3	RCV
		5/10	KRV
	24	4/10	Sendai
Mice	6	--	None positive
	12	--	None positive
	18	--	None positive
	24	--	None positive

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates, Inc. (Bethesda, MD) for the Animal Disease Screening Program.

APPENDIX M

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF DECABROMODIPHENYL OXIDE

TABLE M1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF DECABROMODIPHENYL OXIDE

Week	Control		25,000 ppm				50,000 ppm			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	15	208	18	209	1.2	2,153	16	207	1.1	3,865
2	17	236	17	236	1.0	1,801	17	238	1.0	3,571
3	17	259	18	260	1.1	1,731	18	259	1.1	3,475
4	17	276	18	281	1.1	1,601	19	279	1.1	3,405
5	16	291	17	295	1.1	1,441	17	294	1.1	2,891
6	17	305	17	308	1.0	1,380	17	307	1.0	2,769
7	16	317	19	320	1.2	1,484	17	317	1.1	2,681
8	16	329	19	331	1.2	1,435	17	328	1.1	2,591
9	15	339	16	344	1.1	1,163	16	339	1.1	2,360
10	15	347	16	352	1.1	1,136	16	348	1.1	2,299
11	16	354	16	359	1.0	1,114	16	353	1.0	2,266
12	15	361	16	372	1.1	1,075	16	361	1.1	2,216
17	19	389	18	400	0.9	1,125	18	392	0.9	2,296
21	16	412	17	416	1.1	1,022	18	413	1.1	2,179
25	14	424	15	431	1.1	870	15	422	1.1	1,777
29	17	431	17	440	1.0	966	17	429	1.0	1,981
33	16	438	17	450	1.1	944	18	441	1.1	2,041
37	17	447	18	447	1.1	1,007	18	438	1.1	2,055
41	18	435	18	438	1.0	1,027	20	426	1.1	2,347
45	16	447	18	449	1.1	1,002	17	441	1.1	1,927
49	15	444	16	443	1.1	903	17	441	1.1	1,927
53	15	452	16	456	1.1	877	17	448	1.1	1,897
57	15	449	16	451	1.1	887	16	444	1.1	1,802
61	17	452	18	453	1.1	993	16	445	0.9	1,798
65	16	456	18	449	1.1	1,002	18	452	1.1	1,991
69	12	449	17	440	1.4	966	18	443	1.5	2,032
73	15	452	15	449	1.0	835	16	448	1.1	1,786
77	14	449	16	449	1.1	891	17	451	1.2	1,885
81	14	449	19	442	1.4	1,075	15	441	1.1	1,701
85	16	445	16	440	1.0	909	15	434	0.9	1,728
89	13	436	15	436	1.2	860	15	429	1.2	1,748
93	12	423	17	430	1.4	988	16	419	1.3	1,909
97	12	413	13	412	1.1	789	13	408	1.1	1,593
101	14	413	15	400	1.1	938	16	395	1.1	2,025
103	13	404	16	396	1.2	1,010	16	395	1.2	2,025
104	13	402	14	397	1.1	882	13	389	1.0	1,671
Mean	15.3	390	16.7	391	1.1	1,119	16.6	387	1.1	2,236
SD (d)	1.7		1.4		0.1	311	1.4		0.1	575
CV (e)	11.1		8.4		9.1	27.8	8.4		9.1	25.7

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of decabromodiphenyl oxide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE M2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF DECABROMODIPHENYL OXIDE

Week	Control		25,000 ppm				50,000 ppm			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	10	139	11	139	1.1	1,978	11	138	1.1	3,986
2	11	150	11	151	1.0	1,821	11	150	1.0	3,667
3	10	159	11	159	1.1	1,730	11	157	1.1	3,503
4	11	168	13	169	1.2	1,923	14	167	1.3	4,192
5	10	173	10	174	1.0	1,437	11	173	1.1	3,179
6	10	179	11	182	1.1	1,511	12	177	1.2	3,390
7	10	185	11	186	1.1	1,478	11	184	1.1	2,989
8	10	191	10	191	1.0	1,309	11	188	1.1	2,926
9	10	195	10	196	1.0	1,276	10	192	1.0	2,604
10	10	199	10	198	1.0	1,263	11	198	1.1	2,778
11	11	202	10	199	0.9	1,256	11	198	1.0	2,778
12	9	204	10	205	1.1	1,220	11	203	1.2	2,709
17	10	217	10	214	1.0	1,168	10	209	1.0	2,392
21	10	223	10	219	1.0	1,142	11	217	1.1	2,535
25	9	228	10	227	1.1	1,101	10	223	1.1	2,242
29	11	233	10	229	0.9	1,092	11	227	1.0	2,423
33	10	238	10	234	1.0	1,068	11	234	1.1	2,350
37	11	244	12	240	1.1	1,250	12	239	1.1	2,510
41	11	246	11	240	1.0	1,146	12	240	1.1	2,500
45	11	252	12	251	1.1	1,195	12	247	1.1	2,429
49	12	256	12	255	1.0	1,176	12	254	1.0	2,362
53	12	269	12	268	1.0	1,119	13	268	1.1	2,425
57	12	278	12	276	1.0	1,087	12	272	1.0	2,206
61	12	288	15	286	1.3	1,311	13	283	1.1	2,297
65	13	299	12	297	0.9	1,010	13	294	1.0	2,211
69	12	307	12	303	1.0	990	13	297	1.1	2,189
73	12	315	12	315	1.0	952	13	309	1.1	2,104
77	13	327	12	324	0.9	926	13	314	1.0	2,070
81	12	331	12	327	1.0	917	13	324	1.1	2,006
85	12	337	13	332	1.1	979	13	327	1.1	1,988
89	11	341	12	335	1.1	896	13	329	1.2	1,976
93	11	338	11	334	1.0	823	13	322	1.2	2,019
97	11	338	12	330	1.1	909	12	319	1.1	1,881
101	12	333	11	328	0.9	838	13	318	1.1	2,044
103	11	329	13	334	1.2	973	14	322	1.3	2,174
104	9	333	12	336	1.3	893	12	320	1.3	1,875
Mean	10.9	251	11.3	250	1.0	1,199	11.9	245	1.1	2,553
SD (d)	1.1		1.2		0.1	296	1.1		0.1	588
CV (e)	10.1		10.6		10.0	24.7	9.2		9.1	23.0

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of decabromodiphenyl oxide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE M3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF DECABROMODIPHENYL OXIDE

Week	Control		25,000 ppm				50,000 ppm			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	4	29.4	5	27.9	1.3	4,480	5	29.3	1.3	8,532
2	4	30.6	4	29.7	1.0	3,367	4	29.7	1.0	6,734
3	4	30.9	5	30.2	1.3	4,139	5	30.6	1.3	8,170
4	4	32.0	5	31.6	1.3	3,956	4	32.1	1.0	6,231
5	5	31.8	4	31.5	0.8	3,175	5	31.5	1.0	7,937
6	4	33.2	4	32.3	1.0	3,096	4	31.6	1.0	6,329
7	4	34.0	5	33.3	1.3	3,754	4	33.4	1.0	5,988
8	4	34.8	2	33.6	0.5	1,488	4	34.1	1.0	5,865
9	4	34.7	4	35.1	1.0	2,849	4	34.0	1.0	5,882
10	5	35.4	5	34.1	1.0	3,666	5	34.2	1.0	7,310
11	4	35.2	4	34.5	1.0	2,899	5	34.7	1.3	7,205
12	4	35.6	4	35.2	1.0	2,841	5	35.1	1.3	7,123
16	4	36.5	4	36.5	1.0	2,740	4	35.9	1.0	5,571
20	4	36.9	4	36.4	1.0	2,747	4	35.9	1.0	5,571
24	5	37.0	5	36.4	1.0	3,434	5	36.2	1.0	6,906
28	5	38.5	5	38.4	1.0	3,255	5	37.3	1.0	6,702
32	5	39.9	5	40.1	1.0	3,117	5	39.0	1.0	6,410
36	4	39.0	4	41.0	1.0	2,439	5	40.0	1.3	6,250
40	6	40.4	4	41.1	0.7	2,433	5	39.6	0.8	6,313
42	5	41.0	5	41.0	1.0	3,049	5	40.0	1.0	6,250
44	5	40.2	5	41.7	1.0	2,998	5	40.8	1.0	6,127
46	5	41.5	4	41.7	0.8	2,398	6	40.8	1.2	7,353
48	6	41.0	4	43.0	0.7	2,326	5	42.0	0.8	5,952
50	5	40.0	4	41.0	0.8	2,439	5	41.0	1.0	6,098
52	4	39.6	5	41.1	1.3	3,041	5	41.1	1.3	6,083
54	6	41.2	4	41.3	0.7	2,421	5	39.8	0.8	6,281
56	5	40.0	5	41.4	1.0	3,019	4	40.4	0.8	4,950
58	5	40.1	6	41.5	1.2	3,614	4	41.5	0.8	4,819
60	5	40.7	5	42.1	1.0	2,969	4	41.2	0.8	4,854
62	5	40.1	4	41.3	0.8	2,421	5	41.1	1.0	6,083
64	4	40.9	4	41.6	1.0	2,404	4	41.1	1.0	4,866
66	5	40.0	6	39.0	1.2	3,846	5	40.0	1.0	6,250
68	5	40.0	6	39.7	1.2	3,778	6	39.6	1.2	7,576
70	5	39.0	6	39.0	1.2	3,846	6	39.0	1.2	7,692
72	6	38.6	7	39.9	1.2	4,386	6	38.8	1.0	7,732
74	7	39.3	6	39.7	0.9	3,778	6	39.3	0.9	7,634
76	6	38.3	6	39.5	1.0	3,797	6	38.9	1.0	7,712
80	6	40.0	6	39.0	1.0	3,846	6	39.0	1.0	7,692
84	6	39.0	6	39.0	1.0	3,846	6	38.0	1.0	7,895
88	5	39.1	5	38.8	1.0	3,222	5	39.5	1.0	6,329
92	5	40.0	6	40.0	1.2	3,750	5	39.0	1.0	6,410
96	5	40.0	5	39.0	1.0	3,205	5	38.0	1.0	6,579
100	5	38.0	5	38.0	1.0	3,289	6	37.0	1.2	8,108
102	5	37.0	5	38.0	1.0	3,289	5	37.0	1.0	6,757
103	9	37.0	5	38.0	0.6	3,289	6	38.0	0.7	7,895
Mean	5.0	37.7	4.8	37.8	1.0	3,203	5.0	37.5	1.0	6,645
SD (d)	1.0		0.9		0.2	625	0.7		0.1	955
CV (e)	20.0		18.8		20.0	19.5	14.0		10.0	14.4

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of decabromodiphenyl oxide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE M4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF DECABROMODIPHENYL OXIDE

Week	Control		125 ppm				500 ppm			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	6	21.7	6	21.7	1.0	6,912	6	21.6	1.0	13,889
2	6	22.7	5	22.5	0.8	5,556	5	22.6	0.8	11,062
3	6	23.4	5	23.1	0.8	5,411	5	22.7	0.8	11,013
4	5	23.8	5	24.0	1.0	5,208	5	23.7	1.0	10,549
5	6	24.2	5	24.4	0.8	5,123	5	24.0	0.8	10,417
6	5	25.1	5	24.9	1.0	5,020	5	24.5	1.0	10,204
7	6	25.9	5	25.8	0.8	4,845	6	24.7	1.0	12,146
8	5	25.7	6	25.9	1.2	5,792	6	25.2	1.2	11,905
9	5	26.5	5	26.6	1.0	4,699	5	26.0	1.0	9,615
10	5	27.7	5	27.0	1.0	4,630	5	26.7	1.0	9,363
11	6	27.3	5	27.2	0.8	4,596	5	26.7	0.8	9,363
12	5	28.1	5	27.4	1.0	4,562	5	27.0	1.0	9,259
16	5	28.9	5	28.4	1.0	4,401	5	28.6	1.0	8,741
20	5	30.1	5	30.4	1.0	4,112	5	30.5	1.0	8,197
24	6	30.3	5	30.7	0.8	4,072	5	30.9	0.8	8,091
28	5	32.7	5	32.5	1.0	3,846	5	32.8	1.0	7,622
32	5	34.6	5	34.8	1.0	3,592	5	34.9	1.0	7,163
36	6	36.0	5	37.0	0.8	3,378	6	37.0	1.0	8,108
40	5	35.7	5	37.6	1.0	3,324	5	37.7	1.0	6,631
42	5	38.0	5	36.0	1.0	3,472	5	39.0	1.0	6,410
44	5	37.5	5	38.0	1.0	3,289	4	39.9	0.8	5,013
46	5	38.9	5	39.0	1.0	3,205	5	39.0	1.0	6,410
48	5	38.0	5	39.0	1.0	3,205	5	40.0	1.0	6,250
50	5	38.0	5	39.0	1.0	3,205	5	38.0	1.0	6,579
52	5	38.9	4	40.3	0.8	2,481	5	37.9	1.0	6,596
54	5	39.4	5	40.4	1.0	3,094	5	39.4	1.0	6,345
56	5	39.7	5	39.9	1.0	3,133	5	40.1	1.0	6,234
58	5	40.4	5	39.9	1.0	3,133	5	40.1	1.0	6,234
62	5	40.7	4	40.2	0.8	2,488	4	40.4	0.8	4,950
64	5	40.1	4	40.7	0.8	2,457	5	39.6	1.0	6,313
66	5	39.0	4	41.0	0.8	2,439	5	40.0	1.0	6,250
72	5	39.2	5	40.7	1.0	3,071	5	38.3	1.0	6,527
74	5	39.0	5	40.9	1.0	3,056	5	38.7	1.0	6,460
76	5	39.3	5	40.8	1.0	3,064	6	38.5	1.2	7,792
80	5	41.0	5	41.0	1.0	3,049	5	39.0	1.0	6,410
84	5	41.0	5	42.0	1.0	2,976	4	40.0	0.8	5,000
88	5	42.6	4	42.7	0.8	2,342	5	40.9	1.0	6,112
92	4	41.0	4	43.0	1.0	2,326	5	40.0	1.3	6,250
96	6	41.0	5	44.1	0.8	2,834	5	41.0	0.8	6,098
100	7	40.0	8	42.0	1.1	4,762	5	38.0	0.7	6,579
102	6	42.0	5	43.0	0.8	2,907	5	43.0	0.8	5,814
103	7	41.0	6	43.0	0.9	3,488	7	43.0	1.0	8,140
Mean	5.3	34.6	5.0	35.1	0.9	3,758	5.1	34.5	1.0	7,776
SD (d)	0.6		0.7		0.1	1,088	0.5		0.1	2,145
CV (e)	11.3		14.0		11.1	29.0	9.8		10.0	27.6

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of decabromodiphenyl oxide consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX N

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Meal Diet: June 1980 to July 1982

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

TABLE N1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Brewer's dried yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NIH, 1978; NCI, 1976
(b) Ingredients should be ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE N2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
d-α-Tocopheryl acetate	20,000 IU	
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Folic acid	2.2 g	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
B_{12}	4,000.0 mcg	
Biotin	140.0 mg	*d*-Biotin
K_3	2.8 g	Menadione activity
Choline	560.0 g	Choline chloride
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE N3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	24.20 ± 1.00	22.6-26.3	24
Crude fat (percent by weight)	5.02 ± 0.46	4.2-6.0	24
Crude fiber (percent by weight)	3.48 ± 0.41	2.4-4.3	24
Ash (percent by weight)	6.66 ± 0.41	5.97-7.42	24
Essential amino acids (percent of total diet)			
Arginine	1.260	1.21-1.31	2
Cystine	0.395	0.39-0.40	2
Glycine	1.175	1.15-1.20	2
Histidine	0.553	0.530-0.576	2
Isoleucine	0.908	0.881-0.934	2
Leucine	1.905	1.85-1.96	2
Lysine	1.250	1.20-1.30	2
Methionine	0.310	0.306-0.314	2
Phenylalanine	0.967	0.960-0.974	2
Threonine	0.834	0.827-0.840	2
Tryptophan	0.175	0.171-0.178	2
Tyrosine	0.587	0.566-0.607	2
Valine	1.085	1.05-1.12	2
Essential fatty acids (percent of total diet)			
Linoleic	2.37		1
Linolenic	0.308		1
Arachidonic	0.008		1
Vitamins			
Vitamin A (IU/kg)	11,087 ± 1,723	7,200-17,000	24
Vitamin D (IU/kg)	6,300		1
α-Tocopherol (ppm)	37.6	31.1-44.0	2
Thiamine (ppm)	18.8 ± 0.36	7.4-26.0	(b) 23
Riboflavin (ppm)	6.9	6.1-7.4	2
Niacin (ppm)	75	65-85	2
Pantothenic acid (ppm)	30.2	29.8-30.5	2
Pyridoxine (ppm)	7.2	5.6-8.8	2
Folic acid (ppm)	2.1	1.8-2.4	2
Biotin (ppm)	0.24	0.21-0.27	2
Vitamin B_{12} (ppb)	12.8	10.6-15.0	2
Choline (ppm)	3,315	3,200-3,430	2
Minerals			
Calcium (percent)	1.27 ± 0.19	0.81-1.6	24
Phosphorus (percent)	1.00 ± 0.08	0.84-1.10	24
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.557	0.479-0.635	2
Sodium (percent)	0.304	0.258-0.349	2
Magnesium (percent)	0.172	0.166-0.177	2
Sulfur (percent)	0.278	0.270-0.285	2
Iron (ppm)	418	409-426	2
Manganese (ppm)	90.8	86.0-95.5	2
Zinc (ppm)	55.1	54.2-56.0	2
Copper (ppm)	12.68	9.65-15.70	2
Iodine (ppm)	2.58	1.52-3.64	2
Chromium (ppm)	1.86	1.79-1.93	2
Cobalt (ppm)	0.57	0.49-0.65	2

(a) One or two batches of feed analyzed were manufactured in January and/or April 1983.
(b) One batch (7/22/81) not analyzed for thiamine.

TABLE N4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminant	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.39 ± 0.17	0.13-0.93	24
Cadmium (ppm) (a)	<0.1		24
Lead (ppm)	1.09 ± 0.72	0.33-2.93	24
Mercury (ppm) (a)	<0.05		
Selenium (ppm)	0.30 ± 0.07	0.16-0.48	24
Aflatoxins (ppb) (a, b)	<10		24
Nitrate nitrogen (ppm) (c)	8.50 ± 4.39	0.6-18.0	24
Nitrite nitrogen (ppm) (c)	2.05 ± 1.28	0.4-5.3	24
BHA (ppm) (d, e)	3.68 ± 2.71	0.4-11.0	24
BHT (ppm) (d)	2.65 ± 1.13	1.2-4.9	24
Aerobic plate count (CFU/g)	70,729 ± 49,351	7,000-210,000	21
Coliform (MPN/g) (f)	731 ± 880	<3-2,400	24
E. coli (MPN/g)	7.50 ± 7.68	<3-23	24
Total nitrosamines (ppb) (g, h)	7.24 ± 6.70	1.8-24.5	22
Total nitrosamines (ppb) (g, i)	17.03 ± 28.20	1.8-101.6	24
N-Nitrosodimethylamine (ppb) (g, j)	5.55 ± 6.07	0.7-20.0	22
N-Nitrosodimethylamine (ppb) (g, k)	13.29 ± 26.86	0.7-99	24
N-Nitrosopyrrolidine (ppb)	1.32 ± 0.81	0.3-3.5	24
Pesticides (ppm)			
α-BHC (a, l)	<0.01		24
β-BHC (a)	<0.02		24
γ-BHC-Lindane (a)	<0.01		24
δ-BHC (a)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (a, m)	<0.01	0.05 (7/14/81)	24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (a, m)	<0.05	0.13 (8/25/81)	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCB's (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (a)	<0.1		24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (n)	0.08 ± 0.05	<0.05-0.25	24
Endosulfan I (a)	<0.01		24
Endosulfan II (a)	<0.01		24
Endosulfan sulfate (a)	<0.03		24

TABLE N4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) Detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: Alfalfa, grains, and fish meal
(d) Source of contamination: Soy oil and fish meal
(e) Two batches contained less than 0.5 ppm.
(f) MPN = most probable number
(g) All values were corrected for percent recovery.
(h) Mean, standard deviation, and range exclude two very high values of 101.6 and 100.3 ppb in batches produced on 1/26/81 and 4/27/81.
(i) Mean, standard deviation, and range include the very high values given in footnote h.
(j) Mean, standard deviation, and range exclude two very high values of 97.9 and 99 ppb in batches produced on 1/26/81 and 4/27/81.
(k) Mean, standard deviation, and range include the high values given in footnote j.
(l) BHC = hexachlorocyclohexane or benzene hexachloride
(m) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(n) Nine batches contained more than 0.05 ppm.

APPENDIX O

DISPOSITION OF DECABROMODIPHENYL OXIDE IN F344/N RATS

APPENDIX O. CHEMICAL DISPOSITION

I. Materials and Methods

A. Chemicals: Unlabeled decabromodiphenyl oxide was obtained from Fluka Chemical Corporation, Hauppauge, New York. No purity was specified. [U-^{14}C]decabromodiphenyl oxide (lot no. 83-127-22-25), with a stated specific activity of 16.9 mCi/mmol (0.0176 mCi/mg), was supplied by Midwest Research Institute. No radiochemical purity was indicated.

The radiochemical purity of the ^{14}C-decabromodiphenyl oxide used in the dosing solutions and formulated diets was assessed by high-performance liquid chromatography (HPLC) with a Waters Chromatograph equipped with a model 6000A pump, a model U6K injector, a model 440 absorbance detector, and a model 730 data module. The following conditions were used:

Sample: 0.010-0.020 ml of solution of ^{14}C-decabromodiphenyl oxide in tetrahydrofuran (THF)
Column: Nova-Pak C_{18}
Solvent: 93% methanol, 1 ml/min
UV wavelength: 254 nm

The effluent was collected in vials, as a series of 1-ml samples, starting immediately after injection of the sample and continuing for 30 minutes. Samples were diluted with 15 ml of ScintiVerse I solution and assayed for radioactivity in a Packard Tricarb Scintillation counter. In this chromatographic system, decabromodiphenyl oxide had a retention time of 20-22 minutes. The percent purity was determined by dividing the disintegrations per minute (dpm) present in the major eluted peak by the total dpm eluted from the column. The radiochemical purity of ^{14}C-decabromodiphenyl oxide was 97.9%-99.2%. Unlabeled decabromodiphenyl oxide was assayed similarly, except that the purity was calculated by dividing the area of the major UV peak by the total area of all peaks not in the chromatogram of the blank. Unlabeled decabromodiphenyl oxide was 92% pure, with other components eluting at 12.9 minutes (1%), 17.5 minutes (2%), and 22.5 minutes (5%).

To allow calculation of the specific activity of the ^{14}C-decabromodiphenyl oxide in the dosing solutions and formulated diets, portions were added to the ScintiVerse I scintillation solution and assayed for radioactivity in a Packard Tri-Carb counter. Feed samples were combusted in a Packard 301 sample oxidizer before assay.

Solutions for intravenous injection were prepared at room temperature, with sonication as necessary, and used immediately after preparation. Rats were injected intravenously in the tail vein with 0.1 ml/100 g of body weight. To determine whether the preparations were homogenous and stable, the concentrations of decabromodiphenyl oxide in the dosing solutions and in the formulated diets were determined, before and after dosing, by HPLC analysis with the system described above, except that amounts of 0.003-0.020 ml were injected. The amount present in each sample was calculated from the area under the major UV-absorbing peaks by relating these values to those of a standard curve. To determine the amounts of decabromodiphenyl oxide in the batches of feed, the compound was extracted with THF before HPLC analysis.

B. Study animals: Seven- to eight-week old F344/N rats were purchased from Charles River Laboratories, Stoneridge, New York. The rats, housed five per cage in suspended, solid-bottom, polycarbonate cages lined with hardwood chips, were quarantined for 3-13 days. Food and water were provided at all times, unless otherwise indicated. Before initiation of each feed study, three to six rats were killed, examined and found to have no evidence of ectoparasites or endoparasites and to have no gross abnormalities. For acclimation to the powdered diet, rats to be exposed by feeding were fed powdered chow for 3 days before day 1 of the feeding studies. On study day 1, rats in studies involving more than three animals were randomized by use of a table of random numbers and were identified by inscribing numbers with a felt-tip marker on the dorsal side of the tail. After exposure, the rats, except those in experiment E, were placed in metabolism cages for the duration of the studies and were killed by exsanguination after anesthetization. Those in experiment E were restrained and, at the end of the experiment, killed by an overdose of ether.

C. Procedures

1. **Experiment A--Uptake and disposition of ^{14}C-decabromodiphenyl oxide in F344/N male rats after exposure in the diet:** Formulated diets containing decabromodiphenyl oxide were prepared by mixing decabromodiphenyl oxide and ^{14}C-decabromodiphenyl oxide, in various proportions, with pulverized Wayne Lab-Blox® feed. Mixing was accomplished by placing bottles containing the chemical, feed, and mixing stones on automatic rollers. Each preparation of feed was mixed until homogeneity was attained. Analysis of each was accomplished by extracting samples with THF and assaying by HPLC. The amounts present were calculated by comparison to a standard curve. The feed preparations were determined to be homogenous and stable by assay of quadruplicate samples before and after the feeding periods. Rats were assigned to six groups of three rats each. The rats, 8 weeks old, weighed 156-184 g on study day 1. The feed was provided to the rats in glass beakers inside porcelain jars. The jars had metal screw caps with a center opening 2.5 cm in diameter. Feed consumption was measured daily, beginning with the acclimation period. Fresh feed was supplied each day. Each group of rats was fed the standard diet, which contained unlabeled decabromodiphenyl oxide, on days 1-7 and 9-11 and the study diet, containing ^{14}C-decabromodiphenyl oxide, on day 8. Group I received feed containing the highest concentration of decabromodiphenyl oxide (5.11%) and group VI, the lowest (0.0238%).

   Urine and feces were collected separately each day on study days 9-12. On day 12, tissues were collected separately from each rat. A portion of the collected blood was centrifuged to obtain plasma. The following tissues were collected, blotted on filter paper (if appropriate), wrapped in foil, frozen on dry ice, and stored frozen until analysis: liver, kidney, lung, voluntary muscle, fat, skin (ear), brain, gut contents, and gut tissue.

   For assay of radioactivity, the total collection of feces from each rat was dried at room temperature for 3 days, weighed, and pulverized in a Salton Quick Mill grinder (Salton, Inc., Bronx, New York). Quadruplicate portions of each collection were combusted in the sample oxidizer before assay. Portions of urine, plasma, and whole blood were placed in combustion cups and allowed to dry overnight before combustion and assay for radioactivity. Portions of fat were combusted and assayed without drying. No other types of collected samples were assayed.

2. **Experiment B--Disposition of ^{14}C-decabromodiphenyl oxide after intravenous injection in F344/N male rats:** Five rats, 8.5 weeks old and weighing 150-171 g on study day 1, were used. They were injected intravenously in the tail vein with ^{14}C-decabromodiphenyl oxide in THF:Emulphor:water (1:1:2, v/v/v). Analysis of the dosing solution by HPLC revealed that it was not stable. Before dosing, the concentration was 0.533 ± 0.019 mg/ml (8,150 nCi/ml) and after dosing, 0.429 ± 0.010 mg/ml (6,580 nCi/ml). Although a few tissues were collected and analyzed as described in experiment A, the results were compromised by the instability of the dosing solution.

3. **Experiment C--Uptake and disposition of ^{14}C-decabromodiphenyl oxide in F344/N male rats at 24, 48, and 72 hours after exposure:** Rats were assigned to six groups of three rats each. The 8-week-old rats weighed 149-165 g on study day 1. Feed containing high (4.80%) and low (0.0277%) concentrations of labeled or unlabeled decabromodiphenyl oxide were prepared and characterized as described for experiment A. The preparations were determined to be homogenous and stable. Groups I-III were fed a diet containing the higher amount of decabromodiphenyl oxide, and groups IV-VI were fed a diet containing the lower amount. Rats were killed as follows: group I and IV, on day 10; groups II and V, on day 11; and groups III and VI, on day 12. Tissue and other samples were collected as described in experiment A; in addition, the spleen was collected.

   Samples of whole blood, plasma, urine, and feces were assayed as described for experiment A. Portions of other tissues and gut contents were assayed after homogenization in 9 volumes of water after combustion.

   To determine the extractability of ^{14}C-decabromodiphenyl oxide from feces, a solution (THF:Emulphor:water, 2:1:2, v/v/v) containing this compound was added to feces from F344/N rats, and the feces were dried and pulverized. To quadruplicate 0.5-g portions, 5 ml of water was added, and the preparations were sonicated for 15 minutes. THF (10 ml) and benzene (10 ml) were added, and the preparations were shaken for 60 minutes and centrifuged. The solid material was further extracted, twice with 10 ml of benzene and three times with 10 ml of THF. The pooled benzene extracts were washed with 5 ml of water. The upper phase, the benzene extract, was retained for analysis. The lower phase was added to the combined THF extracts, and 5 ml of benzene was added. The resulting upper layer was retained as the THF extract. The percent extractability was calculated by dividing the amounts in the benzene and THF extracts by the total amount present in all fractions and multiplying by 100. The value derived was 99.7% ± 0.2%.

   To determine the extent of metabolism of ^{14}C-decabromodiphenyl oxide by rats fed decabromodiphenyl oxide and ^{14}C-decabromodiphenyl oxide, pulverized fecal samples collected on days 9-11 were pooled for all rats within a dose group. (There was no appreciable radioactivity in the feces for day 12.) Each of the pooled fecal samples was mixed on a roller apparatus, and portions (0.5 g) were extracted as described above. Of the total radioactivity present, 99.4% ± 0.2% was in the benzene and THF extracts. The extracts were evaporated to dryness. The benzene extracts were dissolved (or suspended) in 4 ml of THF/benzene (1:1, v/v), and the THF extracts in 1 ml of THF. The benzene extracts from rats fed the two highest doses were cloudy with a white substance. Both types of extracts were exposed to the HPLC procedure described above.

To determine the extractability of ^{14}C-decabromodiphenyl oxide from liver, a portion of liver from an F344/N rat was homogenized in 9 volumes of water, ^{14}C-decabromodiphenyl oxide was added, and the preparation was homogenized again and lyophilized to dryness. For each of four portions, extractions were performed with three separate 5-ml portions of THF. The extracts were allowed to evaporate to dryness before radioassay. The remaining pellets were also subjected to radioassay. The percent extractability was calculated by dividing the amount in the extracts by this amount plus the amount in the pellet and multiplying by 100. The value derived was 86.4% ± 1.9%.

In some experiments, a model 1040A photodiode assay spectrophotometric detector (Hewlett-Packard, Palo Alto, California) was used to obtain UV spectra of components eluting from the HPLC column.

4. **Experiment D--Disposition of ^{14}C-decabromodiphenyl oxide in male F344/N rats 72 hours after intravenous injection:** Rats weighed 134-137 g and were 7.5 weeks old. Three rats were injected intravenously with ^{14}C-decabromodiphenyl oxide (1.07 mg/kg, 0.0173 mCi/kg) in THF:Emulphor:water (2:1:2, v/v/v). As determined by the HPLC assay described in I.A., the dosing solution was found to be stable and homogeneous. Urine and feces were collected daily for 3 days. At 72 hours after dosing, tissue and other samples, including spleen and tail, were collected as described in experiment A. Feces (0-48 h and 48-72 h) were pooled separately, extracted, and assayed.

5. **Experiment E--Biliary excretion of ^{14}C-decabromodiphenyl oxide after intravenous administration to F344/N rats:** Six rats (165-181 g, 8.5 weeks old) were anesthetized with pentobarbital (30 mg/kg, intraperitoneally), and their bile ducts were cannulated. The rats were allowed to recover from the anesthesia before ^{14}C-decabromodiphenyl oxide (0.947 mg/kg, 0.016 mCi/kg) in THF:Emulphor:water (2:1:2, v/v/v) was injected intravenously. As determined by the HPLC assay described in I.A., the dosing solution was found to be stable and homogeneous. Bile was collected at designated times over a 4-hour period. During bile collection, each rat was provided water from a bottle placed within reach of the animal. The rats were killed 4 hours after dosing, and their tails were collected and homogenized in 9 volumes of water. Measured portions of each bile sample and portions of the tail homogenates were assayed for radioactivity after combustion.

D. Results

Experiment A: Rats were fed, on days 1-7 and 9-11, unlabeled decabromodiphenyl oxide in amounts ranging from 238 to 51,100 ppm in the diet and on day 8 with ^{14}C-decabromodiphenyl oxide in similar amounts (Table O1). Over the entire period, rats in group I (51,100 ppm) consumed significantly less food ($P < 0.025$) than those in groups III, IV, V, and VI (238 ppm). For day 8, however, the difference in consumption was not significant ($0.10 < P < 0.25$) by a one-way analysis of variance.

In the 72 hours after the diet containing ^{14}C-decabromodiphenyl oxide was removed, recovery of radiolabel in the feces ranged from 91.3% ± 4.0% to 101% ± 4% of the amount ingested (Table O2). Recovery was not related to the dose of decabromodiphenyl oxide. Although the liver contained only small amounts of radioactivity, rats fed the smaller amounts of unlabeled decabromodiphenyl oxide had a greater percentage of radioactivity in this organ. The amounts ranged from 0.008% of the dose for group I to 0.064% for group VI. Although the amount of radioactivity in fat was also low, there was a tendency for rats fed the smaller amounts of unlabeled decabromodiphenyl oxide to have more radioactivity in this tissue. The amounts ranged from 0.072% for group I to 0.157% for group VI, with the value for Group IV (0.090%) being out of line.

A notable result of exposure to decabromodiphenyl oxide was that the liver weights of rats were significantly greater ($P<0.001$) for those consuming diets with large concentrations of decabromodiphenyl oxide (Figure 14). For the two lowest concentrations, the weights were 9.65 ± 0.92 g and 9.80 ± 0.19 g and, for the two highest concentrations, 13.8 ± 0.9 g and 13.7 ± 1.3 g.

Experiment B: As noted above, the results from this experiment were compromised by the instability of the dosing solution. The only results of note were that 15.8% ± 4.3% of the dose (based on the predosing value) was found in the lungs of these rats 72 hours after dosing. Such a large amount in this tissue, which collects particulate material injected into the bloodstream, tends to confirm that precipitation of ^{14}C-decabromodiphenyl oxide in the dosing solution had occurred. This experiment was repeated with a different dosing formulation (experiment D).

TABLE O1. FEED CONSUMPTION, DECABROMODIPHENYL OXIDE CONCENTRATION IN THE DIET, AND DECABROMODIPHENYL OXIDE CONSUMED BY F344/N RATS

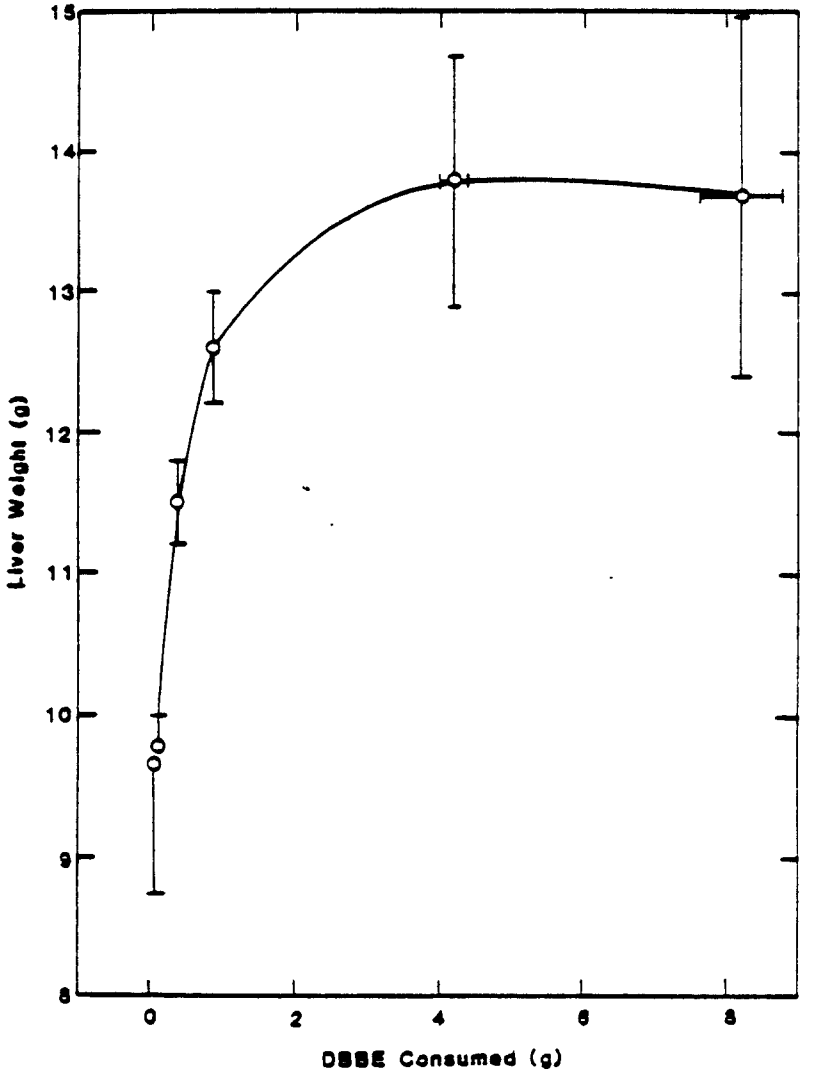
Rat Group	Feed Consumed (g/day)	Concentration of Decabromodiphenyl Oxide in Feed (ppm)			Decabromodiphenyl Oxide Consumed on Day 8	
		Unlabeled (a)	Labeled (b)	nCi/g (b)	mg	nCi[^{14}C]
I	(c,d) 14.4 ± 1.0	51,100	48,600	214 ± 5	716 ± 58	3,150 ± 250
II	15.1 ± 1.1	25,400	24,400	219 ± 28	370 ± 10	3,320 ± 90
III	16.5 ± 0.7	4,730	5,000	232 ± 23	78.0 ± 5.1	3,620 ± 240
IV	17.1 ± 0.3	2,510	2,490	212 ± 19	43.9 ± 2.4	3,740 ± 200
V	16.9 ± 0.5	496	521	206 ± 8	8.72 ± 0.40	3,460 ± 160
VI	17.0 ± 1.5	238	261	215 ± 23	4.37 ± 0.68	3,610 ± 560

(a) Concentration of unlabeled decabromodiphenyl oxide in feed, fed on days 1-7 and 9-11; the values are the averages of those derived by analysis before and after feeding.
(b) Concentration of ^{14}C-decabromodiphenyl oxide in feed, fed on day 8; the values are the averages of those derived by analysis before and after feeding.
(c) Mean ± standard deviation for three rats
(d) Significantly less than ($P < 0.025$) values for groups III, IV, V, and VI

TABLE O2. DISPOSITION OF RADIOACTIVITY IN F344/N RATS 72 HOURS AFTER EXPOSURE TO ^{14}C-DECABROMODIPHENYL OXIDE IN THE DIET ON DAY 8

Rat Group	Feces (days 8-12) (percent of dose)	Liver (day 12) (percent of dose)	Fat (a) (day 12) (percent of dose)
I	(b) 95.5 ± 9.9	0.008 ± 0.002	0.072 ± 0.041
II	93.0 ± 5.0	0.006 ± 0.001	0.088 ± 0.022
III	91.3 ± 4.0	0.011 ± 0.003	0.126 ± 0.017
IV	101 ± 4	0.016 ± 0.003	0.090 ± 0.027
V	100 ± 1	0.043 ± 0.010	0.161 ± 0.026
VI	97.7 ± 5.8	0.064 ± 0.003	0.157 ± 0.007

(a) Considered to be 7% of total body weight
(b) The values are means ± standard deviation for three rats.



The values on the horizontal axis are the total amounts of decabromodiphenyl oxide and ^{14}C-decabromodiphenyl oxide consumed (days 1-12). The points represent the means, and the vertical and horizontal bars, the standard deviations.

FIGURE 14. EFFECT OF EXPOSURE TO DECABROMODIPHENYL OXIDE ON LIVER WEIGHTS OF F344/N RATS

Experiment C: These rats were fed a diet containing unlabeled decabromodiphenyl oxide on days 1-7 and day 9 (groups I and IV), days 1-7 and days 9-10 (groups II and V), or days 1-7 and days 9-11 (groups II and VI) (Table O3). For groups I-III, unlabeled decabromodiphenyl oxide concentration was 48,000 ppm, and for groups IV-VI, 277 ppm unlabeled decabromodiphenyl oxide. A diet containing correspondingly similar amounts of ^{14}C-decabromodiphenyl oxide was fed on day 8. Although for groups I-II the mean values for feed consumption were lower than those for groups IV-VI, the difference was not significantly different. The amount of ^{14}C-decabromodiphenyl oxide consumed was in proportion to the content of the diet. The radioactivity ingested ranged from 3,070 ± 60 nCi to 3,590 ± 140 nCi, but the amounts were not related to the concentrations of decabromodiphenyl oxide in formulated diets.

Recovery of radioactivity in the feces ranged from 82.5% ± 4.7% to 86.4% ± 8.5% and was not related to the dietary concentration of decabromodiphenyl oxide or to the time the rats were killed (24, 48, or 72 hours) after consumption of ^{14}C-decabromodiphenyl oxide (Table O4). For both doses, the percent of the dose remaining in the gut contents (less than 4%) decreased with time the rats were killed after exposure to ^{14}C-decabromodiphenyl oxide. A similar observation was noted for gut tissue, which contained less than 0.04% of the dose.

At 72 hours, the liver contents of radioactivity in rats exposed to decabromodiphenyl oxide in the diet were low (0.016% of the dose for rats fed 48,000 ppm decabromodiphenyl oxide and 0.109% for rats fed 277 ppm decabromodiphenyl oxide). These values are consistent with the values derived in experiment A. For rats fed the low amount of decabromodiphenyl oxide, the liver contained 0.449% ± 0.010% of the dose of ^{14}C-decabromodiphenyl oxide at 24 hours after feeding and 0.213% ± 0.016% at 48 hours. Also consistent with results for experiment A, liver weights for rats receiving the high dose were 12.5 ± 0.7 g; those from rats given the low dose were 8.68 ± 0.69 g.

The maximum percent of dose in other organs and tissues was as follows: kidney, 0.016%; spleen, 0.003%; lungs, 0.011%; brain, less than 0.001%; muscle (considered to be 50% of body weight), 0.248%; fat (considered to be 7% of body weight), 0.077%; and skin (considered to be 16% of body weight), 0.252% (Table O4). For all of these tissues, the maximum value were for rats in groups fed the smaller dose.

The remaining portions of the liver homogenates from rats in group IV were lyophilized to dryness and extracted three times with 5 ml of THF. The extract containing the most radioactivity was purified on a Sep-Pak C_{18} cartridge and analyzed by HPLC under the conditions described above, except that 0.05 ml of sample was injected. Fractions of 1 ml were collected and assayed for radioactivity, revealing that 81% of the radioactivity eluted at the retention time of decabromodiphenyl oxide (23 minutes). The remainder of the sample was further purified by HPLC and passage through a Sep-Pak cartridge. A final HPLC analysis allowed a UV spectrum to be determined for the radioactive material. The spectrum was identical to that for decabromodiphenyl oxide (Figure 15).

In extracts of feces, three main metabolite peaks, eluting at 3-6 minutes, 6-12 minutes, and 12-17 minutes, were evident; decabromodiphenyl oxide eluted at 17-25 min (Table O5). The percent of metabolites present tended to increase as the concentration of decabromodiphenyl oxide in the diet increased. For samples derived from rats fed larger amounts, however, the results are equivocal due to the low recovery of injected radioactivity. Such low recovery was probably due to precipitation of decabromodiphenyl oxide and possibly decabromodiphenyl oxide metabolites when solutions approaching saturation were injected into the HPLC instrument.

TABLE O3. FEED CONSUMPTION, DECABROMODIPHENYL OXIDE CONCENTRATION IN THE DIET, AND DECABROMODIPHENYL OXIDE CONSUMED BY F344/N RATS 24, 48, OR 72 HOURS AFTER EXPOSURE

Rat Group	Food Consumed (g/day)	Concentration of Decabromodiphenyl Oxide in Feed			Decabromodiphenyl Oxide Consumed on Day 8	
		Unlabeled (a)	Labeled (b)	nCi/g (b)	mg	nCi [^{14}C]
I	(c) 12.6 ± 2.3	48,000	48,500	219 ± 9	(c) 755 ± 129	(c) 3,410 ± 580
II	12.9 ± 2.7	48,000	48,500	219 ± 9	794 ± 32	3,590 ± 140
III	13.8 ± 2.5	48,000	48,500	219 ± 9	744 ± 250	3,360 ± 1,130
IV	14.3 ± 2.3	277	259	215 ± 5	3.70 ± 0.07	3,070 ± 60
V	15.7 ± 2.0	277	259	215 ± 5	4.27 ± 0.51	3,540 ± 420
VI	15.8 ± 2.4	277	259	215 ± 5	4.06 ± 1.15	3,370 ± 960

(a) Concentration (ppm) of unlabeled decabromodiphenyl oxide in the feed on days 1-7 and 9 (groups I and IV), days 1-7, and days 9-10 (groups II and V), or days 1-7 and days 9-11 (groups III and VI). Values are the averages of those derived by analysis before and after feeding.
(b) Concentration (ppm) of ^{14}C-decabromodiphenyl oxide in the feed for day 8. Values for ppm decabromodiphenyl oxide are averages of those derived by analysis before and after feeding. The values for nCi/g are the mean ± standard deviation for four separate determinations.
(c) Mean ± standard deviation for three rats

TABLE O4. DISPOSITION OF RADIOACTIVITY IN RATS 24, 48, OR 72 HOURS AFTER EXPOSURE TO ^{14}C-DECABROMODIPHENYL OXIDE IN THE DIET ON DAY 8 (a)

Tissue or Sample	Group I (kill day = 10) Percent of Dose	nCi/g or ml	Group II (kill day = 11) Percent of Dose	nCi/g or ml	Group III (kill day = 12) Percent of Dose	nCi/g or ml
Urine	(b) 0.004 ± 0.002	(c)	0.007 ± 0.003	(c)	0.008 ± 0.005	(c)
Feces	85.3 ± 7.1	(c)	85.6 ± 4.5	(c)	85.1 ± 5.5	(c)
Gut contents	3.32 ± 1.65	(c)	0.552 ± 0.873	(c)	0.059 ± 0.039	(c)
Gut tissue	0.031 ± 0.016	0.255 ± 0.132	0.012 ± 0.011	0.095 ± 0.086	0.001 ± 0.001	0.013 ± 0.011
Liver	0.007 ± 0.001	0.019 ± 0.005	0.007 ± 0.006	0.019 ± 0.015	0.016 ± 0.006	0.040 ± 0.004
Kidneys	<0.001	0.007 ± 0.004	<0.001	0.009 ± 0.003	<0.001	0.009 ± 0.005
Lungs	<0.001	0.015 ± 0.006	<0.001	0.010 ± 0.006	0.001 ± 0.001	0.022 ± 0.005
Spleen	<0.001	0.031 ± 0.018	<0.001	0.038 ± 0.025	<0.001	0.022 ± 0.011
Brain	<0.001	<0.01	<0.001	<0.01	<0.001	<0.01
Muscle (d)	0.015 ± 0.014	0.005 ± 0.005	0.014 ± 0.005	0.006 ± 0.002	0.008 ± 0.012	0.004 ± 0.006
Skin (e)	0.099 ± 0.018	0.115 ± 0.024	0.049 ± 0.017	0.061 ± 0.023	0.036 ± 0.013	0.038 ± 0.008
Fat (f)	0.040 ± 0.015	0.107 ± 0.036	0.018 ± 0.004	0.049 ± 0.010	0.012 ± 0.012	0.025 ± 0.022
Blood (g)	0.003 ± 0.001	0.006 ± 0.002	0.001 ± 0.002	0.003 ± 0.004	0.014 ± 0.011	0.023 ± 0.009
Plasma (h)	0.001 ± 0.001	0.003 ± 0.003	0.001 ± 0.001	0.002 ± 0.002	0.006 ± 0.002	0.019 ± 0.003
Total recovery (percent of dose)	88.8		86.3		85.3	

Tissue or Sample	Group IV (kill day = 10) Percent of Dose	nCi/g or ml	Group V (kill day = 11) Percent of Dose	nCi/g or ml	Group VI (kill day = 12) Percent of Dose	nCi/g or ml
Urine	0.012 ± 0.005	(c)	0.011 ± 0.003	(c)	0.012 ± 0.007	(c)
Feces	86.4 ± 8.5	(c)	83.9 ± 0.9	(c)	82.5 ± 4.7	(c)
Gut contents	1.82 ± 0.36	(c)	0.518 ± 0.413	(c)	0.093 ± 0.029	(c)
Gut tissue	0.038 ± 0.004	0.302 ± 0.023	0.021 ± 0.000	0.181 ± 0.023	0.011 ± 0.001	0.107 ± 0.024
Liver	0.449 ± 0.010	1.62 ± 0.012	0.213 ± 0.016	0.846 ± 0.057	0.109 ± 0.029	0.440 ± 0.203
Kidneys	0.016 ± 0.002	0.407 ± 0.015	0.016 ± 0.000	0.430 ± 0.066	0.013 ± 0.001	0.295 ± 0.059
Lungs	0.011 ± 0.001	0.457 ± 0.022	0.007 ± 0.000	0.321 ± 0.051	0.004 ± 0.001	0.167 ± 0.055
Spleen	0.003 ± 0.001	0.273 ± 0.030	0.002 ± 0.000	0.160 ± 0.017	0.001 ± 0.000	0.074 ± 0.028
Brain	<0.001	<0.01	<0.001	<0.01	<0.001	<0.01
Muscle (d)	0.198 ± 0.024	0.006 ± 0.001	0.244 ± 0.016	0.009 ± 0.001	0.248 ± 0.007	0.008 ± 0.002
Skin (e)	0.252 ± 0.018	0.257 ± 0.022	0.207 ± 0.031	0.232 ± 0.062	0.136 ± 0.018	0.144 ± 0.042
Fat (f)	0.062 ± 0.033	0.145 ± 0.073	0.077 ± 0.022	0.196 ± 0.073	0.048 ± 0.001	0.115 ± 0.026
Blood (g)	0.043 ± 0.006	0.077 ± 0.009	0.024 ± 0.010	0.048 ± 0.023	0.026 ± 0.008	0.050 ± 0.024
Plasma (h)	0.035 ± 0.004	0.112 ± 0.010	0.019 ± 0.006	0.067 ± 0.019	0.021 ± 0.004	0.068 ± 0.007
Total recovery (percent of dose)	89.3		85.3		83.2	

(a) Rats were fed unlabeled decabromodiphenyl oxide in the diet on days 1-7, ^{14}C-decabromodiphenyl oxide in the diet on day 8, and unlabeled decabromodiphenyl oxide in the diet through the kill day.
(b) Tetrahydrofuran extract mean ± standard deviation for three rats
(c) Not calculated
(d) Considered to be 50% of body weight
(e) Considered to be 16% of body weight
(f) Considered to be 7% of body weight
(g) Considered to be 9% of body weight
(h) Considered to be 5% of body weight

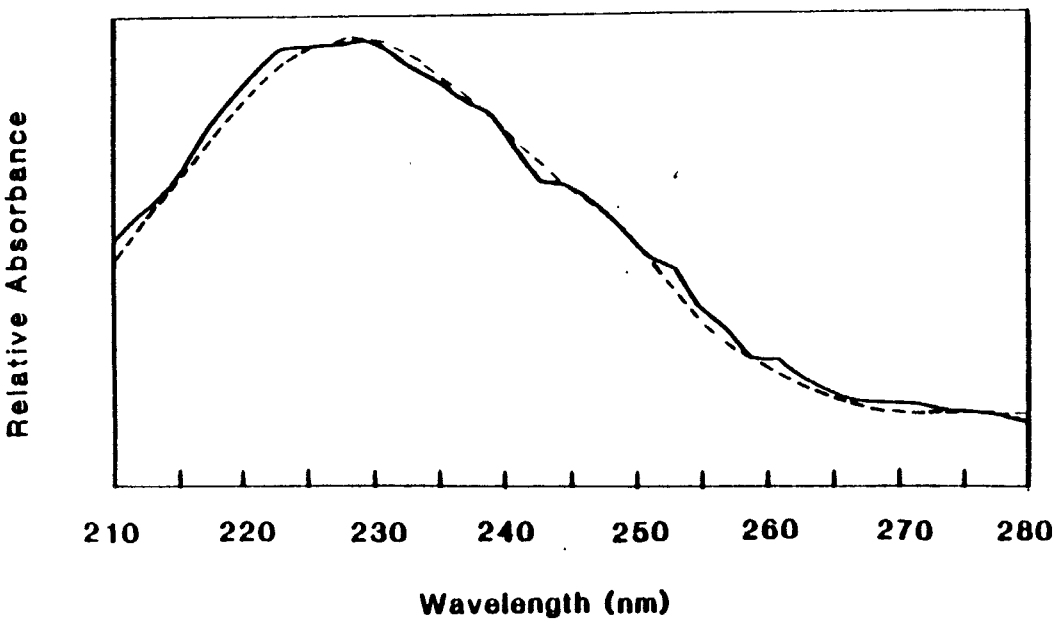


FIGURE 15. ULTRAVIOLET SPECTRA OF REFERENCE DECABROMODIPHENYL OXIDE (——) AND OF THE ISOLATE FROM THE LIVERS (-------) OF F344/N RATS EXPOSED TO DECABROMODIPHENYL OXIDE IN THE DIET

TABLE O5. RECOVERY OF DECABROMODIPHENYL OXIDE AND METABOLITES IN EXTRACTS OF FECES OF RATS FED DIETS CONTAINING DECABROMODIPHENYL OXIDE

Concentration of Decabromodiphenyl Oxide in Diet (ppm)	Extract	Retention Time (a)				Radioactivity (nCi)	
		Metabolites			Decabromodiphenyl Oxide		
		3-6 min	6-12 min	12-17 min	17-25 min	Injected	Recovered
50,000	Benzene (b)	5.47 (4.57)	5.34 (6.04)	9.87 (3.31)	62.2 (69.0)	0.93 (0.37)	0.16 (0.22)
	(c) THF	1.82 (2.50)	2.64 (2.40)	1.80 (2.58)	9.81 (9.52)	0.78 (0.15)	0.11 (0.18)
Total recovery		7.29 (7.07)	7.98 (8.44)	11.7 (5.89)	72.0 (78.5)		
25,000	Benzene	11.7 (8.90)	7.51 (4.65)	5.38 (9.15)	57.8 (59.7)	1.16 (0.46)	0.41 (0.27)
	THF	3.40 (2.11)	1.87 (1.00)	2.08 (2.09)	10.3 (12.4)	0.99 (0.20)	0.29 (0.15)
Total recovery		15.1 (11.0)	9.38 (5.65)	7.46 (11.2)	68.1 (72.1)		
5,000	Benzene	3.26	2.57	2.54	80.4	2.80	1.57
	THF	0.36	0.05	0.17	10.6	0.71	0.44
Total recovery		3.62	2.62	2.71	91.0		
2,500	Benzene	5.28	3.47	2.86	75.5	2.33	2.23
	THF	0.74	0.65	0.54	11.0	0.69	0.67
Total recovery		6.02	4.12	3.40	86.5		
500	Benzene	2.70	2.27	1.55	74.2	4.08	3.78
	THF	0.72	0.56	0.69	16.8	0.95	1.07
Total recovery		3.42	2.83	2.24	91.0		
250	Benzene	0.00	0.00	0.00	86.6	4.51	3.61
	THF	0.67	0.31	0.52	11.8	0.69	0.71
Total recovery		0.67	0.31	0.52	98.4		

(a) High-performance liquid chromatographic analysis
(b) Percent radioactivity in sample; the numbers in parentheses represent values obtained after dilution and reassay of the extracts.
(c) Tetrahydrofuran

To determine if the loss was associated only with decabromodiphenyl oxide or with decabromodiphenyl oxide and its metabolites, the benzene and THF extracts for samples from feces of rats exposed to diets containing 50,000 and 25,000 ppm decabromodiphenyl oxide were diluted by 2.5-fold and fivefold, respectively. The previously insoluble material in the benzene extracts, presumably decabromodiphenyl oxide, went into solution. Although recovery of radioactivity from injected portions of the benzene extracts was higher than the previous recovery, it was still incomplete (Table O5). No further dilutions were possible due to the reduced amount of radioactivity present. The relative amounts of decabromodiphenyl oxide and decabromodiphenyl oxide metabolites did not change drastically, an indication that, on injection of these extracts, both decabromodiphenyl oxide and its metabolites were being lost in equal proportions.

Experiment D: At 72 hours after an intravenous dose of ^{14}C-decabromodiphenyl oxide (1.07 mg/kg), feces plus gut contents contained 74% of the dose (Table O6). The radioactivity appeared to be present in relatively high and approximately equal concentrations in the liver, kidney, and lung. Only traces of radioactivity were in the urine, spleen, and brain. Although the tails contained an average of 9.5% of the dose, there was little difference for the three individual rats, an indication that all three received equivalent amounts in the bloodstream. Muscle and skin retained 12.9% and 7.25% of the dose, respectively. Relative to tissues other than brain and spleen, the concentration of radioactivity in blood was low (1.36 nCi/ml); most of that present was in the plasma (1.97 nCi/ml).

Extraction of the feces of these rats showed that most of the excreted material was decabromodiphenyl oxide metabolites (Table O7). Unchanged decabromodiphenyl oxide constituted 36.5% of the total for the 0- to 48-hour collection period and 40.4% for the 48- to 72-hour period.

Experiment E: Although two of the three rats examined in the initial health check for this experiment had in their livers a single scar-like focus, three additional rats examined had no such defects. Examination of the livers of rats used in the experiment revealed no abnormalities.

As determined by assay of the tail, one of the six rats with biliary cannulas was improperly injected. The values derived for bile from this rat were not used in further calculations. For the remaining five rats, the rate of excretion and the cumulative excretion in the bile of radioactivity from ^{14}C-decabromodiphenyl oxide is shown in Figure 16. Of the dose administered, 7.17% ± 1.01% appeared in the bile in 4 hours. From 1.5 to 4 hours, the rate of excretion was the same, 2.2% of the dose per hour. Tails of the five rats contained 5.38% ± 2.11% of the administered dose, an indication that each received an adequate dose.

TABLE O6. DISTRIBUTION OF RADIOACTIVITY IN F344/N RATS ADMINISTERED ^{14}C-DECABROMODIPHENYL OXIDE BY INTRAVENOUS INJECTION

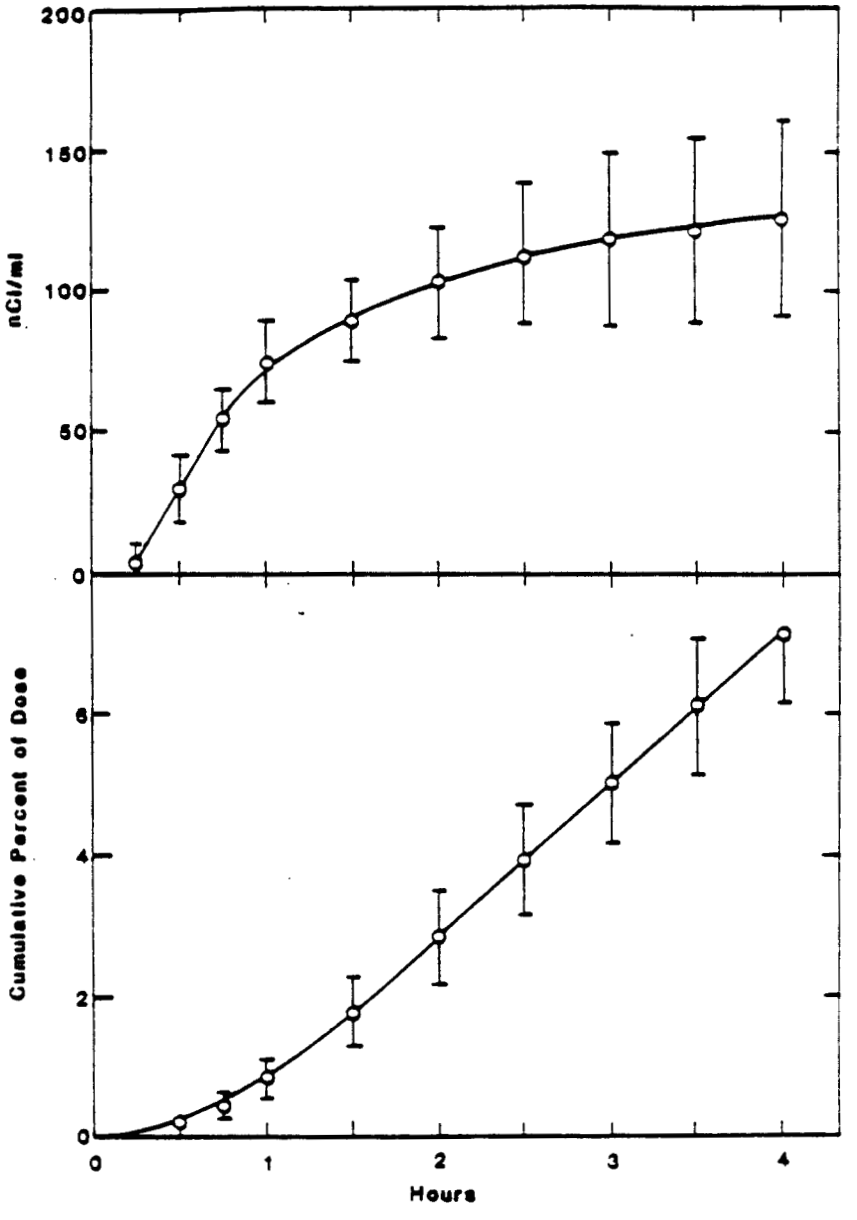
Tissue or Sample	Percent of Dose (a)	nCi/g or ml
Urine	(b) 0.129 ± 0.007	(c) --
Feces	70.0 ± 2.5	--
Gut contents	4.21 ± 1.71	--
Gut tissue	0.853 ± 0.127	--
Tail	9.50 ± 0.89	--
Liver	4.27 ± 1.05	15.1 ± 3.9
Kidney	0.697 ± 0.073	13.7 ± 1.4
Lung	0.361 ± 0.030	13.3 ± 1.4
Spleen	0.027 ± 0.004	1.78 ± 0.42
Brain	0.047 ± 0.003	0.69 ± 0.04
Muscle (d)	12.9 ± 1.1	4.32 ± 0.68
Skin (e)	7.25 ± 0.76	7.53 ± 0.41
Fat (f)	2.99 ± 1.94	7.33 ± 5.25
Blood (g)	0.732 ± 0.053	1.36 ± 0.17
Plasma (h)	0.589 ± 0.068	1.97 ± 0.35

(a) 72 hours after intravenous injection
(b) The numbers are the means ± standard deviation for three rats.
(c) Not calculated
(d) Considered to be 50% of body weight
(e) Considered to be 16% of body weight
(f) Considered to be 7% of body weight
(g) Considered to be 9% of body weight
(h) Considered to be 5% of body weight

TABLE O7. RECOVERY OF DECABROMODIPHENYL OXIDE AND METABOLITES FROM FECES OF F344/N RATS ADMININISTERED DECABROMODIPHENYL OXIDE BY INTRAVENOUS INJECTION

Collection Time	Extract	Retention Time (a)			
		Metabolites			Decabromodiphenyl Oxide
		3-6 min	6-12 min	12-17 min	17-25 min
(b) 0-48	Benzene	4.9 ± 1.6	0.5 ± 0.1	2.1 ± 0.6	28.5 ± 8.2
	(c) THF	(c) 19.7 ± 4.2	1.7 ± 0.5	2.5 ± 0.9	8.0 ± 2.4
	Total	24.6	2.2	4.6	36.5
(b) 48-72	Benzene	10.4	6.9	7.0	36.3
	THF	18.7	4.3	2.5	4.1
	Total	29.1	11.2	9.5	40.4

(a) From HPLC analysis
(b) The 0-48 hour fecal collections for the three rats were assayed separately. The 48-72 hour fecal collections were combined before assay.
(c) The numbers are the means ± standard deviation of the percent of radioactivity in the samples for three rats.



The points represent the means and the vertical bars, the standard deviations. For one point, the standard deviation was too small to be displayed.

FIGURE 16. BILIARY EXCRETION OF RADIOACTIVITY IN F344/N RATS ADMINISTERED DECABROMODIPHENYL OXIDE BY INTRAVENOUS INJECTION

APPENDIX P

DATA AUDIT SUMMARY

APPENDIX P. DATA AUDIT SUMMARY

An audit was conducted on the archival data and pathology materials for the toxicology and carcinogenesis studies of decabromodiphenyl oxide in rats and mice. This study was performed at Hazleton Laboratories America, Vienna, Virginia, under a subcontract with Tracor Jitco, Inc., from the National Cancer Institute. The studies were conducted from July 1980 to July 1982 for mice and from September 1980 to September 1982 for rats and was initiated before the requirement of compliance to Good Laboratory Practice standards by NTP in October 1980. The audit was conducted at Dynamac Corporation and at the NTP Archives in Research Triangle Park, North Carolina. The audit involved the following Dynamac personnel: L. Keifer, Ph.D.; J. Konz, M.S.P.H.; R. Schueler, D.V.M.; M. Perrault, B.S.; C. Sexsmith, B.S.; and Eva Zurek. An additional participant was C. Veigle (Pathology Associates, Inc.).

The full audit report has been reviewed and approved by NTP personnel and is on file at the National Institute of Environmental Sciences, Research Triangle Park, North Carolina. The audit consisted of an indepth review of the data and pathology materials collected during the conduct of these studies as well as review of the correspondence. For the inlife toxicology data, this review involved examination of 100% of the records on animal receipt and husbandry, mortality, environmental conditions, and dosing and examination of body weight and clinical observation data for 10% of the animals. In the review of the chemistry data, all of the available records associated with initial analysis and stability testing by Midwest Research Institute were examined. Records pertaining to bulk chemical analysis and diet preparation and analysis by the study laboratory were examined. The audit of the pathology materials included review of 100% of the Individual Animal Data Records (IADR's) for gross observation to microscopic diagnosis correlation and clerical errors, examination of the wet tissues of 10% of the animals for unidentified lesions, correct animal identification, correlation of slides and tissue blocks for all control and high dose groups, and verification of the reported pathology on a 10% sample of the animals.

Review of the toxicologic data found no problems that affected interpretation of the study. Temperature and humidity readings outside the range specified in the protocol were recorded frequently during several months of the study. No relationship was found between the periods of poor environmental control and mortality. A review of the available chemistry data found no discrepancies.

Although several discrepancies were noted between gross observation and microscopic diagnosis records, these were adequately resolved by subsequent examination of wet tissues and slides.

Overall, the items identified during the audit did not substantially reduce confidence in the data reported. Some problems and discrepancies were identified and discussed in the audit report; most of these were adequately resolved.

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