



EDGEWOOD CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1322

GC-MS/MS ANALYSES OF BIOLOGICAL SAMPLES IN SUPPORT OF DEVELOPMENTAL TOXIC EFFECTS ON SUBCUTANEOUS EXPOSURE OF RATS TO GB

Jeffrey M. McGuire

RESEARCH AND TECHNOLOGY DIRECTORATE

Lucille A. Lumley

U.S. ARMY MEDICAL RESEARCH INSTITUTE
OF CHEMICAL DEFENSE
Aberdeen Proving Ground, MD 21010-5424

Paul S. Demond

LEIDOS, INC.
Gunpowder, MD 21010-0068

Michael W. Busch
Linnzi K. Wright

EXCET, INC.
Springfield, VA 22151-2110

November 2015

Approved for public release; distribution is unlimited.



Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 h per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) XX-11-2015		2. REPORT TYPE Final		3. DATES COVERED (From - To) Jul 2012 – Jan 2015	
4. TITLE AND SUBTITLE GC-MS/MS Analyses of Biological Samples in Support of Developmental Toxic Effects on Subcutaneous Exposure of Rats to GB				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) McGuire, Jeffrey M. (ECBC); Lumley, Lucille A. (MRICD); Demond, Paul S. (Leidos); Busch, Michael W.; and Wright, Linnzi K. (Excet)				5d. PROJECT NUMBER BARDA CBRN OS 75442 ASPR-11-04601	
				5e. TASK NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Director, ECBC, ATTN: RDCB-DRT-T, APG, MD 21010-5424 Director, MRICD, ATTN: MCMR-CDZ, APG, MD 21010-5400 Leidos, Inc.; P.O. Box 68, Gunpowder, MD 21010-0068 Excet, Inc.; 8001 Braddock Road, Suite 303, Springfield, VA 22151-2110				8. PERFORMING ORGANIZATION REPORT NUMBER ECBC-TR-1322	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Biomedical Advanced Research and Development Authority, 330 Independence Avenue, SW, Room G640, Washington, DC 20201-0003				10. SPONSOR/MONITOR'S ACRONYM(S) BARDA	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT: This report documents the results of gas chromatography–tandem mass spectrometry analyses of blood, tissues, and organs (heart, lung, liver, kidney, brain, eye, and diaphragm) that were obtained from rats (postnatal days 7, 14, 21, 42, and 70) after their exposure to various concentrations of sarin (GB) via subcutaneous injection. The amounts of free and regenerated GB present in these biological samples were quantified using chemical ionization mass spectrometry with isotope dilution.					
15. SUBJECT TERMS Gas chromatography–tandem mass spectrometry (GC-MS/MS) Regenerated GB (r-GB) Subcutaneous injection Postnatal day (PND) rats Isotope dilution Sarin (GB)					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Renu B. Rastogi
U	U	U	UU	38	19b. TELEPHONE NUMBER (include area code) (410) 436-7545

Blank

PREFACE

The work described in this report was authorized under project no. BARDA CBRN OS 75442 ASPR-11-04601 and U.S. Army Medical Research Institute of Chemical Defense (MRICD) protocol 1-12-U-1001. The work was started in July 2012 and completed in January 2015, as recorded in ECBC notebooks 09-0088, 13-0001, 13-0080, and 14-0084.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Acknowledgments

The authors acknowledge the following individuals for their hard work and assistance with the execution of this technical program:

- ☐ Richard J. Lawrence and Bernard J. Benton (U.S. Army Edgewood Chemical Biological Center) for their support of the sample preparation task of this program.
- ☐ Personnel from MRICD who aided in the collection of blood and tissue specimens.

Blank

CONTENTS

1.	INTRODUCTION	1
2.	METHODS	2
2.1	Animal Exposures	2
2.2	Sample Preparation and Analysis	3
2.2.1	Chemical Materials	3
2.2.1	Stock Solutions and Calibration Standards	3
2.2.2	Analytical Method	4
2.2.3	Sample Preparation	4
3.	RESULTS AND DISCUSSION	5
	LITERATURE CITED	25
	ACRONYMS AND ABBREVIATIONS	27

TABLES

1.	Body Weight before Exposure.....	2
2.	24 h LD ₅₀ Values for Subcutaneous Exposure to GB.....	2
3.	Results from Regenerated and Free GB Assays of PND 7 Rats.....	7
4.	Results from Regenerated and Free GB Assays of PND 14 Rats.....	11
5.	Results from Regenerated and Free GB Assays of PND 21 Rats.....	13
6.	Results from Regenerated and Free GB Assays of PND 42 Rats.....	15
7.	Results from Regenerated and Free GB Assays of Plasma and Red Blood Cell Fractions from Selected PND 42 Rats.....	19
8.	Results from Regenerated and Free GB Assays of PND 70 Rats.....	20

GC-MS/MS ANALYSES OF BIOLOGICAL SAMPLES IN SUPPORT OF DEVELOPMENTAL TOXIC EFFECTS ON SUBCUTANEOUS EXPOSURE OF RATS TO GB

1. INTRODUCTION

Chemical warfare nerve agents (CWNAs) include G-agents such as tabun (ethyl *N,N*-dimethylphosphoramidocyanidate; GA), sarin (isopropyl methylphosphonofluoridate; GB), soman (pinacolyl methyl phosphonofluoridate; GD), and cyclosarin (cyclohexyl methylphosphonofluoridate; GF), as well as less-volatile V-agents such as VX (*O*-ethyl *S*-[2-diisopropylaminoethyl] methyl phosphonothioate) and Russian VX (*O*-isobutyl *S*-[(2-diethylamino)ethyl] methylphosphonothioate). Like organophosphorus pesticides, CWNAs exert their toxicological effects by inhibiting acetylcholinesterase (AChE), the enzyme responsible for the degradation of the neurotransmitter acetylcholine (ACh) in the central and peripheral nervous systems. ACh accumulation within the synaptic cleft after extensive AChE inhibition increases and prolongs the stimulation of muscarinic and nicotinic receptors on autonomic ganglion, end-organs, myocytes, and postsynaptic neurons, which leads to an acute cholinergic crisis that is characterized by autonomic and cardiac dysfunction, involuntary movements, miosis, muscle fasciculations, respiratory distress, and seizures (Russell and Overstreet, 1987).

In the event of a mass casualty situation involving the release of CWNA amongst a civilian population or the military community, pregnant women, infants, and small children are likely to be exposed. For example, four pregnant women between 9 and 36 weeks of gestation were admitted to the hospital with mild cholinergic symptoms after the release of GB in the Tokyo subway system (Ohbu et al., 1997). In fact, infants and small children may be at greater risk for inhalation and dermal exposures to CWNA than adults because of greater minute ventilation rates (Bloomfield, 2002) and larger surface area to body mass ratios (Guzelian et al., 1992), respectively. Additionally, infants and small children are considered to be at greater risk for seizures relative to adults (Ben-Ari and Holmes, 2006), and early-life seizures increase the susceptibility of seizure-induced brain damage in adulthood (Thompson and Wasterlain, 1997).

Unfortunately, the majority of research on the toxic effects of nerve agent exposure has been focused on the combat soldier, who is typically characterized as a male between 18 and 25 years old. Federal agencies have little data to draw from when making recommendations for pediatric doses of medical countermeasures (Baker, 2007), which may lead to inadequate treatment or even overdose.

Therefore, *Developmental Toxic Effects on Whole-Body and Percutaneous Exposure to Chemical Warfare Nerve Agents (CWNA) in Rats: Effects on Brain and Behavior*, the U.S. Army Medical Research Institute of Chemical Defense protocol number 1-12-U-1001 (MRICD, 2012), provides additional data needed to develop a pediatric animal model of nerve agent exposure to make more accurate human risk assessments. In addition, physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling, which was incorporated into the study, yields a quantitative basis for extrapolating animal-to-human exposure conditions

and predicting the human response to a chemical of interest. An earlier report (McGuire et al., 2015) documented the results of the gas chromatography–tandem mass spectrometry (GC-MS/MS) analyses of blood, tissues, and organs (heart, lung, liver, kidney, brain, eye, and diaphragm) that were used to quantify the amount of free and regenerated GB (r-GB) present in these biological samples from whole-body exposures. This current report details the results of the GC-MS/MS analyses of blood, tissues, and organs to quantify the amount of free GB and r-GB present in these samples from subcutaneous exposures.

2. METHODS

2.1 Animal Exposures

Age-matched male and female Sprague-Dawley rats (CD IGS rats) were purchased from Charles River Laboratories (Kingston, NY). The rats were housed in temperature- and humidity-controlled colony rooms under a 12 h light/dark cycle (lights on at 0600). Food and water were available ad libitum, and the pups remained with their lactating dams until postnatal day (PND) 21. Table 1 shows the pre-exposure body weight in grams (mean $\pm$ standard deviation) for each age group and gender. Males weighed significantly ($p < 0.0001$) more than females on PND 42 and 70.

Table 1. Body Weight before Exposure

PND Age Group	Male (g)	Female (g)
7	17.7 $\pm$ 2.3	17.0 $\pm$ 1.8
14	32.7 $\pm$ 2.2	31.4 $\pm$ 2.1
21	50.5 $\pm$ 5.0	48.2 $\pm$ 5.2
42	226.8 $\pm$ 12.9	172.3 $\pm$ 10.3
70	345.2 $\pm$ 12.5	230.9 $\pm$ 8.8

Rats were exposed to saline or one of three doses of GB: 0.4, 0.6, or 0.8 lethal dose for 50% of the population (LD₅₀) via a subcutaneous injection (2 mL/kg) between the shoulder blades. Table 2 shows the 24 h LD₅₀ values in micrograms per kilogram for each age group and gender.

Table 2. 24 h LD₅₀ Values for Subcutaneous Exposure to GB

PND Age Group	Male (µg/kg)	Female (µg/kg)
7	46.3	51.1
14	59.3	60.5
21	88.9	89.4
42	225	173
70	166	168

At 1, 4, 24, or 48 h post-exposure, the rats were deeply anesthetized with an intraperitoneal injection of a pentobarbital-based solution (FatalPlus; Vortech Pharmaceuticals; Dearborn, MI) in excess of 75 mg/kg. Blood was collected using a cardiac stick, and each rat was perfused through the aorta with normal saline (0.9% sodium chloride) for approximately 5 min. Brain, diaphragm, eye, heart, kidney, liver, and lung tissues were collected from each rat following perfusion, and the tissues were flash-frozen with liquid nitrogen. Blood and tissue samples were stored at 4 and -80°C , respectively, until the samples were processed.

2.2 Sample Preparation and Analysis

2.2.1 Chemical Materials

GB (GB-U-5340-CTF-N) and $^2\text{H}_6$ -GB were obtained from U.S. Army Edgewood Chemical Biological Center (ECBC) chemical agent standard analytical reagent material (CASARM) stock. Before use, the CASARM-grade GB was verified with quantitative ^{31}P nuclear magnetic resonance (NMR) spectrometry as 93.8 ± 2.4 wt % (laboratory notebook [NB] 11-0003-77), and the $^2\text{H}_6$ -GB was verified as 24.86 ± 0.37 wt % (NB 11-0003-113). Potassium fluoride (KF), 2-propanol (IPA), ethyl acetate, glacial acetic acid, and anhydrous sodium sulfate were obtained from Sigma-Aldrich (St. Louis, MO) at $\geq 99\%$ purity. Sodium acetate was purchased from Fischer Chemicals (Fair Lawn, NJ) at $>99\%$ purity. Ammonia and methane were obtained from Sigma-Aldrich, and helium was obtained from Messer Griesheim Industries, Inc. (Malvern, PA) at purities $>99.9\%$.

2.2.1 Stock Solutions and Calibration Standards

Stock solutions of GB and $^2\text{H}_6$ -GB (internal standard [IS]) were prepared in IPA at concentrations of 2.188 mg/mL (NB 11-0003-84-01) and 0.519 mg/mL (NB 11-0003-109-01), respectively, and stored at -20°C until used. Working solutions (5–10 $\mu\text{g/mL}$) were prepared by diluting the stock solutions in ethyl acetate. Calibration standards of GB were prepared by diluting the working solution to obtain the following 12 concentration points: 0.5, 1, 5, 10, 25, 50, 100, 200, 400, 600, 800, and 1000 ng of GB/mL (NB 11-0003-116-04 through 11-0003-116-15). Each calibration standard also contained 200 ng of $^2\text{H}_6$ -GB/mL, which was diluted from the working solution. All calibration standards were stored at -20°C until analysis.

For the GB assays, a calibration curve was constructed using the 12 calibration standards, where *Relative Response* (defined as $\text{Area}_{\text{Analyte}}/\text{Area}_{\text{IS}}$) was plotted against *Relative Concentration* (defined as $\text{Concentration} [\text{ng/mL}]_{\text{Analyte}}/\text{Concentration} [\text{ng/mL}]_{\text{IS}}$). For the GB calibration curve, a quadratic curve fit was used with a $1/x$ weighting factor. Typically, the calibration curve yields a correlation of $R^2 = 0.999$ over 3 orders of magnitude, where R^2 is defined as the coefficient of determination.

2.2.2 Analytical Method

Sample assays were performed using an Agilent Technologies 7000A GC/MS Triple Quad instrument (Santa Clara, CA). Gas chromatographic separations were achieved using a Restek Corporation (Bellefonte, PA) RTx-1701 column (30 m $\times$ 0.25 mm i.d., 0–25 μ m film thickness). The carrier gas was helium with a flow rate of 1 mL/min. Injections of 2.0 μ L were made using an autoinjector (7693 ALS, Agilent Technologies) into a splitless injector port at a temperature of 250 °C. The initial oven temperature of 35 °C was held for 1 min, then ramped at 50 °C/min to 50 °C, ramped again at 2 °C/min to 64 °C, and finally ramped again at 50 °C/min to 200 °C. The column was then backflushed at 280 °C for 4 min at reduced inlet pressure (–6.3 mL/min). Typical retention times for GB and its deuterated standard are 9.8 min.

Samples were ionized by positive-ion chemical ionization (CI) with ammonia reagent gas. CI source conditions were optimized using Fluoroether E3 (Chemical Abstract Service registry number: 3330-16-3, Agilent Technologies) tuning compound with methane reagent gas. Mass spectra were obtained at a dwell time of 0.2 s for each transition in the multiple reaction monitoring mode. Helium was used as the collision gas with a collision energy (CE) of 10 V. The CE was optimized for the mass-to-charge ratio (m/z) 158 > 99 transition for GB and the m/z 164 > 100 transition for $^2\text{H}_6$ -GB. The MassHunter software provided with the 7000A system (Agilent Technologies) was used to process and analyze the data. The software provided automated peak detection, calibration, and quantitation.

2.2.3 Sample Preparation

Sample preparations for this study were similar to those published by McGuire et al. (2015). Upon arrival, all biological samples were stored at –80 °C until analyzed. Whole blood samples were extracted for GB using Sep-Pak C18 3 cc Vac (200 mg) solid-phase extraction (SPE) cartridges (Waters Corporation; Milford, MA), which were first conditioned with 1 mL each of ethyl acetate, IPA, and then pH 3.5 acetate buffer (0.01 M sodium acetate and 0.2 M glacial acetic acid). After the sample of blood in a 2.0 mL microcentrifuge tube (Sigma-Aldrich) was weighed, 1 mL of acetate buffer, 200 μ L of KF solution (6 M), and 1 μ L of $^2\text{H}_6$ -GB IS were added. The mixture was vortexed for 10–20 s and then centrifuged at 15,000 rpm for 5 min using a Micromax Microcentrifuge (Thermo IEC; Needham Heights, MA). The supernatant liquid was transferred to the SPE cartridge, then the sediment at the bottom of the microcentrifuge tube was resuspended with 750 μ L of acetate buffer and 200 μ L of KF solution. This mixture was also vortex-mixed and centrifuged, and the resulting liquid was added to the original solution. After the mixture was added to the SPE cartridge, it was allowed to drain under a gentle vacuum. The analytes were eluted with 1 mL of ethyl acetate, which was collected and dried over anhydrous sodium sulfate. The ethyl acetate was withdrawn from the collection tube, filtered through a 0.2 μ m nylon Acrodisc syringe filter (Pall Gelman Laboratory; Ann Arbor, MI) into a GC autosampler vial (Agilent Technologies), and then concentrated to 50 μ L for analysis.

Tissue and organ sample extracts were prepared in a similar manner, using freeze-fracture pulverization under cryogenic temperatures before SPE extraction. A Tissue Cryoprep system (Covaris, Inc.; Woburn, MA) was used to pulverize 0.5–1 g of tissue. The pulverized

sample was then mixed with 1 mL of acetate buffer, 200 μ L of KF solution, and 1 μ L of IS. This sample was then subjected to focused acoustics using an S-series focused acoustic energy system. This process causes precisely controlled cavitation and acoustic streaming at the focal point within the sample treatment vessel in a noncontact, isothermal process. After centrifugation at 4500 rpm for 15 min, using a Sorvall Legend X1R centrifuge (Thermo Fisher Scientific, Inc.; Waltham, MA), the supernatant liquid was transferred to the SPE cartridge, and the sediment at the bottom of the sample tube was resuspended with 750 μ L of acetate buffer and 200 μ L of KF solution. This mixture was vortex-mixed and centrifuged, and the resulting liquid was added to the original solution. Further sample processing was performed in a manner that was identical to that of the blood samples.

3. RESULTS AND DISCUSSION

The following results have been recorded in ECBC notebooks 09-0088, 13-0001, 13-0080, and 14-0084. Table 3 summarizes the data from the GB assays of whole blood and various tissues and organs that were obtained from PND 7 rats after exposure to various doses of GB through subcutaneous injections. For each type of biological sample, a control sample (subcutaneous injection of saline only) was spiked with GB at a concentration of 100 ng/g to determine the percentage of GB recovered. As indicated, these recoveries ranged from 102 to 126%. Tables 4, 5, 6, and 8 show similar results from the subcutaneous injection exposures to GB for rats from the additional age groups.

The whole blood samples from several PND 42 rats were separated using centrifugation to obtain a plasma fraction and a red blood cell fraction before the samples were submitted for GB assay. This is shown in Table 7, which provides an estimate of the distribution of GB between the plasma and red blood cells in whole blood.

Although a detailed analysis of the pharmacokinetics of absorption, distribution, metabolism, and elimination of GB in rats was beyond the scope of this report, several observations were made: Once GB was in the bloodstream of a rat, the lungs were a major source of accumulated GB, as indicated by the relatively high levels of free GB and r-GB detected in lung tissue. This was not surprising because the lungs are a major organ in the circulatory system. Unlike the whole-body exposures to GB, very little free GB and r-GB were detected in the eyes of rats in this study. An examination of the levels of free GB and r-GB in the blood of the test rats indicates that distribution of the agent from a subcutaneous exposure is very similar to the distribution of GB in blood from a whole-body exposure. For example, 6.7614 ± 2.8802 ng/g of r-GB was measured in the whole blood of PND 42 male rats at 1 h, following a 0.6 LD₅₀ subcutaneous exposure, and 6.2747 ± 1.6818 ng/g of r-GB was measured in the whole blood of PND 42 male rats at 1 h, following a 0.6 lethal concentration with 50% chance of causing death (LC_{t50}) whole-body inhalation exposure for 1 h.

An examination of the free GB and r-GB found in the liver samples of male and female rats at PNDs 7, 21, and 42 after a 0.6 LD₅₀ subcutaneous exposure indicated similar metabolic rates for GB among these groups. A two-way analysis of variance (ANOVA) was done to test whether a significant difference was present between the male and female

populations at the 1, 4, or 24 h sampling points for each of the PND rat groups. Using the means of the free GB and r-GB values for each biological sample from the same sampling time, testing results indicated that no statistically significant difference ($p < 0.05$) was present between the two genders at the three sampling points. Insufficient data sets at additional subcutaneous exposure concentrations (0.4 and 0.8 LD₅₀) precluded further ANOVA tests.

Using the same ANOVA test of the data for the free GB and r-GB found in the kidney samples of male and female rats at PNDs 7, 21, and 42 after a 0.6 LD₅₀ subcutaneous exposure produced results that were similar to those found for the liver samples. Using the means of free GB and r-GB for each biological sample from the same sampling time, testing results indicated that no statistically significant difference ($p < 0.05$) was present between the two genders at the three sampling points.

Because the goal of this report was to document the results of the GC-MS/MS analyses, no further interpretations were attempted. All of the data has been transferred to Dr. Jeffery M. Gearhart of the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. at Wright-Patterson Air Force Base, OH for further efforts to develop the appropriate PBPK/PD models to predict the biological impact of GB exposure in young animals.

Table 3. Results from Regenerated and Free GB Assays of PND 7 Rats

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
333	F	24	30.3	5.1227	1.6331	46.2491	1.1838	1.7390	0.4098	0.2146	SL
337	M	24	27.9	6.4750	2.4200	31.1862	1.0583	1.3902	0.5406	1.0450	0.0995
339	M	24	0	BDL	0.3265	BDL	BDL	BDL	BDL	BDL	SL
342	F	24	0	BDL	0.1184	BDL	BDL	0.4709	NS	BDL	SL
347	M	24	27.7	4.5720	2.7103	47.4792	1.7335	0.8254	0.3709	0.2283	SL
352	F	24	0	102%*	0.1694	BDL	BDL	3.5809	0.0609	BDL	SL
353	F	24	30.7	0.0945	BDL	0.2010	BDL	1.5057	0.0759	BDL	SL
356	M	24	27.6	0.2294	0.1654	0.5689	BDL	0.3452	BDL	BDL	SL
358	M	24	0	BDL	0.1121	BDL	BDL	0.1797	BDL	BDL	SL
502	F	4	40.4	9.9392	6.2969	144.5233	2.2966	SL	4.4557	2.6828	BDL
503	F	24	41.5	10.4468	4.2819	110.4958	7.1125	3.7070	1.8902	1.2360	BDL
509	M	24	36.5	6.5542	2.9817	89.0354	1.5480	0.2962	1.4222	0.8758	BDL
510	M	4	37.4	7.0048	6.4714	140.5682	3.6027	11.9121	3.2276	2.9774	0.6650
511	F	24	40.9	NS	4.4242	123.8078	2.2710	1.8376	1.7343	0.9881	BDL
514	F	1	40.4	NS	11.9393	198.9687	10.0742	11.5209	5.2074	3.0702	BDL
515	F	4	40.7	6.7765	7.3666	188.6554	7.0417	7.4596	3.8508	5.3025	2.1742
516	M	4	37.0	0.0692	BDL	0.0939	1.1952	BDL	BDL	0.0729	BDL
519	M	24	37.4	1.1561	0.2716	13.1240	BDL	BDL	BDL	BDL	BDL
682	F	4	0	118%*	BDL	BDL	BDL	BDL	BDL	BDL	BDL
683	F	4	20.3	3.2547	0.8106	16.4702	0.3958	0.4312	0.5112	0.1267	BDL
684	F	4	41.4	4.5548	1.6203	33.3612	2.5003	1.0896	0.8754	0.6219	0.9885
685	F	4	30.5	5.9475	1.8852	27.5518	2.7824	1.1633	1.1730	0.4486	0.5924
687	M	4	0	0.2969	BDL	0.1590	SL	SL	BDL	BDL	BDL
688	M	4	37.3	6.8400	3.3731	53.6162	1.9015	1.2569	1.4989	0.7168	1.0397
691	F	4	30.9	2.1419	0.2227	70.0040	SL	0.6880	1.0974	0.6356	BDL
692	F	4	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
693	F	4	20.4	1.5190	0.4655	29.4734	SL	SL	0.3420	0.0472	BDL

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

SL: Sample was lost during assay preparation.

Table 3. Results from Regenerated and Free GB Assays of PND 7 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose (µg/kg)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
694	F	4	40.4	6.2815	1.6011	48.4834	1.2889	SL	1.2374	0.5763	BDL
695	F	1	41.1	7.0379	0.1268	41.9894	1.0314	1.2090	1.7558	0.3232	0.6179
697	M	4	37.0	8.4499	2.2198	77.9352	SL	SL	2.0747	0.4572	BDL
700	M	4	40.4	BDL	BDL	BDL	SL	SL	BDL	BDL	BDL
852	F	24	40.9	NS	2.6143	31.9863	1.4321	0.8890	0.8579	0.2580	BDL
858	M	24	36.8	4.0826	2.9394	13.0346	1.6248	1.0785	0.9067	0.1993	1.6539
861	F	24	40.9	7.1134	3.6576	54.1507	2.5840	1.7470	1.3756	0.6364	1.2128
866	M	24	36.5	5.9652	3.2694	SL	2.3823	1.3759	0.9217	0.4059	1.8511
875	F	24	20.5	2.4646	1.1007	29.6021	0.3610	0.1475	0.2669	BDL	BDL
876	M	24	18.4	3.5498	1.0056	19.7442	0.4350	0.1573	0.3125	BDL	BDL
884	F	24	20.1	3.0838	1.3188	53.7707	0.7123	0.3369	0.3268	BDL	BDL
890	M	24	18.6	3.6549	1.5683	43.6765	0.9191	0.2622	0.3898	BDL	BDL
892	F	24	20.3	2.5169	1.4505	23.9213	0.4306	0.2219	0.5075	BDL	BDL
899	M	24	27.5	2.4414	1.3789	28.3929	1.3130	0.3340	0.4951	BDL	BDL
903	F	24	26.0	4.0215	1.3593	56.2971	0.7881	0.4245	0.5594	BDL	0.1523
910	M	24	28.2	0.8593	BDL	11.0760	0.6343	BDL	BDL	BDL	BDL
914	F	24	30.4	3.9071	1.5317	31.4005	2.3000	0.4476	0.6094	BDL	BDL
919	M	24	18.7	2.9494	0.8023	23.9213	3.2601	0.2088	0.2604	BDL	BDL
921	F	24	30.7	4.6155	1.6529	38.5144	2.6338	0.2288	0.4585	BDL	BDL
927	M	24	18.3	1.8149	0.2203	13.2922	0.6001	0.0481	0.0856	BDL	BDL
942	F	4	20.2	NS	1.7103	45.2178	3.0002	1.5820	0.9065	BDL	BDL
943	F	24	0	BDL	BDL	109%*	0.6968	BDL	BDL	BDL	BDL
944	F	4	30.5	NS	3.0890	58.9661	0.7932	1.4147	1.5835	0.2248	BDL
945	F	24	20.3	3.8005	1.9239	17.3379	1.2114	0.6781	0.5887	BDL	BDL
946	F	24	30.4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
947	M	24	18.3	0.3797	BDL	2.0038	BDL	BDL	BDL	BDL	BDL
948	M	4	27.4	NS	4.7740	39.5545	4.0624	5.5847	2.4464	1.0588	0.1706

*Selected control samples (0 µg/kg) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

SL: Sample was lost during assay preparation.

Table 3. Results from Regenerated and Free GB Assays of PND 7 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
949	M	24	0	BDL	BDL	BDL	109%*	BDL	BDL	BDL	BDL
950	M	4	18.3	NS	1.7626	24.3353	0.5182	1.1119	0.5315	BDL	BDL
951	M	24	28.1	5.3341	2.0302	49.1716	0.6332	1.1361	0.5986	BDL	BDL
956	F	24	41.1	6.3426	2.6270	20.6882	1.3692	0.8744	0.8253	BDL	BDL
958	M	24	27.6	3.9609	1.2089	50.7649	0.5584	0.3246	0.2694	SL	BDL
964	F	24	41.5	10.3570	4.0660	68.1186	1.5265	1.7473	1.3873	0.3557	0.2948
966	F	24	28.5	1.9165	1.6384	56.1928	0.3274	0.3682	0.4591	BDL	BDL
967	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	126%*	BDL
968	M	24	18.6	2.3268	0.1302	19.0075	0.1028	0.1612	BDL	BDL	BDL
996	F	4	20.1	2.9781	1.7316	29.6402	1.5620	0.9452	0.5390	BDL	0.3916
997	F	4	30.7	5.4660	2.9852	54.1338	2.2758	1.9812	1.1990	BDL	2.2962
998	F	1	20.3	4.1201	5.4571	67.7039	2.2814	3.1160	1.7722	SL	0.3303
999	F	24	20.4	1.0019	1.3273	48.1835	1.6170	0.3053	SL	BDL	BDL
1000	F	4	40.7	3.8259	4.5073	106.5296	1.7639	1.3285	1.3369	BDL	BDL
1001	M	4	18.6	2.5993	0.4409	28.0308	0.6057	0.7953	0.2123	BDL	BDL
1002	M	4	28.1	2.1585	1.7095	38.2672	0.1658	1.3225	0.3252	BDL	BDL
1003	M	1	18.8	3.2636	2.4065	29.7274	1.7237	4.3342	0.6929	BDL	BDL
1004	M	1	28.1	3.5163	2.6685	61.8596	0.5471	2.9363	1.4356	BDL	1.3726
1005	M	4	37.5	0.9081	3.4406	80.0069	0.3275	1.9767	1.3388	BDL	1.2508
1006	F	4	20.2	2.4248	2.4667	26.9953	1.3921	1.2636	0.9192	BDL	0.7308
1007	F	4	30.5	4.1572	5.7106	91.9094	3.3777	2.5816	3.0970	0.2419	BDL
1008	F	1	20.7	1.4091	1.4767	21.7083	0.9796	1.3932	0.7595	BDL	0.7164
1009	F	1	30.7	2.2883	1.2930	69.5663	2.2647	3.5205	3.1511	0.6626	4.3028
1010	F	4	41.2	NS	5.2375	158.3285	BDL	2.7460	3.4765	1.0228	2.1680
1011	M	4	18.6	3.6222	2.2388	45.2328	BDL	1.2826	1.1754	BDL	0.7125
1012	M	4	28.0	2.5902	3.0118	45.7948	4.5317	1.6966	2.0708	0.3644	SL
1013	M	1	18.7	2.9276	3.0603	20.5446	7.9200	1.0929	1.2454	BDL	BDL

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

SL: Sample was lost during assay preparation.

Table 3. Results from Regenerated and Free GB Assays of PND 7 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
1014	M	4	37.2	2.1256	5.2112	110.1427	1.1721	3.1065	3.3258	0.9576	1.5419
1015	M	24	36.8	BDL	3.8438	79.1449	0.7787	1.5014	1.1337	BDL	BDL
1016	F	4	20.4	SL	0.9883	31.6905	1.6839	0.9092	0.2881	BDL	4.6066
1017	F	4	30.7	2.8918	1.2142	22.8718	0.3713	0.7399	0.5710	BDL	0.5364
1018	F	1	31.0	0.7136	2.5183	20.7938	BDL	0.6252	0.6364	BDL	0.4375
1019	F	1	41.4	3.8311	6.1181	41.2446	9.6513	10.4770	3.5029	0.9156	2.6324
1020	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
1021	M	4	18.6	BDL [†]	0.2667	19.1837	0.5143	0.6658	0.0745	BDL	BDL
1022	M	4	28.1	BDL [†]	1.9233	41.4921	1.7649	1.4260	0.7688	BDL	0.4888
1023	M	1	27.5	4.2846	4.0582	42.7097	3.3647	2.7706	1.4992	0.0680	1.7503
1024	M	24	37.0	BDL [†]	2.0723	45.2929	1.1600	0.6228	0.6155	BDL	0.1174
1025	M	1	36.8	3.7955	2.1712	61.8875	3.8018	4.1810	2.6530	0.2546	2.0449

[†] For these samples <10 μL volume was received.

BDL: Below detection limit (<0.05 ng/g).

SL: Sample was lost during assay preparation.

Table 4. Results from Regenerated and Free GB Assays of PND 14 Rats

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
361	F	24	36.5	6.7608	2.8302	203.6667	1.2704	1.3720	1.5712	0.8040	SL
363	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
372	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	SL
373	F	24	36.1	5.6529	2.5278	74.8346	1.9801	1.3434	1.1252	0.6253	0.4991
382	F	24	36.1	0.3166	BDL	0.8937	BDL	BDL	BDL	BDL	BDL
586	F	4	24.2	3.8548	1.0981	66.5231	1.1544	0.5519	1.7280	0.8671	BDL
588	F	4	48.7	6.1764	2.0384	72.3476	1.6142	2.2144	3.4375	1.3647	BDL
590	M	1	35.6	7.6651	2.9826	8.0423	53.9107	3.9923	4.4828	2.1385	1.8708
592	M	1	23.7	NS	2.1390	47.7550	0.3156	1.0158	2.3326	0.9699	BDL
594	M	4	35.7	5.7648	2.7639	66.2220	1.5666	2.0655	3.5410	1.6218	1.5102
595	F	1	24.4	4.4994	1.5083	56.5111	0.9592	1.0618	1.6988	0.2865	0.6471
600	M	1	35.7	5.5413	2.7547	57.1569	2.8192	3.7892	2.8575	1.3416	1.2774
601	M	4	23.8	4.0904	1.4574	34.8536	0.2554	0.6764	1.5721	0.4220	0.4068
602	M	1	47.4	7.3832	2.7023	64.3003	1.3066	2.1031	6.2017	2.0679	BDL
603	M	4	35.5	4.8885	1.5473	47.0535	0.2212	1.0777	2.8278	1.1088	0.8890
701	F	24	24.0	3.0198	1.7889	33.3859	0.7070	0.8239	0.8078	0.5523	0.8184
702	F	24	36.1	13.2226	1.9155	37.0493	1.7971	1.4007	1.4624	0.8095	0.8490
703	F	24	48.1	5.1476	2.2182	60.7241	1.2421	1.6100	2.5025	0.8661	BDL
705	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	115%*	BDL
706	M	24	0	BDL	BDL	0.1116	BDL	BDL	BDL	BDL	BDL
707	M	24	23.6	2.8997	0.9229	35.7346	0.7663	0.5933	0.4868	0.2340	0.2533
708	M	24	47.4	7.4130	2.8397	64.8903	3.0450	1.6302	2.5297	0.9836	0.8045
710	M	24	35.7	5.5027	2.5996	38.4886	1.6187	1.3672	2.0362	0.9965	1.1552
711	F	4	48.4	6.7958	4.4133	50.8774	10.8672	6.5092	5.2702	1.9058	0.3946
712	F	24	48.6	7.7168	3.2700	45.9285	5.0604	1.7433	2.3022	1.1715	1.3664

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

SL: Sample was lost during assay preparation.

Table 4. Results from Regenerated and Free GB Assays of PND 14 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
713	F	24	24.4	3.4658	1.8218	40.4717	1.4187	0.9273	1.0710	0.2504	0.4451
714	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
716	F	24	35.4	6.5059	1.3787	78.7613	3.0352	1.3623	1.3869	0.3314	0.6996
718	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
720	M	24	23.7	4.3849	1.5712	51.8771	0.8852	0.8949	0.7685	0.1312	0.4500
823	F	24	24.0	3.1774	1.3367	10.4901	1.4004	0.4944	0.6267	0.2241	0.3401
826	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
827	M	24	35.8	4.2445	1.9226	27.6324	2.9290	1.1358	1.2028	0.7037	1.2615
828	M	24	23.8	2.7839	1.5621	13.4143	1.0043	0.7827	0.6836	0.3380	0.8937
830	M	24	47.2	6.6475	2.8911	35.8701	1.7972	1.8313	2.3312	1.1497	1.7597
831	F	24	24.2	3.0581	1.7530	42.5253	0.4447	0.8193	0.5975	0.1047	0.3862
835	F	24	48.7	6.3242	3.6368	46.1098	1.8935	2.3296	2.5162	1.4742	1.2744
836	M	24	0	2.2670	BDL	BDL	BDL	BDL	BDL	BDL	84%*
837	M	24	23.8	3.1246	0.8001	37.7088	0.3198	1.0401	0.5932	0.1355	0.7148
838	M	24	35.6	4.1957	1.4732	37.3759	0.5875	0.8886	1.1092	0.5100	0.8418
839	M	4	47.4	7.9432	6.7805	145.1309	3.0819	7.3678	5.4743	2.0777	3.9034
840	M	24	47.7	3.3671	1.4582	18.9544	0.3164	0.6904	0.9273	0.3545	0.3589

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

Table 5. Results from Regenerated and Free GB Assays of PND 21 Rats

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
256	F	24	13.33	0.2840	0.4365	29.3393	0.3526	BDL	0.1426	0.8244	0.1456
257	F	24	0	BDL	BDL	0.4061	BDL	BDL	BDL	0.5350	0.3458
260	M	24	13.4	0.2039	0.3908	27.6135	0.5500	2.3464‡	0.1338	BDL	BDL
264	M	24	0	BDL	0.3533	BDL	BDL	BDL	BDL	0.1859	BDL
265	M	24	13.33	0.1882	BDL	68.2324	0.4187	1.5282‡	0.2270	1.5476	0.3358
266	M	24	0	BDL	0.2868	0.1129	BDL	BDL	102%*	BDL	0.9966
272	M	24	13.4	0.1785	1.0285	55.1428	0.6334	2.2709‡	0.7534	0.2804	3.4762
274	M	24	0	BDL	BDL	BDL	BDL	0.3880‡	BDL	0.2273	BDL
283	M	24	13.4	0.1854	0.4546	43.9602	0.5496	1.5317‡	0.4406	0.2719	0.1388
391	F	24	53.6	2.4988	0.1979	27.7298	0.6216	0.2330	0.0635	BDL	SL
394	F	24	0	BDL	BDL	BDL	BDL	0.1366	BDL	BDL	SL
398	M	24	0	BDL	BDL	BDL	BDL	0.2926	BDL	BDL	BDL
399	M	24	53.3	2.2043	0.6580	29.1715	0.2865	0.1564	0.0581	BDL	SL
401	F	24	0	BDL	0.1182	BDL	BDL	BDL	BDL	BDL	SL
405	F	24	53.8	3.5605	0.3429	46.5360	0.9725	1.6498	0.2637	BDL	SL
407	M	24	0	BDL	0.0780	BDL	BDL	0.1649	BDL	BDL	BDL
410	M	24	53.2	3.1011	0.4015	52.9197	0.1477	0.6281	0.1189	BDL	SL
412	F	24	53.6	2.9723	0.4235	33.6554	0.4462	0.6770	0.3189	BDL	SL
415	F	24	0	BDL	0.1434	BDL	BDL	0.5718	BDL	BDL	SL
418	M	24	53.6	0.6813	0.0851	2.0195	0.1270	0.4210	BDL	BDL	SL
420	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	SL
541	F	4	53.6	4.7079	2.0840	94.8603	1.5436	1.0812	3.7720	1.5117	1.4873
542	F	4	35.8	3.3418	0.7302	53.5288	1.1642	0.5843	1.5861	0.6133	BDL
543	F	4	71.5	6.6446	3.4173	143.0693	3.3076	2.5822	8.0148	2.9937	2.3754
544	F	1	71.5	4.5549	4.7429	289.1706	3.1660	SL	10.6292	3.1968	6.4447
545	F	1	53.6	5.4854	5.0812	69.8820	2.2029	5.9177	7.4400	1.0572	2.3717
546	M	1	53.3	6.0510	5.1871	113.8615	3.7240	9.1965	7.3230	3.3518	9.2511

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

‡These samples were compromised during assay preparation, and the results may be suspect.

BDL: Below detection limit (<0.05 ng/g).

SL: Sample was lost during assay preparation.

Table 5. Results from Regenerated and Free GB Assays of PND 21 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
547	M	1	71.1	4.9451	3.1881	83.2027	3.9562	5.2612	6.2258	1.7344	2.9624
548	M	1	35.6	3.4454	0.9061	29.6869	1.6179	2.0843	0.7041	0.7315	6.3492
549	M	4	53.3	3.9900	3.6972	144.2203	3.0264	1.5946	3.8558	1.6016	3.0104
550	M	4	71.1	7.3113	5.7867	308.0959	4.4806	5.3949	9.7346	1.0314	5.5698
551	F	1	35.8	2.3227	4.0681	78.1322	0.9929	1.8831	0.9720	0.4028	1.7945
552	F	4	71.5	5.0710	1.9879	165.2091	1.2715	5.2367	4.2301	2.2747	2.3457
553	F	4	53.6	5.8343	4.7409	102.0629	4.2422	5.1675	8.7328	2.8937	3.1247
554	F	1	53.6	3.6013	4.5665	145.2757	1.7803	SL	8.2459	0.6808	3.1027
555	F	1	71.5	4.3709	5.8561	239.2641	0.7871	21.3603	6.0880	0.4100	3.1797
556	M	1	53.3	5.3311	3.5604	116.9568	2.5997	2.6413	4.4557	1.6235	1.6051
557	M	1	71.1	5.2501	4.0605	237.8733	1.7938	10.1393	7.9466	0.8804	5.3149
558	M	4	53.3	4.0110	0.7969	132.3209	2.1308	1.6369	1.1042	0.5897	1.6733
559	M	4	35.6	2.0576	0.6001	30.5984	0.1600	0.2966	0.2137	0.1810	NS
560	M	4	71.1	7.9714	4.7749	155.3793	1.5984	6.8167	7.0977	1.4475	0.8524
1172	F	24	53.6	4.8155	0.6237	49.3236	1.0840	0.3511	0.6361	0.1038	0.2505
1176	F	4	0	0.2765	46.5475	0.9328	0.2000	0.4634	NS	0.0734	0.2765
1177	M	4	53.2	0.2170	25.6066	0.3752	0.1069	0.3233	0.0691	0.8772	0.2170
1185	F	4	53.4	0.8533	97.8236	0.6084	0.5867	1.4622	0.4623	0.4405	0.8533
1191	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL: Below detection limit (<0.05 ng/g).

SL: Sample was lost during assay preparation.

NS: No sample was received.

Table 6. Results from Regenerated and Free GB Assays of PND 42 Rats

Rat No.	Sex	Sample Time (h)	Dose (µg/kg)	Regenerated and Free GB (ng/g)							
				Plasma	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
225	F	24	51.8	1.1912	0.7180	99.4757	1.6601	1.4911‡	1.0336	0.2747	0.3201
226	F	24	0	BDL	BDL	0.1121	0.1153	77%*	102%*	119%*	BDL
227	F	24	51.8	1.0103	0.5937	86.8540	1.4776	0.3570‡	0.2438	0.4739	0.1226
228	F	24	0	BDL	BDL	89%*	90%*	BDL	BDL	0.2142	0.0782
229	F	24	51.8	1.2380	0.0958	18.9175	0.8176	0.5242‡	BDL	0.3973	BDL
230	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
231	M	24	67.6	0.9269	1.8521	181.6104	0.6533	0.1849‡	1.0193	BDL	0.1516
232	M	24	67.6	1.2678	3.9168	270.5482	1.3110	2.6220‡	2.2498	1.9698	0.9200
233	M	24	0	1.4402	101%*	151.4812	0.9866	0.3437‡	0.4042	0.0908	0.0923
234	M	24	67.6	BDL	BDL	BDL	BDL	BDL	BDL	0.4087	BDL
290	M	4	90.1	70.3848	7.3520	208.4826	4.2208	33.5337	4.5776	1.5302	10.8794
291	M	4	67.6	61.3952	0.8517	125.6293	2.1113	4.0349	0.4656	0.5648	0.6111
292	M	4	67.6	52.4993	1.9494	83.1288	1.8215	2.1115	0.2080	BDL	0.0445
293	M	4	67.6	67.2651	3.1267	256.7950	1.7647	14.5112	2.0156	0.5801	1.8744
294	M	4	90.1	77.9211	2.2280	318.5955	2.7766	18.0944	3.4029	0.8747	0.6116
300	M	4	90.1	97.1221	2.9097	246.9332	1.8490	17.0737	5.1609	1.7627	0.6429
301	M	4	45.05	37.3204	1.5376	27.9671	0.6661	0.3693	0.0751	0.3328	0.3017
302	M	4	67.6	64.1937	7.7738	231.4457	3.3664	54.5027	8.3100	1.5735	1.4110
303	M	4	90.1	70.9100	1.0999	184.3352	3.3666	3.3977	2.0982	0.8613	0.2187
304	M	4	45.05	42.9910	4.7328	92.7269	1.2142	6.8692	0.3289	0.2890	1.0054
310	M	4	67.6	53.1547	4.1459	60.8503	0.7739	1.6264	0.3545	1.4432	0.6439
312	M	4	45.05	61.9125	1.5151	17.9141	1.5398	6.0176	0.1292	0.4362	2.6146
313	M	4	45.05	50.1410	0.6347	17.3817	1.5727	1.8369	0.1240	0.0656	0.1782
314	M	4	45.05	59.7563	4.2405	31.1227	0.5509	10.7411	0.5238	0.2322	1.8730
				Whole Blood							
428	M	1	178.6	6.5738	6.8234	146.4314	3.6429	16.1301	3.4455	1.1164	4.6969
429	M	1	89.3	4.9550	2.5884	15.8504	3.2729	5.8360	0.0612	0.2715	5.7961

*Selected control samples (0 µg/kg) were spiked with GB to determine percentage recovered.

‡These samples were compromised during assay preparation, and the results may be suspect.

BDL: Below detection limit (<0.05 ng/g).

Table 6. Results from Regenerated and Free GB Assays of PND 42 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
430	M	1	179.3	NS	16.5145	230.0588	3.0970	8.3239	3.9921	1.5539	4.3381
435	M	Unknown		4.9252	1.9182	153.2559	1.0734	1.1612	0.5979	0.0877	BDL
436	M	1	136.4	9.4863	13.9858	328.5808	2.9908	16.0114	10.4400	2.5830	20.8609
437	M	1	90.5	NS	1.7080	5.5848	1.7172	4.7237	0.0778	0.5117	2.4469
438	M	1	90.1	4.8254	2.1501	138.2909	2.3555	1.3852	0.1009	1.4683	1.3736
439	M	1	180.2	NS	5.8600	SL	SL	2.7862	0.6871	0.0794	9.1580
440	M	1	90.1	NS	6.9506	57.9832	1.6271	1.2151	0.2994	0.1481	6.4690
446	M	1	90.1	4.0789	4.2108	27.9709	0.3181	0.8173	0.0755	BDL	0.2204
447	M	1	135.8	9.3052	7.0433	300.1590	4.5260	10.5937	7.7327	1.0309	9.3718
448	M	1	180.2	NS	13.8796	96.6658	3.0326	12.5560	0.7780	1.5732	9.1950
449	M	1	180.2	NS	13.7627	14.1036	4.6881	6.5369	0.5310	0.3527	4.2665
450	M	1	181.0	NS	4.9800	150.6946	0.5288	7.7285	1.2357	0.2536	0.5355
456	M	1	90.1	4.2195	5.7278	23.0896	1.7610	5.9506	0.0888	0.1731	10.6384
457	M	1	181.0	NS	4.1812	83.6079	1.7128	4.0901	0.7544	0.5125	2.0075
458	M	1	135.8	NS	12.7856	319.5039	4.4174	8.7203	4.1285	0.3705	4.3262
459	M	1	134.6	2.8721	8.6003	226.6240	1.2485	10.8147	7.9848	1.6508	4.3854
460	M	1	178.6	NS	10.4479	306.0882	1.5942	17.9030	6.8833	1.3293	7.5986
721	F	4	138.0	14.9678	6.2375	54.5286	3.5251	5.5852	3.0945	1.5191	1.7835
726	M	4	181.0	22.6821	12.2919	402.2281	SL	27.0707	5.9839	1.6441	1.1330
751	M	24	89.3	5.7073	0.2920	13.6441	0.2298	0.4065	BDL	BDL	BDL
752	M	24	181.7	12.0007	5.1599	144.3169	1.1538	4.3748	3.1979	0.7586	1.0626
753	M	24	181.0	16.2001	8.2180	138.9223	8.2861	5.6873	2.8509	0.8040	0.7338
754	M	24	89.4	5.3545	0.3153	9.9519	0.2326	0.2208	BDL	0.1151	BDL
755	M	24	89.7	6.2580	0.2292	9.4526	0.5694	0.2230	BDL	0.0842	0.0610
756	M	24	181.8	13.7616	4.5243	285.3524	1.7515	4.6204	2.6330	0.8033	3.8261
757	M	24	90.9	4.5142	0.1943	3.0377	0.6586	0.2103	BDL	BDL	BDL
758	M	24	181.9	NS	3.7578	237.5802	0.9701	5.3916	2.8495	0.7023	0.7169
759	M	24	180.9	13.5043	6.3221	286.5475	1.2082	4.5071	2.4292	0.6107	1.0832

BDL: Below detection limit (<0.05 ng/g).

SL: Sample was lost during assay preparation.

NS: No sample was received.

Table 6. Results from Regenerated and Free GB Assays of PND 42 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
760	M	24	90.9	4.0789	0.1964	2.3692	0.6238	0.1843	BDL	BDL	0.0466
841	F	24	0	BDL	BDL	BDL	BDL	BDL	96%*	BDL	BDL
842	F	24	135.8	8.9666	2.4801	141.1326	0.8852	2.0542	1.9450	1.2646	0.8373
843	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.0823
844	M	24	0	BDL	BDL	120%*	BDL	BDL	BDL	BDL	BDL
845	F	24	0	BDL	BDL	BDL	BDL	0.1298	BDL	BDL	BDL
846	F	24	89.7	4.5226	0.4325	7.7185	0.3940	0.2834	BDL	0.2394	0.0704
848	M	24	135.2	8.8358	0.8279	112.1905	1.1469	1.1815	0.3386	0.6340	0.4380
849	M	24	136.4	7.4594	4.0280	132.2805	1.4033	6.7086	4.5724	1.4895	3.4676
850	F	24	179.4	10.3717	6.1236	419.0639	1.0890	6.7516	3.3278	0.5680	2.0110
931	F	4	137.3	3.1252	0.3976	14.5015	1.1684	0.2775	0.0762	0.1976	0.2790
932	F	1	69.0	1.7964	0.3658	1.6071	0.3962	0.7375	BDL	BDL	0.5680
933	F	4	69.0	1.3105	0.4759	0.4739	0.3518	0.6385	BDL	BDL	BDL
934	F	24	0	113%*	BDL	BDL	0.4122	BDL	101%*	BDL	120%*
935	F	24	0	BDL	95%*	BDL	0.2682	92%*	BDL	BDL	BDL
936	F	24	104.2	0.9582	0.0585	0.7189	0.5729	0.2146	BDL	BDL	0.1737
937	F	24	137.3	2.7960	0.1131	5.4252	0.1682	0.3467	BDL	BDL	BDL
938	F	1	136.5	2.8324	0.1277	34.9810	0.6288	0.3920	0.0792	BDL	0.2329
939	F	24	68.2	0.8713	BDL	0.5363	0.8587	0.2231	BDL	BDL	BDL
940	F	1	103.6	1.3080	0.4763	0.2183	0.8942	0.2761	BDL	BDL	0.5698
941	F	4	103.6	0.9257	0.2163	0.2921	0.1448	0.2420	BDL	BDL	BDL
972	F	4	47.4	4.9164	1.4615	217.1614	0.6078	1.1119	3.0983	2.1231	0.2059
973	F	24	104.8	4.7533	0.9857	155.6597	0.7121	0.3462	0.9052	BDL	BDL
974	F	24	138.1	4.2197	1.1707	105.5244	0.5116	1.8479	1.0308	0.1050	0.1645
975	F	1	69.8	3.4856	3.0097	2.5186	0.8834	0.1315	0.1736	BDL	0.1159
976	F	4	69.4	2.7639	0.2897	24.8783	0.2074	BDL	BDL	BDL	BDL
977	F	24	138.9	4.6614	1.6804	0.8985	0.5489	0.5221	2.4465	0.3026	SL
978	M	1	134.1	4.8052	0.8038	46.5516	0.3568	0.4554	0.2141	0.2912	0.2911

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

SL: Sample was lost during assay preparation.

Table 6. Results from Regenerated and Free GB Assays of PND 42 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
979	M	4	90.4	3.2872	2.5992	34.4187	0.2326	0.2076	0.1822	BDL	0.0584
980	M	4	179.5	8.9555	6.2790	170.1139	3.9053	5.0329	9.6972	4.4411	2.4900
981	M	1	90.1	3.0313	0.5630	39.6483	0.4967	0.6964	0.1238	BDL	BDL
982	M	4	135.7	7.1804	5.2107	292.8341	1.0762	5.2051	7.2168	3.4819	1.7681
983	M	1	134.2	7.3383	6.4180	273.5803	0.6700	5.8117	10.3670	2.2421	2.0775
984	M	24	68.6	2.9486	0.2331	25.0827	0.5730	0.1713	BDL	BDL	BDL
985	F	1	103.0	4.7264	3.2962	208.0982	0.3618	2.6774	4.7709	0.5033	1.3363
986	F	24	69.4	2.9025	0.6594	73.0111	0.6832	0.3356	0.1610	BDL	0.1186
987	F	4	104.2	2.3856	2.2761	3.4228	0.7155	0.4018	BDL	BDL	0.6743
988	F	1	138.9	4.6156	1.7855	31.6034	2.3359	0.5589	0.1210	BDL	0.2104
989	F	24	103.0	3.3938	1.2017	124.1337	1.5048	0.9721	0.7548	BDL	0.1196
990	M	48	135.2	5.4803	0.7963	82.2639	0.8959	0.6573	0.5265	BDL	0.1321
991	M	48	89.2	4.1579	7.5250	125.2688	0.4340	1.4708	1.2989	BDL	0.3275
992	M	48	179.4	6.3534	2.6527	168.4783	1.1144	2.8533	2.6786	0.2871	0.5737
993	M	48	89.7	2.5606	0.1608	18.7300	0.4764	0.2101	BDL	BDL	BDL
994	M	48	181.9	6.4192	3.0086	176.0417	0.5898	2.9464	3.1582	0.4835	0.5754
995	M	48	134.0	4.5087	1.8600	89.3288	0.3418	0.9226	1.1506	BDL	0.1463
1112	F	1	104.8	25.8935	0.7300	65.0610	0.8912	0.2841	0.4621	BDL	BDL
1113	F	1	69.4	13.9873	0.1966	2.9019	0.2390	0.3135	BDL	BDL	BDL
1114	F	1	138.9	24.9995	0.8919	84.6538	0.9622	0.5917	0.8849	0.0858	0.1869
1115	F	4	103.6	17.7616	0.9195	86.6735	0.8440	0.4078	0.3630	0.0970	BDL
1116	F	4	68.6	11.2093	0.1282	15.0158	0.8171	0.0685	BDL	BDL	0.1070
1117	F	4	139.7	25.9885	3.7741	255.2327	3.8363	3.6441	5.1701	0.9971	1.0966
1118	F	4	103.6	16.0703	0.3473	32.5126	1.6514	0.4919	BDL	BDL	0.1166
1119	F	1	138.1	24.2331	0.1400	28.8509	0.2641	0.0612	BDL	BDL	0.1067
1120	F	4	68.2	16.7351	2.5143	226.3347	0.5516	2.0361	3.0184	0.3021	0.7390
1121	F	1	103.0	29.6897	4.8911	99.8535	1.4695	2.4612	5.6104	1.1636	2.1689

BDL: Below detection limit (<0.05 ng/g).

Table 6. Results from Regenerated and Free GB Assays of PND 42 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose (µg/kg)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
1252	F	24	0	NS	BDL	NS	BDL	BDL	BDL	BDL	BDL
1255	F	4	103.0	10.3274	0.5096	60.9171	0.8201	0.1358	0.1157	0.9646	0.0483
1256	F	24	0	0.0582	BDL	BDL	BDL	BDL	BDL	BDL	BDL
1259	F	4	102.4	12.5313	0.9418	18.9409	0.6592	0.0962	BDL	BDL	0.1211
1472	F	1	104.3	10.2386	4.3640	160.4737	0.2919	2.5533	3.8668	1.1086	1.8257
1477	F	1	102.4	6.6552	0.6331	77.5858	0.1713	0.2171	0.4954	0.8915	0.0776

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

Table 7. Results from Regenerated and Free GB Assays of Plasma and Red Blood Cell Fractions from Selected PND 42 Rats

Rat No.	Sex	Sample Time (h)	Dose (µg/kg)	Regenerated and Free GB (ng/g)		
				Whole Blood	Plasma	Red Blood Cell
1112	F	1	104.8	25.8935	48.8766	22.1947
1113	F	1	69.4	13.9873	25.9370	10.1119
1114	F	1	138.9	24.9995	50.1841	19.7203
1115	F	4	103.6	17.7616	24.1184	20.1280
1116	F	4	68.6	11.2093	15.7571	12.9941
1117	F	4	139.7	25.9885	22.6987	25.8916
1118	F	4	103.6	16.0703	43.4036	17.4515
1119	F	1	138.1	24.2331	33.1267	25.7097
1120	F	4	68.2	16.7351	33.4011	11.3546
1121	F	1	103.0	29.6897	55.5093	24.4399

Table 8. Results from Regenerated and Free GB Assays of PND 70 Rats

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
319	M	24	49.9	2.6055	2.0162	170.7761	1.1470	3.2965	0.9803	BDL	0.3029
320	M	24	49.9	1.5486	2.3193	164.4164	0.8311	3.4072	1.0364	0.2922	0.3548
321	M	24	49.9	1.0705	3.4981	139.3031	0.9632	6.2298	2.4797	0.3266	1.1916
322	M	24	0	BDL	BDL	BDL	BDL	103%*	BDL	BDL	BDL
327	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	0.4493	BDL
328	M	24	49.9	3.2085	2.7864	168.3040	0.8710	6.9275	2.5607	0.3192	0.6656
329	M	24	49.9	2.2598	0.2576	17.2355	0.8739	0.3633	BDL	BDL	0.1174
330	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	1.3148	102%*
461	F	4	101.0	NS	1.5550	53.4891	1.6117	4.1945	0.3466	1.2074	0.6488
462	F	4	135.9	NS	9.4680	268.3919	5.4100	20.7558	8.4459	1.5774	4.3993
463	F	4	67.7	NS	2.0208	1.4535	0.7090	0.5852	BDL	BDL	0.3695
464	F	4	133.6	10.6719	11.2664	270.7126	6.3794	6.4959	9.0059	2.2418	5.3040
465	F	4	66.8	NS	0.4329	12.1183	1.4559	1.0408	BDL	0.7160	0.5655
466	M	4	99.8	6.4837	5.5638	220.9467	1.7266	13.6709	3.9015	1.1931	1.1174
467	M	4	133.8	NS	5.4875	234.5909	1.7584	8.2177	5.0427	1.0573	0.9905
468	M	4	66.3	NS	1.0097	10.2543	0.7157	0.7613	0.0997	0.2301	0.5967
469	M	4	66.3	NS	2.3891	4.8432	0.9991	0.9643	BDL	0.2604	1.7674
470	M	4	99.2	5.2791	1.2405	41.9795	1.1644	1.8470	0.1679	0.1977	1.2527
471	F	4	100.1	NS	5.0423	262.7584	1.7101	2.5062	2.1213	1.1981	3.5566
472	F	4	66.7	NS	1.3337	13.3759	0.5443	3.2929	BDL	0.3221	0.1507
473	F	4	135.9	11.0680	6.4555	242.1052	6.6768	6.2643	8.1416	1.8141	2.8407
475	F	4	100.1	5.5843	1.4184	36.0002	2.385	1.7343	0.1041	1.0946	0.7808
476	M	4	133.8	7.1045	9.2388	234.1778	3.9734	15.1269	4.4991	1.7332	2.2506
477	M	4	132.6	6.6959	1.5492	185.3235	2.1716	6.0627	1.2656	2.131	0.6123
478	M	4	66.9	NS	0.1209	5.5869	0.6765	1.0435	BDL	0.8256	0.7541
479	M	4	132.6	NS	7.543	152.7187	3.6866	4.2392	1.2097	1.3223	0.2724
480	M	4	100.1	4.6867	3.1281	25.5375	1.2708	1.8460	0.1152	0.2749	0.9506

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

Table 8. Results from Regenerated and Free GB Assays of PND 70 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
606	M	1	134.3	9.0661	0.9263	203.3525	0.2425	SL	1.2973	0.5147	0.2600
607	M	24	134.7	6.9225	0.7549	107.6341	0.2413	SL	0.2329	0.6726	0.0499
608	M	1	135.5	9.6508	1.7463	70.3438	0.2160	0.9676	0.5166	0.3744	0.1399
609	M	24	135.5	NS	0.7499	88.4100	0.5836	1.2317	0.1602	0.2338	0.1901
610	M	124	133.8	10.8727	5.5763	309.1224	0.5622	3.3733	5.7441	1.1424	0.4248
611	F	1	133.6	10.1046	2.6315	215.1262	2.4753	3.2644	2.4360	0.5690	0.6856
612	F	1	133.6	15.2928	6.3972	278.8942	0.5554	7.3555	5.0072	1.5213	2.4622
613	F	24	133.0	15.3002	4.9922	281.2365	0.1144	4.4624	9.1159	2.0136	5.4463
614	F	1	133.0	12.6118	4.4684	203.8459	2.7340	2.5332	3.1383	0.7797	1.7891
615	F	24	134.1	11.5246	4.3273	299.5718	0.6821	4.1250	6.6536	1.6567	2.6549
616	M	1	135.1	8.6321	2.7408	154.1596	SL	SL	1.441	0.4576	0.0732
617	M	24	133.9	11.8753	1.9644	247.7984	0.3332	2.5276	3.0928	1.1450	0.4706
618	M	24	133.8	10.8951	1.7333	267.2500	0.1222	2.2469	1.584	0.9378	0.4488
619	F	24	133.6	10.6716	2.7248	158.9066	0.5378	1.8379	2.0053	0.8820	0.0815
620	F	24	133.0	8.9487	1.6446	100.0195	1.4847	0.8634	0.7765	0.2521	0.3782
651	F	24	67.7	5.9626	0.1643	7.8216	0.1826	0.0946	BDL	0.0512	0.2643
652	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
653	F	24	67.4	5.5480	0.1765	7.8874	0.4470	0.6225	0.0615	BDL	BDL
654	F	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
655	F	24	67.1	3.9263	1.1827	5.8347	0.0810	0.0978	BDL	BDL	BDL
658	M	24	0	BDL	BDL	0.1358	BDL	BDL	BDL	BDL	BDL
660	M	24	66.7	5.5175	0.0795	7.1281	0.2232	0.1087	BDL	0.0493	BDL
665	F	24	68.0	6.4162	0.2542	41.7426	0.8160	0.1643	BDL	BDL	0.0582
666	M	24	66.9	6.1482	0.2161	7.8057	BDL	SL	BDL	BDL	BDL
668	M	24	0	BDL	BDL	BDL	SL	BDL	BDL	BDL	BDL
670	M	24	66.5	3.9325	0.128	1.7312	0.2014	0.0938	BDL	BDL	0.0456
671	F	24	0	BDL	BDL	BDL	BDL	SL	BDL	BDL	BDL
672	F	24	68.0	3.9114	0.0652	2.6548	0.1644	0.0601	BDL	BDL	BDL

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

SL: Sample was lost during assay preparation.

Table 8. Results from Regenerated and Free GB Assays of PND 70 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose ($\mu\text{g/kg}$)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
673	F	24	67.7	4.5007	0.5835	83.3820	0.2094	0.4424	0.2047	BDL	0.0511
675	F	24	134.1	8.7979	1.537	267.7802	1.1016	1.2048	1.6668	0.3602	0.5052
676	M	24	132.2	9.8339	4.2939	125.8592	0.3692	2.2754	1.6627	0.3225	0.9232
677	M	24	66.5	4.9575	0.0949	14.2620	0.2888	0.2278	BDL	BDL	BDL
678	M	24	64.4	6.1876	0.1829	20.7656	0.2470	SL	BDL	BDL	0.0753
679	M	24	66.9	4.7289	0.1337	2.6927	0.3485	0.1159	BDL	BDL	BDL
680	M	24	0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
656	M	4	133.0	12.5695	1.795	165.2859	SL	3.5188	2.3233	0.3727	0.9818
657	M	4	133.8	16.5421	6.0459	455.0249	0.2953	5.0468	4.5058	1.2150	1.3072
659	M	4	100.4	8.3490	0.5033	66.3055	0.1319	0.4342	0.2443	0.0598	BDL
661	F	4	101.4	9.7485	0.3037	73.4396	0.8383	SL	0.1062	BDL	BDL
662	F	4	133.5	16.6860	8.1823	236.2522	1.8850	7.6117	7.0603	1.8230	3.0314
663	F	4	101.9	7.7238	0.3387	19.3155	0.6254	0.1600	BDL	BDL	BDL
667	M	4	99.5	7.2298	0.6381	40.6727	0.3488	0.5961	0.169	BDL	BDL
669	M	4	99.2	8.6995	0.7748	127.2952	0.3357	1.6379	0.4511	BDL	BDL
674	M	4	101.0	11.3298	1.0395	154.7854	0.8308	SL	1.2755	0.3887	BDL
781	F	24	0	BDL	117%*	BDL	BDL	BDL	BDL	BDL	BDL
782	F	24	0	BDL	BDL	BDL	BDL	0.1326	BDL	BDL	BDL
783	F	24	100.1	8.3172	1.0528	169.8808	0.9820	1.0342	0.4579	0.6121	0.6138
784	F	24	0	BDL	BDL	BDL	BDL	97%*	BDL	BDL	BDL
785	F	24	101.8	5.4218	0.1722	21.3899	2.2523	0.1963	BDL	BDL	BDL
786	M	1	66.3	6.6809	6.3784	13.4106	0.4570	3.3629	0.0938	1.1281	3.5054
787	M	1	99.5	9.8738	5.9516	48.6277	1.0923	4.9523	0.1357	0.4419	0.4344
788	M	1	66.5	6.3750	13.9927	10.2695	1.7358	2.2485	0.0583	0.8275	0.6921
789	M	1	99.8	8.3490	8.3084	11.2525	1.3994	9.1921	0.1078	BDL	0.7970
790	M	1	100.1	7.5533	9.0326	18.1298	0.7572	0.7695	0.1643	1.8372	5.8858
791	F	24	100.1	3.6732	2.1424	24.9708	1.5759	0.3104	BDL	BDL	0.1108
792	F	24	101.4	7.9150	4.7925	123.8381	0.9296	2.2991	2.5050	0.7102	0.8365

*Selected control samples (0 $\mu\text{g/kg}$) were spiked with GB to determine percentage recovered.

BDL: Below detection limit (<0.05 ng/g).

SL: Sample was lost during assay preparation.

Table 8. Results from Regenerated and Free GB Assays of PND 70 Rats (continued)

Rat No.	Sex	Sample Time (h)	Dose (µg/kg)	Regenerated and Free GB (ng/g)							
				Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm
793	F	24	134.7	10.0078	4.1515	143.0936	1.7179	3.3043	1.6694	0.4466	0.7635
794	F	24	101.9	5.8208	0.3557	18.8464	1.4948	0.2561	BDL	0.0826	0.1859
795	F	24	101.8	7.7560	1.6033	102.1208	1.9775	1.6019	1.0307	0.2950	0.4538
796	M	1	66.5	7.0529	4.6518	NS	0.6713	2.8887	0.0742	1.6843	0.0796
797	M	1	66.7	6.1761	3.0504	5.5842	0.2845	1.4374	0.0676	0.4007	0.4519
798	M	1	99.2	8.3638	6.2219	28.2571	1.0927	4.7436	0.1371	1.5974	1.0499
799	M	1	66.5	6.2920	5.3570	40.7552	1.0035	1.2909	0.1613	1.5813	3.8529
800	M	1	100.1	8.2162	9.0441	106.8710	1.0122	7.4188	1.3553	2.4636	2.5642
1392	F	1	101	17.7739	0.5404	15.4906	0.1676	0.2171	0.3611	0.1152	0.6269
1394	F	1	101	20.4192	1.7174	164.0401	0.2572	0.6919	1.4230	0.3945	0.2976
1395	F	1	101	NS	3.3401	87.9056	1.0717	1.7052	1.6816	0.4942	0.8372
1397	F	1	101	19.3689	5.3100	143.6014	0.4459	2.1354	2.8070	1.1085	2.1975
1399	F	1	101	17.1892	0.6983	103.5906	0.3555	0.4142	0.4826	0.1903	0.1231
1401	F	1	101	14.3184	0.8985	112.0169	0.2587	0.2544	0.4367	0.0591	0.1138
1484	M	4	100.1	8.8444	0.2853	NS	0.1984	0.1771	0.0887	0.0780	0.0648
1487	M	4	99.8	7.1969	0.1599	NS	0.1328	0.1259	0.0483	BDL	BDL

BDL: Below detection limit (<0.05 ng/g).

NS: No sample was received.

Blank

LITERATURE CITED

Baker, M.D. Antidotes for Nerve Agent Poisoning: Should We Differentiate Children from Adults? *Curr. Opin. Pediatr.* **2007**, *19*, 211–215.

Ben-Ari, Y.; Holmes, G.L. Effects of Seizures on Developmental Processes in the Immature Brain. *Lancet Neurol.* **2006**, *5*, 1055–1063.

Bloomfield, D. Tachypnea. *Pediatr. Rev.* **2002**, *23*, 294–295.

Guzelian, P.S.; Henry, C.J.; Farland, W.H.; Olin, S.S. Developmental Toxicants Risk Assessment. *Pediatrics* **1992**, *89*, 353.

McGuire, J.M.; Demond, P.S.; Busch, M.W. *GC-MS/MS Analyses of Biological Samples in Support of Developmental Toxic Effects on Whole-Body Exposure of Rats to GB*; ECBC-TR-1286; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2015; UNCLASSIFIED Report.

Ohbu, S.; Yamashina, A.; Takasu, N.; Yamaguchi, T.; Murai, T.; Nakano, K.; Matsui, R.; Sakurai, K.; Hinohara, S. Sarin Poisoning on Tokyo Subway. *South. Med. J.* **1997**, *90*, 587–593.

Russell, R.W.; Overstreet, D.H. Mechanisms Underlying Sensitivity to Organophosphorous Anticholinesterase Compounds. *Prog. Neurobiol.* **1987**, *28*, 97–129.

Thompson, K.; Wasterlain, C. Lithium-Pilocarpine Status Epilepticus in the Immature Rabbit. *Brain Res. Dev. Brain Res.* **1997**, *100*, 1–4.

U.S. Army Medical Research Institute of Chemical Defense (MRICD). *Developmental Toxic Effects on Whole-Body and Percutaneous Exposure to Chemical Warfare Nerve Agents (CWNA) in Rats: Effects on Brain and Behavior*; Protocol no.1-12-U-1001; MRICD: Aberdeen Proving Ground, MD, 2012; UNCLASSIFIED Protocol.

Blank

ACRONYMS AND ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase enzyme
ANOVA	analysis of variance
BARDA	Biomedical Advanced Research and Development Authority
BDL	below detection limit
CASARM	chemical agent standard analytical reagent material
CE	collision energy
CI	chemical ionization
CWNA	chemical warfare nerve agent
ECBC	U.S. Army Edgewood Chemical Biological Center
GA	ethyl <i>N,N</i> -dimethylphosphoramidocyanidate; tabun
GB	isopropyl methylphosphonofluoridate; sarin
GC-MS/MS	gas chromatography–tandem mass spectrometry
GD	pinacolyl methyl phosphonofluoridate; soman
GF	cyclohexyl methylphosphonofluoridate; cyclosarin
IPA	2-propanol
IS	internal standard
LC _{t50}	lethal concentration with 50% chance of causing death
LD ₅₀	lethal dose for 50% of the population
<i>m/z</i>	mass-to-charge ratio
MRICD	U.S. Army Medical Research Institute of Chemical Defense
NB	notebook
NMR	nuclear magnetic resonance
PBPK/PD	physiologically based pharmacokinetic and pharmacodynamic (modeling)
PND	postnatal day
<i>R</i> ²	coefficient of determination
r-GB	regenerated GB
SPE	solid-phase extraction
VX	O-ethyl S-(2-diisopropylaminoethyl) methyl phosphonothioate

DISTRIBUTION LIST

The following individuals and organizations were provided with one Adobe portable document format (pdf) electronic version of this report:

ECBC Analytical Toxicology Branch
RDCB-DRT-T
ATTN: McGuire, J.
Evans, R.

G-3 History Office
U.S. Army RDECOM
ATTN: Smart, J.

U.S. Army Medical Research Institute of
Chemical Defense
MCMR-CDZ
ATTN: Lumley, L.

ECBC Technical Library
RDCB-DRB-BL
ATTN: Foppiano, S.
Stein, J.

Defense Threat Reduction Agency
DTRA/RD-CBD T
ATTN: Ward, T.
J9-CBS
ATTN: Moore, E.

Office of the Chief Counsel
AMSRD-CC
ATTN: Upchurch, V.

Defense Technical Information Center
ATTN: DTIC OA

Department of Homeland Security
DHS ORD CSAC
ATTN: Famini, G.

ECBC Rock Island
RDCB-DES
ATTN: Lee, K.

