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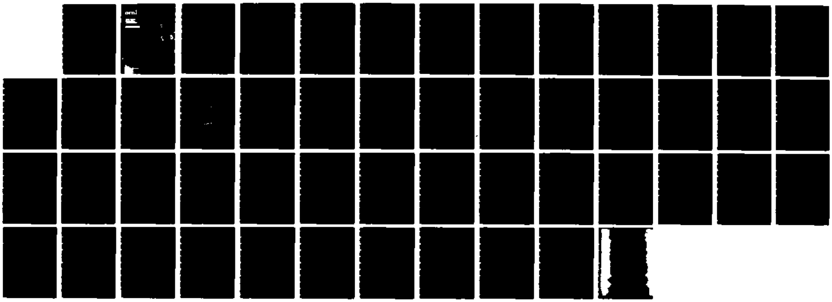

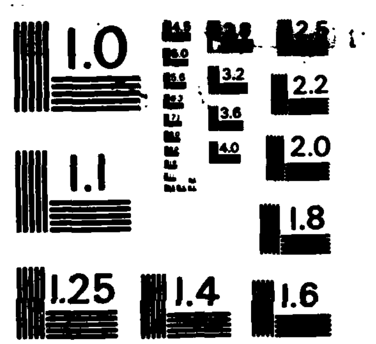

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Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures

DIESEL FUEL SMOKE PARTICULATE DOSIMETRY IN SPRAGUE-DAWLEY RATS

R. A. Jenkins
D. L. Manning
M. P. Maskarinec
J. H. Moneyhun
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S. Lock

SEPTEMBER 1983

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

Army Project Order Nos. 9600 and 0027

Project Officer: James C. Eaton

Health Effects Research Division
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The use of decachlorobiphenyl (DCBP) as a dosimetric tracer for a diesel fuel smoke aerosol is described. DCBP added to the fuel prior to aerosolization is distributed solely into the particle phase of the resulting smoke. DCBP is distributed uniformly throughout the aerosol particles, regardless of particle size. Determinations of DCBP in tissue extracts of rats exposed to DCBP-containing smoke were performed by purifying the extracts on activated Florisil, and subsequently analyzing them by gas/liquid chromatography with electron capture detection.

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20. The largest internal amounts of smoke tracer were found in the lungs. Animals exposed at concentration-time products (Ct) of 8 had between 2 and 4 mg smoke in their lungs, while animals exposed at Ct=12 had between 3 and 6 mg smoke in their lungs. At a given exposure duration, animals exposed at high smoke concentrations had greater levels of tracer deposited in their lungs. Tracer found in the upper respiratory tract accounted for less than 1.5 percent of the total internal dose, while that found in the digestive system accounted for approximately 30% of the total internal dose. Deposition fractions, the fraction of inhaled particles which is actually retained, ranged from 4 to 8 percent, values which were similar to those determined in other studies.

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Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures

DIESEL FUEL SMOKE PARTICULATE DOSIMETRY IN SPRAGUE-DAWLEY RATS

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EXECUTIVE SUMMARY

The use of decachlorobiphenyl (DCBP) as a dosimetric tracer for a diesel fuel smoke aerosol is described. DCBP added to the fuel prior to aerosolization is distributed solely into the particle phase of the resulting smoke. DCBP is distributed uniformly throughout the aerosol particles, regardless of particle size. Determinations of DCBP in tissue extracts of rats exposed to DCBP-containing smoke were performed by purifying the extracts on activated Florisil®, and subsequently analyzing them by gas/liquid chromatography with electron capture detection.

The largest internal amounts of smoke were found in the lungs. Smoke levels in the upper respiratory tract were only a very small fraction of those found in the lungs. Significant amounts of DCBP tracer were found in the digestive tract following smoke exposure. At a given exposure duration, animals exposed at higher smoke concentrations had greater levels of smoke deposited in their lungs.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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INTRODUCTION

Aerosolized diesel fuel, deployed from the exhaust manifold of armored vehicles, is used by the Army to create obscurant smokes. As part of a program to investigate the health effects of battlefield smokes, the US Army Medical Research and Development Command has supported a study of the inhalation toxicology of diesel fuel aerosols in Sprague-Dawley rats (1,2,3,4). In that study, as in all inhalation toxicology studies, the "dose" to the animals cannot be readily measured. The product (Ct) of the concentration of the airborne material and the duration of exposure has been related to dose (5), but this relationship ignores changes in breathing frequency and volumetric breathing rate, as measured by the minute volume, that may occur as a result of exposure, as well as retention and clearance mechanisms which are dependent upon the physical and chemical properties of the aerosol or vapor, and it has no relation to the site of deposition of the inhaled substance. The dosimetry study was designed to supply some of the missing information concerning the site and quantity of disposition of inhaled diesel fuel aerosol (DFA) at selected combinations of aerosol concentration and exposure duration in the range of those used in the inhalation toxicology experiments. This information could be applied to the interpretation of the biological changes associated with the exposure regimes of the inhalation toxicology experiment.

In order to determine the sites and quantities of DFA deposition an inert tracer, decachlorobiphenyl (DCBP), was added to the diesel fuel prior to aerosolization. Because of the non-volatile nature of the DCBP, the dosimetry experiments dealt with deposition of aerosol particles only. It has been shown that the diesel fuel does not completely condense, either in the field deployment systems or in the generation system employed in the inhalation toxicology exposures, but fractionates into a gaseous phase and an aerosol phase. The vapor phase is richer in the more volatile components of the fuel, and the aerosol contains more of the larger, less volatile compounds of this complex mixture of hydrocarbons. The distribution of the fuel components between the two phases is a function of ambient conditions, DFA concentration, and the makeup of the fuel. In the inhalation toxicology exposures and in the dosimetry experiments reported here, ambient conditions were kept uniform, and a standard reference grade of diesel fuel was used in both cases. The DFA concentration was varied during these experiments, however, and consequently the ratio of vapor to aerosol was not held completely constant. In the range of concentrations used in the dosimetry experiments, however, the fraction of diesel fuel vapor in the chamber did not vary greatly from 20 percent of the aerosol concentration.

The study was accomplished in three phases. First, the suitability of DCBP as a tracer was determined. DCBP has been employed in tobacco smoke dosimetry studies (6-9) and offered the desirable qualities of preferential distribution into the smoke particle phase and high analytical sensitivity with few interferences (gas/liquid chromatography with electron capture detection). While radiolabeled tracers such as

^{14}C-dotriacontane (10-11) offered these same qualities, the amounts required for lengthy (several hours) exposures would make the experiments cost-prohibitive. In addition, contamination of the animal exposure chambers with radionuclides would be unacceptable. Questions had to be answered concerning DCBP's stability under the conditions of diesel fuel smoke generation, its distribution into the liquid phase, and its distribution among the various particle sizes. Next, preliminary experiments were performed to trace the location of the DCBP in the animal after it had been taken into the respiratory tract. Rapid translocation of the DCBP would have precluded its use as a tracer, since it could not be established that the same mechanisms of translocation would operate upon the diesel fuel particles. The final task was to determine the distribution of the DCBP, and thus that of the DFA, under a variety of conditions in the range of those used in the inhalation toxicology experiments.

EXPERIMENTAL

Decachlorobiphenyl was obtained from Aldrich Chemical (Milwaukee, WI). Standard solutions were prepared by dissolving known quantities of DCBP in hexane. The diesel fuel used for dosimetric measurements was spiked with DCBP to concentrations of 600-1000 ppm (wt/wt). Analyses of standard solutions, spiked fuel or smoke, or tissue extracts were performed by gas/liquid chromatography (Hewlett Packard 5730A GC equipped with Ni^{63} electron capture detector and HP 3390A reporting integrator--GC/ECD). Analysis conditions were as follows: Column, 1 m x 6 mm o.d. 10 percent OV-1 on an 80-100 mesh Chromosorb G-HP, isothermal at 220°C; detector temperature, ∿300°C; injector temperature, 250 C; Carrier gas, 90 percent Argon - 10 percent methane. With a flow rate of 12 mL min^{-1}, retention time of the DCBP was approximately 12 minutes. DCBP levels were determined by comparison with a calibration curve generated from a series of standard solutions. Separate high resolution gas chromatographic analysis of DFA extracts for the stability studies were performed on a 30-m SE-52 coated fused silica capillary column, using hydrogen as a carrier gas, or a 50 m Quadrex 007 column, using helium carrier gas.

The aerosol generator has been described in detail elsewhere (1). Briefly, nitrogen carrier gas is heated to 600°C as it sweeps the surface of a Vycor heater, so that diesel fuel pumped onto the end of the heater is volatilized and carried into a diluent air stream. The saturated vapors cool and condense, forming an obscurant cloud. This aerosol cloud flows through a New York University (NYU)-type 1.4 m^3 animal inhalation exposure chamber.

In order to determine the actual concentration of the DCBP in the DFA particles, samples of the aerosol were collected on 45-mm Cambridge glass fiber filter pads (12). At low sampling flows, these filters are 99.5% efficient at collection of particles larger than 0.3 μm diameter. Generally, sampling flows less than 2.0 L min^{-1} were employed for collection.

Particle size distribution of the DFA was determined by using a Mercer-Lovelace cascade impactor, operated at a flow rate of 1.0 L min^{-1} (13). Stages of the impactor were rinsed with carbon disulfide (CS_2), and an aliquot of the CS_2 solution was analyzed gas chromatographically to determine mass of diesel fuel collected on each stage, as described in detail elsewhere (1). Since the CS_2 was incompatible with the DCBP analysis, the CS_2 was evaporated and the residue taken up in hexane. The resulting solutions, one for each impactor stage, were analyzed for DCBP.

Procedures for the actual exposure of the animals have been described in detail elsewhere (1). In order to determine deposition of the DCBP-containing smoke in the tissue samples, animals were killed by carbon dioxide asphyxiation, and the various tissues were removed and placed in pre-weighed vials. The samples were stored frozen until analysis. Tissue samples were processed as follows: Each sample was placed in a glass tube (10 cm x 2 cm o.d.), and 15 mL of n-hexane and 4 g anhydrous Na_2SO_4 were added. The sample was ground for one minute (except for the digestive tracts, which required 2 minutes) using a Brinkman tissue homogenizer equipped with a Model PT10-ST grinding head. The head was continually bathed with small amounts of hexane during the grinding to prevent the drying of the tissue extract on the head during the processing. Following grinding, the homogenizer head was cleaned with hexane in a sonicating bath. The wash solution was retained and added to the tissue extract. Next, the hexane-ground tissue-sodium sulfate suspension was centrifuged for one minute at 660 rpm. The supernatant was poured off, and approximately 5-10 mL of fresh hexane was added to the tissue/sodium sulfate mixture. The resulting suspension was mixed throughly, recentrifuged, and the supernatant poured off. The procedure was repeated once more. The combined portions of supernatant comprised the tissue extract. Centrifugation, rather than filtration, was required because ordinary filter paper was found to retain approximately 25 percent of the DCBP in spiked hexane solutions passed through it. The final volume of the filtrate was adjusted to 25.0 mL.

Polar constituents extracted from the tissue homogenates tended to interfere with the DCBP analysis. In order to separate the DCBP from these constituents, the tissue extracts were purified on Florisil® columns. The procedure was as follows: Columns were prepared by loading 25-mL burets -- to which 200-mL solvent reservoirs had been attached -- with approximately 6.5 g activated Florisil® (volume approximately 16 mL). The Florisil® had been activated at 150°C for 6 hours and allowed to stand overnight at room temperature in a vacuum dessicator. The Florisil® activation temperature is critical in this application, since activation at 500-600°C resulted in complete retention of the DCBP by the Florisil®. Freshly conditioned Florisil® was made up daily. An aliquot, usually 10 mL, of the tissue extract was loaded onto the column and elution with hexane was initiated. The first 8 mL of eluate was discarded. The DCBP elutes in the next approximately 23 mL. This eluate was adjusted to a final volume of 25.0 mL with hexane. Following determination of the recovery for each sample (see below), the Florisil® was discarded and replaced with fresh material. An aliquot of the 25.0 mL eluate was injected directly into the gas chromatograph, or concentrated under a stream of dry nitrogen prior to GC analysis, the method of analysis being dependent upon the expected DCBP concentration.

Despite exacting activation and elution procedures, there was still some daily variation in the volume of hexane required to elute all of the DCBP from the Florisil® columns. Because of this, recovery determinations were made for each tissue samples analyzed. To accomplish this, occasional tissues from unexposed animals were spiked with a known amount of DCBP, and processed identically to the exposed tissues. However, to do this for every sample would have required an unacceptably large number of unexposed animals. To avoid this problem, "simulated recoveries" were determined for most samples by adding 1.00 mL of a DCBP stock solution to 15 mL hexane and 4 g Na_2SO_4. This mixture was then ground and treated identically to a genuine tissue. The observed DCBP concentration was compared with the calculated DCBP concentration in the final eluate in order to estimate recovery of the DCBP in the tissue sample which had been purified on the column in question. The concentration of the original DCBP stock solution was adjusted so that the DCBP concentration in the final recovery solution would be similar to that of the sample. The simulated recoveries of DCBP for a batch of 40 lung samples averaged 93 ± 4 percent. Recoveries for 80 turbinate and trachea plus larynx samples averaged 96 ± 17 percent. The greater scatter observed with the latter series of samples was believed to be due to the uncertainties associated with evaporating the samples to dryness and redissolving in 500 μL of hexane.

CHEMICAL VALIDATION OF DCBP AS A TRACER FOR DFA SMOKE PARTICLES

DCBP was chosen as a dosimetric marker in this application because of its analytical sensitivity and thermal stability during aerosolization. However, data from polychlorinated-biphenyl incineration studies had indicated that DCBP decomposes above 800°C (14). Thus, it was necessary to determine the stability in our application, in which temperatures of 600°C are maintained inside the aerosol generator. For these tests, spiked fuel samples were aerosolized, and the resulting smoke particulates were collected on Cambridge filter pads backed with Tenax® traps. Analysis of the pad extracts by GC/ECD indicated no chlorine- containing constituents other than the DCBP. Subjecting the extract to high resolution gas chromatography with flame ionization detection (sensitive to all hydrocarbon constituents) resulted in the usual pattern of fuel constituents. There were no additional constituents in the aerosol, other than the DCBP, indicating that there were no chromatographable DCBP decomposition products in the smoke particles (see Figure 1).

The Tenax® was examined to determine if DCBP, or any of its decomposition products, might be present in the gas phase of the smoke. Normally, Tenax® is desorbed by thermal means. However, the boiling

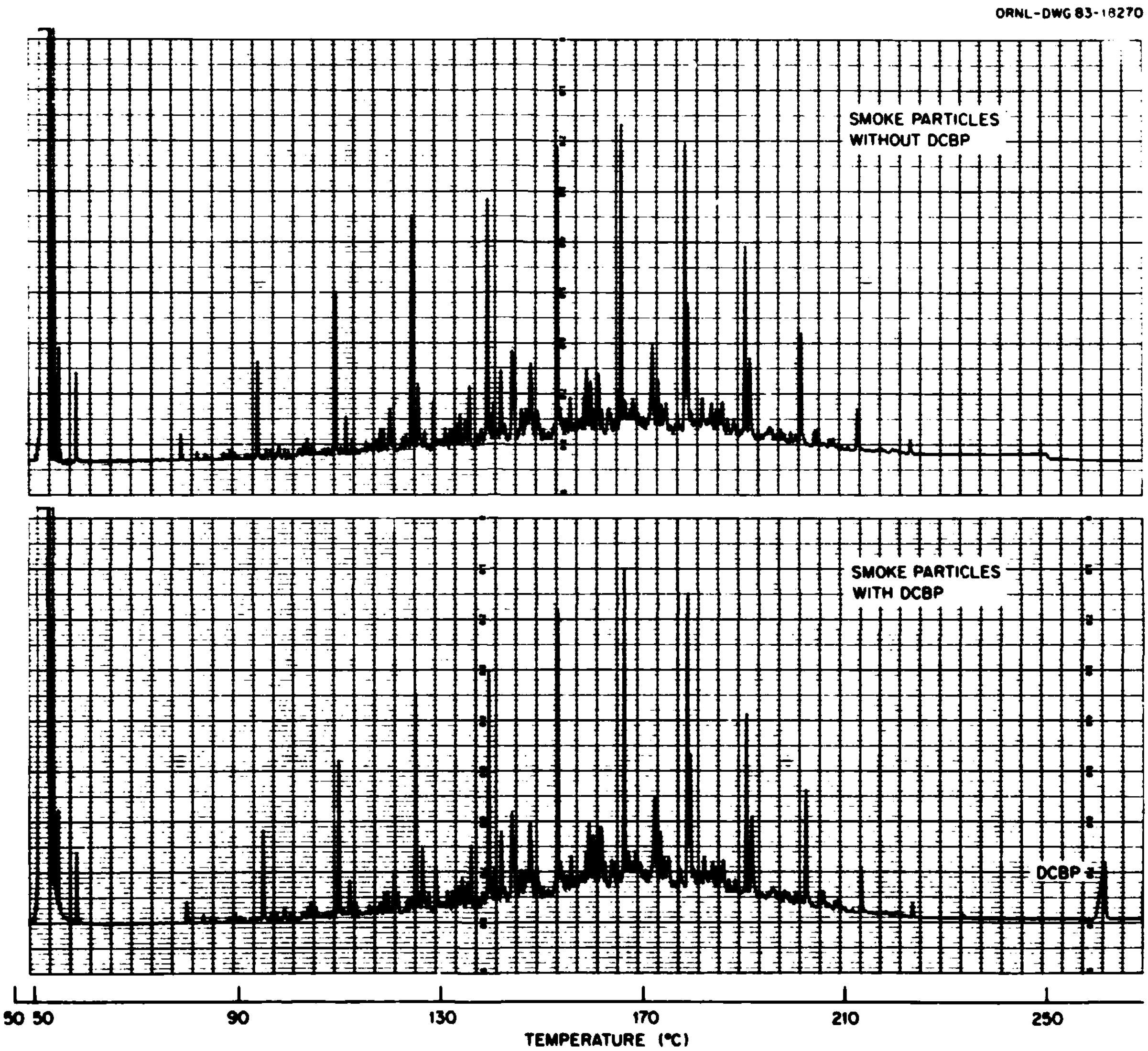

Figure 1

Comparison of High Resolution Chromatograms: Diesel Fuel Smoke Particles, With and Without Added DCBP point of DCBP was expected to be so high that the Tenax® would have to be heated beyond its decomposition point before DCBP would thermally desorb. Instead, the Tenax® was exhaustively washed with hexane, and the resulting solution concentrated and subjected to GC/ECD. While elution of Tenax® with hexane is not necessarily a quantitative method for removal of DCBP, the sensitivity of the analysis is sufficient to detect traces of chlorine containing compounds in the eluate. There was no evidence of DCBP, or any other chlorine containing constituent, in the gas phase. These findings indicated that, in the presence of nitrogen carrier gas, the DCBP is stable to 600°C, and, upon aerosolization, is transferred into the particulate phase of the aerosol without decomposition.

During aerosolization of the diesel fuel, some of the more volatile constituents in the fuel do not recondense. This has the effect of increasing the DCBP concentration in the particle phase of the aerosol, relative to that in the original fuel. The extent of this increase, as a function of particle concentration of the aerosol in a small test chamber, is presented in Table 1 for a DCBP concentration of 850 $\mu g\ g^{-1}$ fuel.

TABLE 1. EFFECT OF AEROSOL PARTICLE CONCENTRATION ON RELATIVE CONCENTRATION OF DCBP IN LIQUID PHASE OF THE DIESEL FUEL AEROSOL

Aerosol Particle Concentration mg L^{-1}	DCBP Concentration Ratio[a]
0.43	1.25 ± 0.03
1.0	1.23 ± 0.04
3.0	1.21 ± 0.06
5.0	1.17 ± 0.05
8.0	1.08 ± 0.03

[a]Ratio of DCBP concentration in particle phase of aerosol to that in fuel prior to aerosolization. (Mean ± standard deviation for triplicate analyses)

The data indicate that, in this particular concentration range, the relative concentration of DCBP in the particles increases as particle concentration decreases. This is due to an increasingly larger proportion of the fuel remaining uncondensed in the vapor phase, as the aerosol becomes more dilute. This finding points to the necessity of determining DCBP in the aerosol being tested, rather than that in the fuel prior to aerosolization.

Since aerosol particle size can affect the site of deposition in the respiratory tract (15), the tracer should be distributed evenly through the particles, regardless of particle size. To determine if this was the case for DCBP, the diesel fuel was spiked with DCBP to a concentration of 1000 $\mu g\ g^{-1}$ fuel and aerosolized so as to produce a particle concentration of 5.3 $mg\ L^{-1}$. Comparison of the average ratios (four runs) of diesel fuel to DCBP collected on each impactor stage is made in Table 2, and is graphically portrayed in Figure 2.

TABLE 2. RATIO OF DCBP CONTENT AND DIESEL FUEL SMOKE CONTENT OF IMPACTOR STAGES AS A FUNCTION OF PARTICLE SIZE

Diameter of Particles Deposited on Impactor Stages, μm	$\frac{\text{DCBP}}{\text{Diesel Fuel}} \times 1000$[a]
<0.31	1.51
0.31 - 0.70	1.09
0.70 - 1.21	0.86
1.21 - 1.75	0.80

[a]As determined from cascade impactor stages. Absolute ratio values determined from this procedure tend to be lower than those determined from filter pad analyses.

The data in Table 2 indicate that, as expected, the DCBP is enriched in the smaller particles. However, the degree of enrichment is important only in the smallest particles. The data in Figure 2 indicate that these particles comprise only 3 percent or less of the mass of the smoke particles collected. Indeed, the mass median particle diameter (MMD) determined from DCBP on the impactor stages was 1.1 μm, whereas the MMD determined from diesel fuel analysis was 1.25 μm. Thus, the small preference of the DCBP for the smaller particles should have no effect on the conclusions of the experiment.

PRELIMINARY ANIMAL EXPERIMENTS

Studies on the effects of inhalation exposure of rats to diesel fuel aerosol have included single exposures, repeated exposures and a 13-week subchronic study. Animals have been exposed to a series of concentration levels for times ranging from 2 to 6 hours. There has been some question over whether animals exposed to equivalent concentration-time (Ct) products are really being subjected to the same insult.

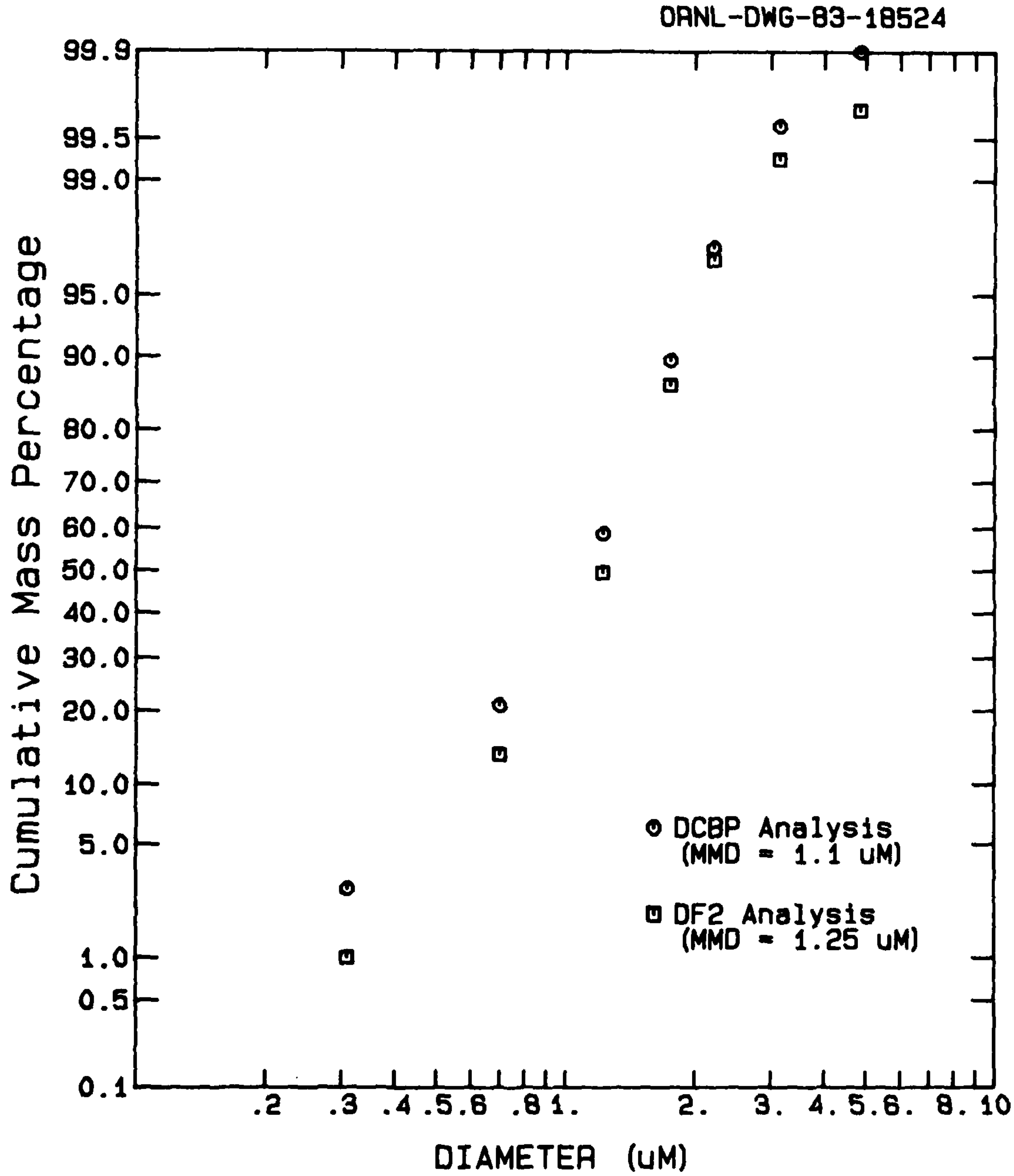

Figure 2

Particle Size Distribution of Decachlorobiphenyl in
a Diesel Fuel Based Aerosol

In this study, tracer dosimetry was employed in order to address this question. However, cognizance of the inherent limitations of tracer dosimetry in such a complex organic matrix as diesel fuel is critical to the appropriate evaluation of the data obtained. While the diesel fuel smoke particle impacts the tissue as a single entity (i.e., liquid droplet containing a small amount of tracer), it is not likely to remain in that state for long. The diesel fuel is a complex organic material comprised of hundreds of individual constituents, each with different solubilities in the various tissues. The efficacy of the DCBP tracer in this study is tied not to its ability to track individual fuel constituents or particles, but rather to its tendency remain in the particular location where it initially deposits. One of the reasons why DCBP was chosen as a tracer was its relative biological inertness. That is, its chemical structure did not suggest rapid metabolism. Nevertheless, DCBP can undergo translocation - or "clearance" - either on its own accord or dissolved in the remaining organic material which was once the fuel smoke particle which originally impacted the tissue. Mechanisms of clearance of fuel smoke constituents or DCBP might include dissolution into the target tissue followed by transport into the circulatory system and/or removal of the material from the lower airways by mucociliary action. This latter action would be followed by swallowing of the ejected material. Rapid clearance from the lungs would mean that only a fraction of the DCBP originally deposited in the lungs would remain after several hours, and thus, concentrations measured at the end of a long exposure would not be indicative of the total amount of smoke deposited in the respiratory tract. In order to gauge whether such clearance might adversely affect the ability to interpret experimental data, two sets of dosimetry experiments were performed. The experiments were essentially identical in design, with some minor differences in termination times for the animals. Individually caged male Sprague-Dawley rats were exposed to a diesel fuel smoke concentration of 5.3 mg L^{-1} for one hour. Following exposure, one group of four animals was terminated (CO_2 asphyxiation) immediately (0-30 minutes). Another group was killed 60-100 minutes following exposure, and a third group was killed approximately 5 hours following exposure. Immediately after termination, the animals were weighed and skinned in such a way as to avoid contact with other organs and subsequent transfer of DCBP. Next, the lungs, trachea plus larynx, turbinates, liver, and digestive tracts were removed and subjected to DCBP analysis. Because of the similarity of the two experiments, not all of the organ systems were examined in both experiments.

Results of the experiments are summarized in Tables 3 and 4. (More detailed information is appended in Tables A-1 and A-2). In both experiments, the animal-to-animal variation was considerable. For example, while the mean initial tracer deposition (Group 1) in the first experiment appear to be twice that of Group 1 in the identical second experiment, the difference was not significant ($p > 0.05$). Thus, the mean initial deposition for both experiments was determined to be 1.85 ± 0.84 mg, or 46 percent relative standard deviation. There were decreases in the amount of tracer in the lung with time. In

TABLE 3. APPARENT SMOKE DEPOSITION AS A FUNCTION OF TIME OF TERMINATION EXPERIMENT NO. 1[d]

			Smoke Particle Deposition[b], in mg (Mean ± one standard deviation)				
Group[a]	Mean Animal Weight (g)	Approximate Time of Termination[c], min	Lung	Turbirates	Trachea + Larynx	Digestive Tract	Fur and Skin
1	376 ± 11	0-30	2.40 ± 0.73	ND	0.12 ± .05	0.59 ± 0.60	2.24 ± 0.56
2	352 ± 22	60-90	1.16 ± 0.57	0.02 ± .01	0.06 ± .02	1.28 ± 0.66	2.81 ± 0.66
3	342 ± 16	300-340	1.16 ± 0.11	ND	0.04 ± .02	1.60 ± 0.24	3.78 ± 2.75

[a]Four animals per group
ND: Below limit of detection (∿ 0.02 μg per organ)

[b]Based on a concentration of DCBP in smoke particles of 1.19 $\mu g/mg^{-1}$

[c]Following termination of exposure

[d]Smoke concentration: 5.3 mg L^{-1}

TABLE 4. APPARENT SMOKE DEPOSITION AS A FUNCTION OF TIME OF TERMINATION EXPERIMENT NO. 2[d]

			Smoke Particle Deposition[b], in mg (Mean ± one standard deviation)			
Group[a]	Mean Animal Weight (g)	Approximate Time of Termination[c], min	Lung	Liver	Digestive Tract	Fur and Skin
1	432 ± 26	6	1.30 ± 0.57	*	0.59 ± .35	3.25 ± .53
2	427 ± 11	60	1.08 ± 0.41	*	0.70 ± .35	5.79 ± 1.38
3	433 ± 10	300	0.60 ± 0.26	0.06 ± .05	0.62 ± .29	5.39 ± 2.32

[a]Four animals per group

[b]Based on a measured DCBP concentration of 1.31 ± 0.04 μg DCBP per mg smoke particles.

[c]Following termination of exposure.

[d]Smoke concentration: 5.3 mg L^{-1}.

*Analyses of liver levels of DCBP performed only on two animals of Group #3.

experiment #1, these differences were somewhat more significant ($p < 0.05$) than in experiment #2, ($p < 0.10$). In experiment #1, there was a significant ($p < 0.05$) increase in the amount of tracer in the digestive tract with time, but not in experiment #2. However, in both experiments, there were sizable amounts of tracer in the lung after the only one hour exposure. In many of the individual groups, the level of tracer in the digestive tract was comparable to that in the lung. However, while occasional preening and hand washing were observed in about half of the rats following exposure (see Tables A-1 and A-2), there did not appear to be consistently more tracer in the tracts of animals which had preened than in those of animals which did not preen. While there was a considerable amount of tracer on the fur and skin - a result presumably of whole body exposure, the only significant ($p < 0.05$) difference between various groups was that found between groups 1 and 2 in experiment #2. Given the amounts of tracer found on the fur relative to those in the digestive tract, in order to have altered the levels in the digestive tracts markedly, the animals would have had to remove tracer from a substantial portion of their fur. Compared to the fur, lungs, and digestive tracts, there were only small amounts of tracer present in the liver, turbinates trachea, and larynx.

The magnitude of individual variations precluded the drawing of a conclusive picture of the movement of the tracer. In both experiments, there was the suggestion of movement of the tracer from the lungs at a rate that approximately halved the level of tracer in that region in 5-6 hours. A possible mechanism of DCBP movement into the digestive tract might be muco-ciliary transport from the respiratory tract into the throat, followed by swallowing. In experiment #1, the concentration of tracer in the digestive tract appeared to increase as that in the lung decreased, as would be expected if this mechanism was operative. However, experiment #2 did not show any change. Elucidation of the precise mechanisms of DCBP transport was beyond the scope of this effort. However, the impact of potential movement from lung to digestive tract with a 6-hour half life on the efficacy of the DCBP tracer in this application was assessed as follows. If the increase in DCBP deposition in the lung is presumed to be linear with time, and a competing exponential decay acts to decrease lung concentration, then the integrated rate equation is given by:

$$\mathrm{DCBP} = \frac{k_1}{k_2}(1 - e^{-k_2 t})$$

where k_1 is the deposition rate
k_2 is the rate of removal from the lung = $0.17\ \mathrm{hr}^{-1}$
($t_{1/2} \sim 6$ hours)

Under these conditions, the amount of DCBP found in the lung would underestimate that actually deposited there by 11 percent after 2 hours and 28 percent after 6 hours. These underestimations were judged to be small in comparison to the animal-to-animal variation initially observed in experiments 1 and 2.

SMOKE DEPOSITION STUDY

The purpose of the experiments conducted in this phase of the study was to determine the deposition of diesel fuel smoke particulates in the animals' respiratory tracts under conditions similar to those employed during the repeated inhalation exposures. Thus, four smoke concentrations (approximately 1.3, 2, 4, and 6 mg L^{-1}) and two exposure durations (2 and 6 hours) were used to achieve two distinct concentration/time products (Ct = 8, 12). For each of the four experiments, twenty male Sprague-Dawley rats were exposed in the NYU chambers in the Biology Divison. In the three middle rack positions, individually caged animals were exposed in layers of seven, six, and seven (top, middle, and bottom positions, respectively). During each exposure, a minimum of two pairs of cascade impactor and three pairs of Cambridge filter pad samples were taken to document the particle size distribution and concentration of DCBP in the smoke, respectively. In addition, chamber smoke concentrations were continuously monitored with an instrumental aerosol sensor (1). Animals were killed immediately after the exposure. Animal weights were recorded, the animals skinned, and the lungs, trachea plus larynx, turbinates (skinned head less brains, external nares, and lower jaw) and digestive tracts were removed and weighed. The tissues were then frozen for later analysis. Eventually, half the animals° tissues (selected at random) were chosen for analysis, while half were retained in case of analytical problems or high animal-to-animal variation with the first batch of tissues.

The exposure parameters for the dosimetry experiments are summarized in Table 5. In all cases, the Ct achieved was close to the target value. Analysis of the cascade impactor stages indicated that the particle size distribution of the DCBP varied with smoke concentration as expected. That is, the mass median particle diameter increased with increasing smoke concentration, from a low of 0.7 μm to a high of 1.1 μm.

A summary of the respiratory tract smoke deposition levels is found in Table 6. More detailed data are appended (Tables A-3 through A-14). Several conclusions are evident. First, animal-to-animal variation within a given exposure group was considerable (10-30 percent relative standard deviation for lungs) but not unexpectedly high. Second, the amounts of particulates deposited in the upper respiratory tract are only small fractions of those deposited in the lungs. In all cases, the sum of the smoke deposition in the turbinates, trachea, and larynx amounted to less than 1.5 percent of that found in the lungs. Third, as expected, for a given exposure duration, animals exposed at higher smoke concentrations had greater levels of smoke deposited in their lungs. For the two hour exposures, the deposition was approximately proportional to the smoke concentrations. For the longer exposures, the relationship was not ($p > 0.05$). At a given Ct product, rats exposed for longer durations seemed to have less smoke in their lungs than animals exposed for shorter durations. This may be due, in part, to a greater extent of translocation of the DCBP tracer either on its own, or dissolved in the fuel matrix, from the lungs at longer exposure

TABLE 5. SUMMARY
EXPOSURE PARAMETERS OF DOSIMETRY EXPERIMENTS

Smoke Concentration mg/l	Exposure Duration, hrs	Ct ($mg \cdot hr \cdot L^{-1}$) Target	Ct ($mg \cdot hr \cdot L^{-1}$) Actual	[DCBP][a] in smoke, μg/mg	Particle size[b] MMAD
6.01 ± 0.12	2.0	12	12.0	1.13	1.1
4.06 ± 0.28	2.0	8	8.1	1.20	1.0
1.38 ± 0.08	6.0	8	8.3	1.36	0.68
2.33 ± 0.12	6.0	12	14.0	1.30	0.75

[a]Determined from a set of separate experiments, as some of the pad samples from the original dosimetry runs were inadvertantly destroyed.

[b]Mass median aerodynamic diameter as determined from DCBP levels on cascade impactor stages.

TABLE 6. SUMMARY OF DIESEL FUEL SMOKE PARTICLE DEPOSITION IN THE RESPIRATORY TRACE OF RATS

Aerosol Concentration $mg \cdot L^{-1}$	Exposure Time, hrs	Target Ct $mg \cdot hr \cdot L^{-1}$	Deposition, mg smoke particles per tissue (mean ± one standard deviation)		
			Lungs	Trachea + Larynx[a]	Turbinates[a]
6.01	2	12	5.96 ± 1.22	0.031 ± .012	0.031 ± .020
4.06	2	8	3.68 ± 1.11	0.024 ± .013	0.013 ± .007
1.38	6	8	2.17 ± 0.42	0.008 ± .005	0.022 ± .011
2.33	6	12	4.60 ± 0.41	0.013 ± .007	0.029 ± .013

[a] Levels of 5 ng DCBP per tissue were used for samples which were found to have DCBP levels at or below this lower limit of detection.

times, or to a progressive change in breathing rate as smoke exposure continued. However, while analysis of the digestive tracts (Table A-15) of a few of the exposed animals showed that the amount of tracer present was a sizable fraction (20-65 percent) of that present in the lungs, there were no clear differences in tracer levels in the digestive tracts between groups of animals at different exposure durations - which would have been expected if mucociliary transport and swallowing were the predominant route of clearance. For these animals, the tracer found in the digestive tract averaged about 30 percent of the total internal tracer deposition. In summary, at shorter exposure times, observed mean tracer levels were proportional to Ct, but at longer times, they were not.

In order to compare the lung deposition levels observed in these experiments with those of other studies, deposition fractions, taken as the fraction of inhaled particles which was actually deposited (17), were computed. Estimated average minute volumes of 100 mL min^{-1} - observed for male Sprague Dawley rats undergoing diesel fuel smoke exposure at smoke concentrations greater than 0.5 mg L^{-1} (16) - were used. Deposition fractions, F, are reported in Table 7, and ranged from a low of 4.4 percent to a high of 8.3 percent. The values obtained for the longer duration exposures were lower than those for the shorter exposures, perhaps a result of greater cumulative clearance at longer exposure times. In general, our values were in relatively good agreement with others found for small rodents exposed to monodisperse aerosols (17). Raabe, et al., *found deposition fractions of* 6-8 percent for particles in the size range of the diesel fuel smoke studied in these experiments (0.6 - 2.0 μm). Wolff, et al., found somewhat higher deposition fractions, approximately 15 percent for Fischer 344 rats exposed to 0.1 μm diameter $^{67}Ga_2O_3$ aggregates (18).

As a whole, the data indicated that at the longer exposure times, the rats had lower levels of tracer in the lung than would have been predicted from a Ct proportionality. This may be due, in part, to translocation of the tracer or other constituents from the lungs to the digestive system. Correcting the lung deposition levels for the tracer translocation (11% underestimation at 2 hour exposure and 28% underestimation at 6 hour exposures) as suggested from the preliminary animal experiments, leads to the estimates in Table 8. These "corrected" data are more in keeping with the expected proportionality to smoke concentration and exposure duration. As stated previously, it is unknown whether the tracer remains dissolved in the diesel fuel or fuel constituents after deposition, in which case the tracer movement would parallel that of the fuel particulates, or if the tracer migrates out of the fuel and moves on its own. If the former is true, then a significant portion of the systemic load of fuel in the animal may enter the circulatory system through the digestive tract, rather than the respiratory tract.

TABLE 7. OBSERVED DEPOSITION FRACTION, F,[a] COMPUTED FROM DIESEL FUEL SMOKE EXPOSURE

Aerosol Concentration $mg \cdot L^{-1}$	Exposure Time, hours	Smoke Concentration/ Time Product (c·t)	Mean Smoke Deposition, mg per animal	Deposition Fraction, F[b] in percent
6.0	2	12.0	6.0	8.3
4.0	2	8.1	3.7	7.6
1.4	6	8.3	2.2	4.4
2.3	6	14.0	4.6	5.5

[a]Deposition Fraction $F = \dfrac{\text{Amount of Particles Deposited}}{\text{Amount of Particles Inhaled}} = \dfrac{D}{(c \cdot t)\ R}$

Where D = Smoke particles deposited, from DCBP tracer data
C = Smoke particle concentration, $mg \cdot L^{-1}$
t = Exposure time, in hours
R = Animal respiration rate, in $L \cdot hr^{-1}$

[b]Values calculated using an average minute volume of 100 mL. Male Sprague-Dawley rats decrease their respiratory minute volume to between 80 and 110 mL upon exposure to diesel fuel smoke at concentrations above 0.5 $mg \cdot L^{-1}$. (Reference 16).

TABLE 8. ESTIMATED LUNG LEVELS OF SMOKE PARTICLE DEPOSITION CORRECTED FOR AN ESTIMATED DCBP LUNG HALF-LIFE OF SIX HOURS

Aerosol Concentration $mg \cdot L^{-1}$	Exposure Time, hrs	Target Ct	Estimate Corrected Lung Deposition mg per lung mean ± one standard deviation
6.0	2	12	6.7 ± 1.4
4.1	2	8	4.1 ± 1.2
1.4	6	8	3.0 ± 0.6
2.3	6	12	6.4 ± 0.6

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PERSONNEL

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PUBLICATIONS

The following publications resulted from the work described in this report:

Jenkins, R. A., Gayle, T. M., Wike, J. S., and Manning, D. L. 1982. Sampling and Chemical Characterization of Concentrated Smokes. Toxic Materials in the Atmosphere, ASTM STP 786, American Society for Testing and Materials, pp. 153-166.

Jenkins, R. A., Holmberg, R. W., Wike, J. S., and Moneyhun, J. H. The Chemical and Physical Characterization of the Diesel Fuel Smoke for Inhalation Exposure and Toxicology Studies. Task Summary Report to U.S. Medical Bioengineering Research and Development Laboratory, Ft. Detrick, Frederick, MD, in preparation.

Table A-1

DCBP Clearance Experiment No. 1: Individual Deposition Values

Animal Designation	Weight at Sacrifice (g)	Time of Sacrifice, min	Amount of Diesel Fuel Smoke Particulates[a,b] Deposited per Organ, in mg: Lung	Turbinates	Trachea + Larynx	Digestive Tract	Fur and Skin
DC-A-1	368	0	2.94	ND	.18	.40	2.48
DC-A-2	369	5	1.94	ND	.13	.20	—
DC-A-3	391	15	1.63	ND	.06	.28	2.63
DC-A-4	378	24	3.10	ND	.09	1.49P	1.60
DC-A-5	349	61	1.21	.02	.08	.55	2.69
DC-A-6	364	72	0.68	.02	.05	2.15P	2.73
DC-A-7	373	87	0.79	.03	.07	1.13	3.70
DC-A-8	322	96	1.94	ND	.05	1.27	2.10
DC-A-9	360	300	1.16	ND	.03	1.52P	6.98
DC-A-10	336	312	1.05	ND	.08	1.93	1.79
DC-A-11	322	326	1.31	ND	.04	1.58P	5.13
DC-A-12	349	341	1.10	ND	.03	1.37	1.20

P: Animal observed to be preening while in fresh air.
ND: None detected - level of DCBP in final analyte solution was <1 ng mL^{-1}.
a: Based on a DCBP concentration of 1.19 μg/mg smoke particles
b: Smoke concentration: 5.3 mg L^{-1}.

Table A-2

DCBP Clearance Experiment No. 2: Individual Deposition Values

Animal Designation	Weight at Sacrifice (g)	Time of Sacrifice, min	Amount of Diesel Fuel Smoke Particulates[a,b] Deposited per Organ, in mg: Lung	Liver	Digestive Tract	Fur and Skin
DC-B-1	419	6	2.02		1.07	3.73
DC-B-2	468	6	1.49		0.43	3.48
DC-B-3	409	6	0.81		0.61	3.28
DC-B-4	433	6	0.89		0.25	2.50
DC-B-5	439	60	1.17		0.89P	7.24
DC-B-6	418	60	0.70		0.97	3.94
DC-B-7	417	60	1.62		0.20	5.72
DC-B-8	433	60	0.83		0.72	6.25
DC-B-9	421	300	0.74	.02	0.97P	3.83
DC-B-10	439	300	0.40	.09	0.70P	8.50
DC-B-11	429	300	0.89		0.28P	5.81
DC-B-12	444	300	0.35		0.53P	3.43

P: Animal observed to be preening while in fresh air.
a: Based on a DCBP concentration of 1.32 ± 0.04 μg DCBP per mg smoke particulates.
b: Smoke concentration: 5.3 mg L^{-1}.

Table A-3

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Lungs
C Series
Ct=12

Animal Designation	Tissue Weight, g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue μg	Percent Recovery of 1.6 μg portion of DCBP	Corrected Amount of DCBP in Tissue (μg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-C-1	1.437	142.2	8.89	99.5	8.93	7.90
DC-C-3	1.422	103.1	6.44	96.6	6.67	5.90
DC-C-5	1.231	116.7	7.29	97.5	7.48	6.62
DC-C-6	1.252	111.1	6.94	95.9	7.24	6.41
DC-C-9	1.423	135.2	8.45	100.6	8.40	7.43
DC-C-12	1.433	92.2	5.81	93.9	6.19	5.48
DC-C-14	1.336	94.9	5.93	95.2	6.23	5.51
DC-C-15	1.486	94.4	5.90	93.3	6.32	5.59
DC-C-18	1.432	89.2	5.58	93.3	5.98	5.29
DC-C-20	1.322	59.8	3.74	93.6	4.00	3.54
Mean (± 1 std. dev.)	1.377 ± 0.087					5.96 ± 1.22

[a]Exposure conditions: 6.01 ± 0.12 mg L^{-1} for 2.0 hours.
[b]Based on a DCBP concentration of 1.13 μg per mg smoke.

Table A-4

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Trachea & Larynx
C Series[a]
Ct=12

Animal Designation	Tissue Weight, g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-C-1	0.216	251.6	.314	124	.253	.224
DC-C-3	0.185	38.9	.049	91.2	.054	.048
DC-C-5	0.234	19.4	.024	85.2	.028	.025
DC-C-6	0.199	39.3	.049	95.0	.052	.046
DC-C-9	0.221	38.3	.048	99.8	.048	.042
DC-C-12	0.200	16.7	.021	93.9	.022	.019
DC-C-14	0.191	20.8	.026	99.7	.026	.023
DC-C-15	0.162	13.7	.017	99.5	.017	.015
DC-C-18	0.199	23.3	.035	95.9	.036	.032
DC-C-20	0.162	21.6	.027	94.8	.028	.025
Mean (± 1 std. dev.)	0.197 ± 0.023					.031 ± .012

[a]Exposure conditions: 6.01 ± 0.12 mg L^{-1} for 2.0 hours.
[b]Based on a DCBP concentration of 1.13 µg per mg smoke.

Table A-5

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Turbinates
C Series[a]
Ct=12

Animal Designation	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-C-1	33.1	.041	112	.037	.033
DC-C-3	79.4	.099	119	.083	.073
DC-C-5	19.4	.024	90.2	.027	.024
DC-C-6	20.9	.026	92.0	.028	.025
DC-C-9	35.1	.044	103	.043	.038
DC-C-12	*	—	107	—	c
DC-C-14	31.3	.039	72.5	.054	.048
DC-C-15	*	—	105.3	—	c
DC-C-18	20.0	.025	92.7	.027	.024
DC-C-20	25.9	.032	85.3	.038	.034
Mean (± 1 std. dev.)					.031 ± .020

[a]Exposure conditions: 6.01 ± 0.12 mg L^{-1} for 2.0 hours.
*Below limit of good quantitation: less than 5 ng mL^{-1}.
[b]Based on a DCBP concentration of 1.13 µg per mg smoke.
[c]Levels of 5 ng DCBP per tissue were used for samples which were found to have DCBP levels at or below this lower limit of detection.

Table A-6

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Lungs

D Series[a]

Ct=8

Animal Designation	Tissue wt, g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 1.6 µg Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-D-3	1.308	94.3	5.89	82.3	7.16	5.97
DC-D-4	1.506	79.8	4.99	84.2	5.92	4.93
DC-D-6	1.341	48.9	3.06	87.7	3.49	2.91
DC-D-8	1.315	58.4	3.65	90.8	4.02	3.35
DC-D-9	1.130	58.0	3.62	94.2	3.85	3.21
DC-D-12	1.491	80.2	5.01	103	4.85	4.04
DC-D-14	1.329	59.3	3.71	92.8	3.99	3.32
DC-D-15	1.409	35.5	2.22	93.4	2.37	1.98
DC-D-17	1.467	57.3	3.58	90.6	3.95	3.29
DC-D-18	1.497	64.8	4.05	89.8	4.51	3.76
Mean ($\pm$ 1 std. dev.)	1.349 ± 0.118					3.68 ± 1.11

[a]Exposure conditions: 4.06 ± 0.28 mgL^{-1} for 2.0 hours.
[b]Based on a DCBP concentration of 1.20 µg per mg smoke.

Table A-7

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure

Tissue: Trachea & Larynx
D Series[a]
Ct=8

Animal Designation	Tissue wt, g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-D-3	0.207	*	—	94.7	--	c
DC-D-4	0.181	24.6	.031	92.2	.034	.028
DC-D-6	0.229	24.0	.030	83.6	.036	.030
DC-D-8	0.157	17.4	.022	90.0	.024	.020
DC-D-9	0.122	11.5	.014	87.0	.016	.013
DC-D-12	0.238	39.5	.049	85.9	.057	.048
DC-D-14	0.178	18.8	.024	117	.021	.018
DC-D-15	0.171	14.8	.018	114	.016	.013
DC-D-17	0.158	23.1	.029	109	.027	.022
DC-D-18	0.225	40.1	.050	102	.049	.041
Mean (± 1 std. dev.)	0.187 ± 0.037					.024 ± .013

[a]Exposure conditions: 4.06 ± 0.28 mg L^{-1} for 2.0 hours.
*Below limit of good quantitation: less than 5 ng mL^{-1}.
[b]Based on a DCBP concentration of 1.20 µg per mg smoke.
[c]Levels of 5 ng DCBP per tissue were used for samples which were found to have DCBP levels at or below this lower limit of detection.

Table A-8

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure

Tissue: Turbinates

D Series[a]

Ct=8

Animal Designation	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-D-1	17.1	.021	92.0	.023	.019
DC-D-5	15.8	.020	91.7	.022	.018
DC-D-6	13.6	.017	97.3	.017	.014
DC-D-8	19.1	.024	88.8	.027	.022
DC-D-11	*	—	86.4	—	c
DC-D-13	13.3	.017	91.2	.019	.016
DC-D-15	*	—	91.6	—	c
DC-D-16	*	--	94.5	—	c
DC-D-18	14.8	.018	99.8	.018	.015
DC-D-19	12.6	.016	85.5	.019	.016
Mean (± 1 std. dev.)					.013 ± .007

[a]Exposure conditions: 4.06 ± 0.28 mg L^{-1} for 2.0 hours.
*Below limit of good quantitation: less than 5 ng mL^{-1}.
[b]Based on a DCBP concentration of 1.20 µg per mg smoke.
[c]Levels of 5 ng DCBP per tissue were used for samples which were found to have DCBP levels at or below this lower limit of detection.

Table A-9

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Lungs
E Series[a]
Ct=8

Animal Designation	Tissue wt, g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 1.6 µg Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-E-3	1.352	52.7	3.29	92.0	3.58	2.63
DC-E-5	1.331	41.2	2.58	91.9	2.80	2.06
DC-E-7	1.555	53.9	3.37	94.5	3.56	2.62
DC-E-8	1.380	56.6	3.54	93.1	3.80	2.79
DC-E-12	1.225	42.8	2.68	89.4	2.99	2.20
DC-E-13	1.353	33.7	2.11	95.6	2.20	1.62
DC-E-16	1.431	45.4	2.84	95.8	2.96	2.18
DC-E-17	1.322	32.3	2.02	96.6	2.09	1.54
DC-E-18	1.268	38.6	2.41	93.9	2.57	1.89
DC-E-19	1.558	43.2	2.70	92.7	2.91	2.14
Mean (± 1 std. dev.)	1.378 ± 0.110					2.17 ± .042

[a]Exposure conditions: 1.38 ± 0.08 mg L^{-1} for six hours.
[b]Based on a DCBP concentration of 1.36 µg per mg smoke.

Table A-10

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Trachea & Larynx
E Series[a]
Ct=8

Animal Designation	Tissue wt, g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-E-3	0.145	*	—	107	—	c
DC-E-5	0.173	10.6	0.013	94.4	.014	.010
DC-E-7	0.173	*	—	103	—	c
DC-E-8	0.197	*	—	99.4	—	c
DC-E-12	0.170	11.1	0.014	105	.013	.010
DC-E-13	0.152	14.9	.019	72	.026	.019
DC-E-16	0.187	14.6	.018	101	.018	.013
DC-E-17	0.162	*	—	86.1	—	c
DC-E-18	0.179	*	—	1.21	—	c
DC-E-19	0.199	8.0	.010	97.3	.010	.007
Mean (± 1 std. dev.)	0.174 ± 0.018					.008 ± .005

[a]Exposure conditions: 1.38 ± 0.08 mg L^{-1} for six hours.
*Below limit of good quantitation: less than 5 ng mL^{-1}.
[b]Based on a DCBP concentration of 1.36 µg per mg smoke.
[c]Levels of 5 ng DCBP per tissue were used for samples which were found to have DCBP levels at or below this lower limit of detection.

Table A-11

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Turbinates
E Series[a]
Ct=8

Animal Designation	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-E-3	21.3	.027	73.6	.037	.027
DC-E-5	33.3	.042	85.3	.049	.036
DC-E-7	34.9	.044	80.5	.055	.040
DC-E-8	24.8	.031	73.0	.042	.031
DC-E-12	11.9	.015	106	.014	.010
DC-E-13	23.2	.029	107	.027	.020
DC-E-16	15.8	.020	101	.020	.015
DC-E-17	15.2	.019	174	.019	.014
DC-E-18	11.3	.014	103	.014	.010
DC-E-19	22.6	.028	103	.027	.020
Mean ($\pm$ 1 std. dev.)					.022 $\pm$ .011

[a]Exposure conditions: 1.38 $\pm$ 0.08 mg L^{-1} for six hours.
[b]Based on a DCBP concentration of 1.36 µg per mg smoke.

Table A-12

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Lungs
F Series[a]
Ct=12

Animal Designation	Tissue wt., g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 1.6 µg Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-F-1	1.204	117.4	7.34	103	7.13	5.48
DC-F-5	1.258	90.0	5.63	98.0	5.74	4.42
DC-F-6	1.469	76.6	4.79	89.1	5.38	4.14
DC-F-8	1.203	84.9	5.31	93.8	5.66	4.35
DC-F-11	1.388	97.3	6.08	93.3	6.52	5.02
DC-F-13	1.487	90.0	5.62	93.3	6.03	4.64
DC-F-15	1.609	92.6	5.79	92.7	6.25	4.81
DC-F-16	1.566	88.7	5.54	94.4	5.87	4.52
DC-F-18	1.779	77.5	4.84	86.4	5.60	4.31
DC-F-19	1.529	77.8	4.86	86.4	5.63	4.33
Mean (± 1 std. dev.)	1.449 ± 0.188					4.60 ± 0.41

[a]Exposure conditions: 2.33 ± 0.12 mg L^{-1} for six hours.
[b]Based on a DCBP concentration of 1.30 µg per mg smoke.

Table A-13

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure[a]

Tissue: Trachea & Larynx

F Series[a]

Ct=12

Animal Designation	Tissue wt., g	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-F-1	0.121	15.3	.019	97.8	0.019	.015
DC-F-5	0.147	21.7	.027	102	.026	.020
DC-F-6	0.214	*	-	104	—	c
DC-F-8	0.202	16.1	0.20	89.3	.022	.017
DC-F-11	0.164	19.2	.024	99.4	.024	.018
DC-F-13	0.184	15.6	.020	82.2	.024	.018
DC-F-15	0.150	8.6	.011	105	.010	.008
DC-F-16	0.234	*	—	102	—	c
DC-F-18	0.178	19.4	.024	102	.024	.018
DC-F-19	0.151	*	--	100	—	c
Mean (± 1 std. dev.)	0.175 ± 0.035					.013 ± .007

[a]Exposure conditions: 2.33 ± 0.12 mg L^{-1} for six hours.
*Below limit of good quantitation: less than 5 ng mL^{-1}.
[b]Based on a DCBP concentration of 1.30 µg per mg smoke.
[c]Levels of 5 ng DCBP per tissue were used for samples which were found to have DCBP levels at or below this lower limit of detection.

Table A-14

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure

Tissue: Turbinates
F Series[a]
Ct=12

Animal Designation	Concentration of DCBP in Analyte Soln, ng mL^{-1}	Apparent Amount of DCBP in Tissue µg	Percent Recovery of 32 ng Portion of DCBP	Corrected Amount of DCBP in Tissue (µg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[b]
DC-F-1	23.3	.029	92.8	.031	.024
DC-F-5	60.7	.076	101	.075	.058
DC-F-6	44.9	.056	98.3	.057	.044
DC-F-8	19.3	.024	94.7	.025	.019
DC-F-11	19.0	.024	99.7	.024	.018
DC-F-13	19.8	.025	100	.025	.019
DC-F-15	27.4	.034	97.7	.035	.027
DC-F-16	21.7	.027	97.8	.028	.022
DC-F-18	30.0	.038	99.7	.038	.029
DC-F-19	30.5	.038	94.8	.040	.031
Mean (± 1 std. dev.)					.029 ± .013

[a]Exposure conditions: 2.33 ± 0.12 mg L^{-1} for six hours.
[b]Based on a DCBP concentration of 1.30 µg per mg smoke.

Table A-15

Particle Deposition in Rats Following Diesel Fuel Smoke Exposure

Tissue: Digestive Tract

Animal Designation	Smoke Concentration, mg L^{-1}	Exposure Time, hr	Apparent Amount of DCBP in Tissue μg	Percent Recovery of 1.6 μg Portion of DCBP	Corrected Amount of DCBP in Tissue (μg)	Equivalent Amount of Diesel Fuel Smoke in Tissue, mg[a,b]
DC-C-6	6	2	1.24	91.2	1.36	1.20
DC-C-9	6	2	4.43	98.9	4.48	3.96
DC-C-14	6	2	2.54	94.1	2.70	2.39
DC-C-20	6	2	1.18	95.8	1.23	1.09
DC-F-5	2.3	6	3.26	88.3	3.69	2.84
DC-F-8	2.3	6	2.94	85.0	3.46	2.66
DC-F-13	2.3	6	1.38	88.9	1.55	1.19
DC-F-15	2.3	6	3.15	82.4	3.82	2.94

[a] C series (6 mg L^{-1} for 2 hours) data based on a DCBP concentration of 1.13 μg/mg smoke.
[b] F series (2.3 mg L^{-1} for 6 hours) data based on a DCBP concentration of 1.30 μg/mg smoke.

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