National Institute of Environmental Health Sciences

NIEHS Report on the In Vivo Repeat Dose Biological Potency Studies of 1,2-Dichlorobenzene (CASRN 95-50-1) in Female Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6D2F1/Crl Mice (Whole-body Inhalation Studies)

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Foreword

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The environmental health sciences research described in this series is conducted primarily by the Division of Translational Toxicology (DTT) at NIEHS. NIEHS/DTT scientists conduct innovative toxicology research that aligns with real-world public health needs and translates scientific evidence into knowledge that can inform individual and public health decision-making.

This report is available free of charge on the NIEHS website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health).

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No.	Role	Definition
1	Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
2	Data Curation, Formal Analysis, and Software	Management activities to annotate (produce metadata), scrub, and maintain research data (including software code, when it is necessary for interpreting the data) for initial use and later reuse or Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data or Programming and software development; design of computer programs; implementation of computer code and supporting algorithms; testing of existing code components
3	Investigation	Conduct of the research/investigation process, specifically the performance of experiments or the collection of data/evidence
4	Methodology	Development or design of methodology; creation of models
5	Project Administration	Management and coordination responsibility for research planning and execution
6	Resources for Study Conduct	Provision of study materials, reagents, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
7	Validation	Verification, whether as a part of the activity or separately, of the overall replication/reproducibility of results/experiments and other research outputs
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12	Peer Review and Production	Coordination and management of external peer review and publication, including identification of experts, conflict-of-interest screening, correspondence with reviewers, preparation of review documents, and publication activities

[a]Developed using the Contributor Roles Taxonomy (CRediT) framework.[1]

Peer Review

This report was modeled after the *NTP Research Report on In Vivo Repeat Dose Biological Potency Study of Triphenyl Phosphate (CAS No. 115-86-6) in Male Sprague Dawley Rats (Hsd:Sprague Dawley SD) (Gavage Studies)* (https://doi.org/10.22427/NTP-RR-8), which was reviewed internally at the National Institute of Environmental Health Sciences and peer reviewed by external experts. Importantly, these reports employ mathematical model-based approaches to identify and report potency of dose-responsive effects and do not attempt more subjective interpretation (i.e., make calls or reach conclusions on hazard). The peer reviewers of the initial 5-day research report determined that the study design, analysis methods, and results presentation were appropriate. The study design, analysis methods, and results presentation employed for this study are identical to those previously reviewed, approved, and reported; therefore, following internal review, the *NIEHS Report on the In Vivo Repeat Dose Biological Potency Studies of 1,2-Dichlorobenzene (CASRN 95-50-1) in Female Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6D2F1/Crl Mice (Whole-body Inhalation Studies)* was not subjected to further external peer review.

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Abstract

Background: 1,2-Dichlorobenzene (1,2-DCB) is a chlorinated aromatic hydrocarbon that is widely used in industrial applications, including as a solvent for waxes, resins, and paints, and it serves as an ingredient in the production of disinfectants and deodorants. Short-term in vivo transcriptomic studies were used to assess the biological potency of 1,2-DCB. The data from these studies are intended to support risk assessment and establishment of acceptable exposure levels of 1,2-DCB in environmental and occupational settings.

Methods: Short-term in vivo biological potency studies on 1,2-DCB in adult female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6D2F1/Crl mice were conducted. Animals were exposed via whole-body inhalation to 1,2-DCB vapor for 6 hours plus the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) per day for 5 consecutive days (study days 0–4) at exposure concentrations of 0, 1, 10, 30, 100, 250, or 500 ppm for rats and 0, 1, 10, 30, 100, or 250 ppm for mice. Blood was collected from animals dedicated to internal concentration assessment in all groups. On study day 5, the day after the final day of exposure, animals were euthanized, standard toxicological measures were assessed, and the heart, kidney, liver, lung, and ovary were assayed in gene expression studies using the TempO-Seq assay. Modeling was conducted to identify the benchmark doses (BMDs) associated with apical toxicological endpoints and transcriptional changes in the heart, kidney, liver, lung, and ovary. A benchmark response of 1 standard deviation from the mean was used to model all apical endpoints, whereas a benchmark response set to a 25% change in the median response was used to model the gene expression data.

Results: Several clinical pathology, body weight, and organ weight measurements showed exposure-related changes from which BMD values were calculated. In rats, the effects, and their BMDs and benchmark dose lower confidence limits [BMD_{LS}] in ppm, included significantly increased relative liver weight (57.9 [29.9]), increased relative right kidney weight (93.1 [43.2]), increased relative left kidney weight (101.4 [40.9]), increased absolute liver weight (114.0 [41.8]), increased bile salt/acid concentration (143.8 [45.9]), increased albumin/globulin ratio (182.3 [99.4]), decreased terminal body weight (227.1 [116.4]), increased cholesterol concentration (238.4 [147.3]), decreased body weight gain over study duration (273.4 [215.6]), decreased body weight on study day 4 (298.0 [137.9]), and decreased glucose concentration (360.3 [183.9]). In mice, the effects, and their BMDs (BMD_{LS}) in ppm, included significantly decreased alkaline phosphatase activity (33.4 [10.9]), increased absolute liver weight (57.4 [36.4]), increased relative liver weight (60.5 [37.7]), increased sorbitol dehydrogenase activity (93.0 [56.8]), decreased body weight on study day 2 (107.8 [62.4]), decreased body weight on study day 3 (118.5 [69.5]), decreased body weight on study day 4 (133.3 [82.8]), increased alanine aminotransferase activity (157.8 [108.9]), decreased body weight gain over study duration (159.8 [115.4]), and increased relative right kidney weight (161.5 [82.3]).

In rats and mice, 1,2-DCB blood, liver, and lung concentrations following the last exposure (study day 4) increased proportionally to the exposure concentration up to 100 ppm. At higher exposure concentrations, blood and tissue concentrations increased more than proportionally to the exposure concentration, demonstrating saturation of metabolism and/or clearance processes above 100 ppm. In general, tissue concentrations were similar to blood concentrations, demonstrating low tissue distribution and/or retention. Blood and tissue concentrations on the day after the last exposure (study day 5) were much lower than those on the last day of exposure

(study day 4), demonstrating rapid elimination of 1,2-DCB. In general, when normalized to the theoretical inhaled dose, there were no apparent species differences in blood and tissue concentrations of 1,2-DCB.

In both rats and mice, no Gene Ontology (GO) biological process in the heart, kidney, liver, lung, or ovary had BMD values below the lower limit of extrapolation (<0.333 ppm). The most sensitive gene sets for which a reliable estimate of the BMD could be made are given below for each tissue and species, with their BMDs (BMD_{LS}) in ppm.

In the heart, the most sensitive gene set in rats was cellular response to hydrogen peroxide (148.7 [58.2]), whereas in mice, the most sensitive gene set was cellular modified amino acid metabolic process (4.4 [1.7]). In the kidney, the most sensitive gene sets in rats and mice were embryonic organ development (114.0 [73.9]) and organic hydroxy compound transport (2.3 [0.6]), respectively. In the liver, the most sensitive gene set in rats was xenobiotic metabolic process (29.8 [25.3]) and the most sensitive gene set in mice was positive regulation of lipid biosynthetic process (1.5 [0.5]). In the lung, the most sensitive gene sets in rats were detoxification (175.9 [109.3]), cellular oxidant detoxification (175.9 [109.3]), and cellular detoxification (175.9 [109.3]), whereas in mice, the most sensitive gene set was unsaturated fatty acid metabolic process (2.1 [0.7]). In the ovary, the most sensitive gene sets in rats were kidney development (156.7 [50.8]), gonad development (156.7 [50.8]), and male gonad development (156.7 [50.8]), and the most sensitive gene set in mice was hemopoiesis (108.0 [35.9]).

Summary: Taken together, in rats, the most sensitive gene set and apical endpoint BMD (BMD_L) values that could be reliably determined occurred at 29.8 (25.3) and 57.9 (29.9) ppm, respectively. In mice, the most sensitive gene set and apical endpoint BMD (BMD_L) values that could be reliably determined occurred at 1.5 (0.5) and 33.4 (10.9) ppm, respectively.

Background

1,2-Dichlorobenzene (1,2-DCB) (CASRN: 95-50-1, U.S. Environmental Protection Agency [U.S. EPA] Chemical Dashboard: DTXSID6020430, PubChem CID: 7239, European Committee Number: 202-425-9), also known as ortho-DCB, is an organochlorine compound classified under chlorinated aromatic hydrocarbons.[2] 1,2-DCB is widely used in industrial applications, including as a solvent for waxes, resins, and paints, as well as a precursor in the manufacture of agrochemicals and dyestuffs.[3; 4] Additionally, it serves as an ingredient in the production of disinfectants and deodorants.[3; 4]

Both the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health have determined that exposure to 1,2-DCB should not exceed a ceiling of 50 ppm for an 8-hour workday[5; 6] whereas the American Conference of Governmental Industrial Hygienists advises that the average airborne exposure should not exceed 25 ppm for an 8-hour work shift.[7] Under the Safe Drinking Water Act, both the federal maximum contaminant level goal and the maximum contaminant level for 1,2-DCB are set at 600 µg/L.[4; 8] The chronic oral reference dose from the U.S. EPA's Integrated Risk Information System is 0.09 mg/kg body weight/day (mg/kg/day) and is based on a no-observed-adverse-effect level (NOAEL) of 120 mg/kg/day (adjusted to 85.7 mg/kg/day based on 5 days/week gavage), the highest dose of the National Toxicology Program 2-year carcinogenicity studies of 1,2-DCB,[9] and a 1,000-fold uncertainty factor.[10] The International Agency for Research on Cancer concluded that 1,2-DCB is not classifiable as to its carcinogenicity to humans (Group 3).[11]

Exposure to 1,2-DCB can occur through various routes, including inhalation, ingestion, and dermal contact.[2; 3; 5] Inhalation exposure is particularly significant because of the volatility of 1,2-DCB, making occupational exposure a concern in industrial settings.[2; 3] Upon exposure, 1,2-DCB is absorbed efficiently via the lungs and gastrointestinal tract.[2] It is distributed widely in the body, with a preference for adipose tissues given its lipophilicity.[2] The metabolism of 1,2-DCB involves hepatic cytochrome P450 enzymes, mainly CYP2E1, leading to the formation of potentially toxic metabolites, including chlorophenols, dichlorocatechols, quinones, and epoxides.[2; 12; 13] These metabolites are subsequently conjugated to glutathione and excreted primarily via the urine.[2]

1,2-DCB has demonstrated several toxicological effects that vary depending on the duration of exposure. Acute exposure can result in central nervous system depression, with symptoms including dizziness, headaches, and in severe instances, unconsciousness; it also causes irritation to the eyes, skin, and respiratory tract.[3; 5] Subacute or subchronic exposure to 1,2-DCB in animal models has been linked to liver and kidney damage; hepatotoxicity is indicated by elevated liver enzymes and histopathological changes such as liver cell hypertrophy and necrosis, and nephrotoxicity manifests as proximal tubular damage and altered renal function markers.[2-4; 10] Chronic exposure studies in rats and mice concluded that there was no evidence of carcinogenicity; however, there were positive trends for alveolar/bronchiolar carcinoma in the lungs of male mice and malignant histiocytic lymphoma in male and female mice. Increases in the incidences of renal tubular regeneration in the kidney were also observed in male mice.[9]

The toxic effects of 1,2-DCB are primarily mediated through its metabolism to reactive intermediates such as epoxides that can induce oxidative stress and cellular damage.[2; 12-14] The metabolites formed during detoxification by the liver can covalently bind to cellular macromolecules, leading to lipid peroxidation, protein damage, and DNA adduct formation that can subsequently disrupt normal cellular functions and lead to organ toxicity.[2; 12] Additionally, the induction of inflammatory pathways by 1,2-DCB may contribute to the observed toxic effects, particularly in hepatic tissues.[12; 13]

The primary purpose of these short-term toxicity studies is to characterize the biological potency of 1,2-DCB via inhalation exposure. The data from these studies are intended to support risk assessment and establishment of acceptable exposure levels of 1,2-DCB in environmental and occupational settings.

Materials and Methods

Chemistry

Procurement and Characterization of 1,2-Dichlorobenzene

1,2-Dichlorobenzene (1,2-DCB) was obtained from Oakwood Products, Inc. (Estill, SC) in a single lot (216310R29M). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH). Reports on analyses performed in support of the 1,2-DCB studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The identity and purity of lot 216310R29M, a clear, colorless liquid at room temperature, was evaluated using gas chromatography (GC) with mass spectrometry (MS) detection. The MS spectrum was consistent with the National Institute of Standards and Technology library spectrum for 1,2-DCB. The overall purity of the test article was estimated at approximately 100%.

Bulk 1,2-DCB was stored in the original shipping container at room temperature. Reanalysis of the bulk chemical was performed by the analytical chemistry laboratory within 30 days of study termination and no degradation was detected.

Vapor Generation and Exposure System

A diagram of the generation and distribution system is shown in Figure A-3. 1,2-DCB was pumped from a stainless-steel reservoir into a heated glass vaporizer column filled with glass beads and wrapped with heat tape. A waste collection flask was connected to the bottom of the column for collection of residual 1,2-DCB not completely vaporized within the vaporizer column. Preheated nitrogen entered the column from below, vaporized 1,2-DCB, and carried the vapor from the generator cabinet located in the control room to the distribution manifold located in the exposure room through a heated Teflon® transport line. The nitrogen-test article mixture was diluted with heated air before it entered the distribution manifold. Concentration in the manifold was determined by the chemical pump rate, dilution air flow rate, nitrogen flow rate, and special modifications to the distribution manifold (Appendix A).

Individual heated Teflon delivery lines carried the vapor from the exposure valves in the distribution cabinet to the chamber inlets. The exposure valves diverted vapor delivery to the manifold exhaust until the generation system was stable, and exposures were ready to proceed. The rate of 1,2-DCB vapor delivery to each chamber was controlled by precision metering valves at the manifold. When the exposure started, the exposure valves actuated, directing the vapor into the chamber inlet, where it was diluted with conditioned air to achieve the desired exposure concentration. Conditioned air was a temperature-controlled and filtered mix of air derived from each exposure chamber's wet and dry air duct supplies.

The exposure system consisted of seven exposure chambers with target test article concentrations of 0 (control group), 1, 10, 30, 100, 250, and 500 ppm. The inhalation exposure chamber (Lab Products, Inc., Seaford, DE) was designed so that uniform vapor concentrations could be maintained throughout the chamber with catch pans in place. The total active mixing volume of

each chamber was 1.7 m^3. A small particle detector (Model 3022A; TSI, Inc., Shoreview, MN) was used in the exposure chambers, both with and without animals, to ensure vapor (not aerosol) was produced. No particle counts above the minimum resolvable level were detected.

Vapor Concentration Monitoring

Exposure chamber and room concentrations of 1,2-DCB were monitored using an online GC equipped with a flame ionization detector (FID) (Table A-1). All chambers were sampled at approximately twice per hour during exposure through Teflon tubing connected to each exposure chamber's relative-humidity sampling lines at a location close to the GC/FID. The samples flowed into a 16-port Hastelloy®-C stream-select valve that directed a continuous stream of sampled atmosphere to a 6-port Hastelloy-C gas-sampling valve with a 1 mL sample loop. Valves were mounted in a dedicated valve oven to maintain temperature. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and GC allowed for digital measurement of sample flow. The concentration measurements and relative standard deviations were all within the acceptance criteria of 10% for all exposure chambers and below the limit of detection for the control chambers and exposure rooms in both studies. Additional details are provided in Appendix A.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined prior to (without animals) and during (with animals) the studies. The time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) were estimated from the concentration versus time curves. At a chamber airflow rate of 15 ft^3/min, the theoretical T_{90} value was 9.2 minutes. The estimated values in both rat and mouse studies ranged from 8 to 11 minutes, and a value of 12 minutes was used for the studies. Estimated T_{10} for both studies ranged from 10 to 13 minutes.

Prior to the studies, the persistence of 1,2-DCB was monitored in the 250 and 500 ppm chambers after exposure without animals present. Although T_{10} values were acceptable, the concentration of 1,2-DCB never reached 0 ppm in the ≥10 ppm chambers and required ≥144 minutes to reach 1% of the starting concentration (T_1) without animals present. During the studies and with animals present, T_1 was 79 (500 ppm rat chamber) and 192 minutes (250 ppm mouse chamber). The reason for the prolonged T_1 values is unknown, but the prolonged T_1 values were considered to have no effect on study findings given the low levels compared to the target chamber concentrations.

The uniformity of 1,2-DCB vapor concentration was evaluated in all exposure chambers without animals present and repeated during the studies with animals present in the lowest (1 ppm) and highest (500 or 250 ppm for rats and mice, respectively) exposure concentration chambers. Concentrations were measured at 12 chamber positions, one in the front and one in the back, for each of the six possible animal cage positions per chamber. Chamber concentration uniformity was maintained throughout the studies.

To measure the stability and purity of 1,2-DCB in the generation and delivery system, samples of the test atmosphere were collected from the distribution line, generator reservoir, and the highest, lowest, and control exposure concentration chambers for both species at the beginning and end of the exposure day. Exposure atmosphere samples were collected with sorbent gas-sampling tubes in series with a silica gel sorbent tube. Samples were extracted with methanol for analysis. In addition, analysis was performed on a second set of samples collected from the same locations and exposure times and extracted in acetone to determine whether any impurities in 1,2-DCB were obscured by methanol. No impurity peaks were present in any samples. 1,2-DCB was not detected in the silica gel samples, demonstrating 100% capture of the inhalation exposure atmosphere onto sorbent media. The stability and purity of 1,2-DCB were maintained throughout the exposure system.

Study Design for Rats

Female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Inotiv (Envigo, at time of procurement, Indianapolis, IN). Sprague Dawley rats were employed to represent the Division of Translational Toxicology (DTT)'s typical rat strain of choice. Females were chosen instead of males to reduce the overall study size and limit the complexity of comparisons between the mouse data set (see further justification below) and the rat data set. On receipt, the rats were 6 weeks of age. Animals were quarantined for 9 days and then randomly assigned to one of seven exposure groups. The rats in each exposure group were exposed to 1,2-DCB via whole-body inhalation for 6 hours plus T_{90} per day for 5 consecutive days (study days 0–4) at exposure concentrations of 0, 1, 10, 30, 100, 250, or 500 ppm. There were 5 core female rats in each exposed group and 10 in the 0 ppm group to establish a more confident estimate of variance in the control group; an additional 3 rats were added to each group for internal concentration assessment. Immediately following the final exposure on study day 4, blood, lung, and liver samples were collected from the internal concentration assessment animals once chamber concentrations were at or below the regulatory limit of 12 ppm without additional health and safety considerations. Euthanasia, blood/serum collection, and tissue sample collection for all core animals were completed on study day 5, the day following the final exposure. In addition, blood, lung, and liver samples were collected from three core animals in each group on study day 5 for internal concentration assessment. Animal identification numbers and FASTQ data file names for each animal are presented in Appendix C.

Study Design for Mice

Female B6D2F1/Crl mice were obtained from Charles River Laboratory (Raleigh, NC). Given previously observed carcinogenic effects in the lung in female mice and in the liver in male and female mice with a close structural analog (1,4-dichlorobenzene),[15] these studies employed the same strain/sex in which these effects were observed to allow for phenotypic anchoring of the biological interpretation of the data generated in the proposed studies. Females accounted for both carcinogenic responses (liver and lung) with exposure to the structural analog, hence they were chosen instead of males. This decision reduced the overall study size and limited the complexity of comparisons between the mouse and rat data sets. On receipt, the mice were 8 weeks of age. Animals were quarantined for 9 days and then randomly assigned to one of six exposure groups. The mice in each exposure group were exposed to 1,2-DCB via whole-body inhalation for 6 hours plus T_{90} per day for 5 consecutive days (study days 0–4) at exposure

concentrations of 0, 1, 10, 30, 100, or 250 ppm. There were 5 core female mice in the 1, 10, 30, and 100 ppm groups, 8 in the 250 ppm group, and 10 in the 0 ppm group to establish a more confident estimate of variance in the control group. For internal concentration assessment, an additional 3 female mice were added to the 0, 1, 10, 30, and 100 ppm groups, and an additional 5 female mice were added to the 250 ppm group to account for potential mortality or moribundity. Immediately following the final exposure on study day 4, blood, lung, and liver samples were collected from the internal concentration assessment animals once chamber concentrations fell below the regulatory limit of 12 ppm without additional health and safety considerations. Euthanasia, blood/serum collection, and tissue sample collection for all surviving core animals were completed on study day 5, the day following the final exposure. In addition, blood, lung, and liver samples were collected from up to three core females in each group on study day 5 for internal concentration assessment. Animal identification numbers and FASTQ data file names for each animal are presented in Appendix C.

Exposure Concentration Selection Rationale

The exposure concentrations evaluated in these studies were based on data in the published literature as described in the background of this report. In addition, a pilot study was conducted during the prestudy exposure engineering phase to confirm concentrations would be well tolerated for the duration of the planned study. Informed by the pilot exposure in a small number of animals, the highest concentrations were selected to be 500 ppm and 250 ppm for rats and mice, respectively. The lowest concentration of 1 ppm was selected to be tenfold less than the second lowest nonzero exposure concentration to produce a no-effect level at the transcriptome level.

Clinical Examinations and Sample Collection

Clinical Observations

All animals were observed twice daily for signs of mortality or moribundity, except for the day of receipt (rats) and at removal (rats and mice) when animals were observed once. Clinical observations were performed once prior to exposure on study day 0 and at study termination.

Body and Organ Weights

Animals were weighed during quarantine for randomization, on the first day of exposure (study day 0), daily thereafter (prior to exposure), and on the day of necropsy (study day 4 or 5). During necropsy for all core animals, the heart, liver, kidneys, and lungs were removed, and organ weights were recorded; bilateral organs were weighed separately.

Clinical Pathology

Animals were anesthetized with a 70% carbon dioxide (CO_2)/30% oxygen (O_2) mixture and bled in random order 1 day after the final day of exposure. After blood collection, animals were euthanized by exsanguination (rats) or 100% CO_2 followed by exsanguination (mice). Blood samples were collected within approximately a 2-hour window. Blood was taken via retro orbital plexus (rats) or retro orbital sinus (mice) and collected into tubes containing tripotassium ethylenediaminetetraacetic acid (K_3 EDTA) for hematology analysis (rats only) and into serum collection tubes without anticoagulant for clinical chemistry (rats and mice). The following

hematology parameters were measured on a Sysmex XN-2000V (Sysmex America, Lincolnshire, IL) for rats: erythrocyte count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, white blood cell count and differential, reticulocyte count, and platelet count. Manual hematocrit was determined using a microcentrifuge and capillary reader. Blood smears were prepared, and qualitative evaluation of cellular morphology was performed per study protocol. The following clinical chemistry parameters were measured on a Roche cobas® c501 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) for rats and mice: alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, total bile acids, total bilirubin, direct bilirubin, cholesterol, creatine kinase, creatinine, glucose, sorbitol dehydrogenase, total protein, triglycerides, and blood urea nitrogen. Globulin, albumin/globulin ratio, and indirect bilirubin were calculated based on direct measurements (e.g., indirect bilirubin = total bilirubin − direct bilirubin). Individual animal and summary clinical chemistry and hematology data are available in Appendix G.

Internal Concentration Assessment

An assessment was conducted to determine systemic exposure and tissue distribution and evaluate whether the test chemical had bioaccumulative properties (i.e., whether the half-life was >24 hours). Blood, lung, and liver samples were collected from the internal concentration assessment animals immediately following the last exposure on study day 4 (once chamber concentrations were at or below the regulatory limit of 12 ppm without additional health and safety considerations) and on study day 5 (approximately 18 hours following the last exposure) from core animals designated for internal concentration assessment. On study day 4, blood was collected via cardiac puncture for all surviving rats and mice (up to 3/exposure group) while animals were anesthetized with CO_2/O_2 (70%/30%). On study day 5, blood samples from designated core animals (3/exposure group) were taken via retro orbital plexus (rats) or retro orbital sinus (mice) while animals were anesthetized with CO_2/O_2 (70%/30%). Blood samples were collected within a 2-hour window. Blood was collected into tubes containing K_3 EDTA and three aliquots of 100 µL were transferred into headspace vials and kept on wet ice. Internal standard, $^{13}C_6$ 1,2-DCB (added as a mixture of $^{13}C_6$ 1,2-DCB and $^{13}C_6$ 1,4-DCB) was added to aliquoted samples and then stored frozen (−85°C to −60°C). All samples were frozen within 1 hour of collection. After blood collection, animals were euthanized by exsanguination and lung and liver tissues were collected (following organ weight measurements for core animals designated for internal concentration assessment) from each animal within 1 hour of each other. Up to three aliquots of approximately 100 mg (rats) or 50 mg (mice) lung and liver tissue were collected from each animal and flash frozen. Samples were stored frozen (−85°C to −60°C) until analysis as described in Appendix B.

Transcriptomics

Sample Collection for Transcriptomics

Within approximately 20 minutes of euthanasia, tissue samples were collected following organ weight measurements in the following order: lung, heart, liver (left lobe), kidney, and ovary (no ovary weights were taken) from all remaining animals on study day 5 for transcriptomic analysis. Two samples of lung, heart, liver, and kidney tissue (approximately 5 mm^3) were collected and placed into cryotubes containing RNA*later*™ (Thermo Fisher Scientific, Waltham, MA). The ovaries were collected whole bilaterally (one sample) and placed into cryotubes containing

RNA*later*. The tissue samples were stored at 2°C to 8°C for approximately 55 hours for rats or 72 hours for mice. The RNA*later* was then removed and the samples were stored in a −85°C to −60°C freezer until processed for RNA isolation.

RNA Isolation, Library Creation, and Sequencing

RNA isolation was performed on tissue samples preserved in RNA*later*. Tissues were homogenized in QIAzol lysis buffer (Qiagen Inc., Valencia, CA) using the TissueLyser II bead-beating system followed by RNA extraction using the RNeasy 96 QIAcube HT kits (Qiagen Inc., Valencia, CA) with a DNA digestion step. The concentration and purity of all isolated samples were determined from absorbency readings taken at 260 and 280 nm using a NanoDrop ND-2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). The readings accurately determined the concentration of each sample while ensuring that an acceptable purity (A_{260}/A_{280} ratio) between 1.80 and 2.20 was achieved. Further quality control (QC) evaluation of each RNA sample was performed using the RNA 6000 Nano kit and analyzed with a 2100 Bioanalyzer (Agilent Technology, Foster City, CA), which evaluates the RNA Integrity Number (RIN). RIN must be >3.0 to meet acceptable quality for TempO-Seq analysis. All samples were divided into at least two aliquots. One aliquot was used for BioSpyder TempO-Seq S1500+ analysis. Any tissue samples remaining after RNA isolation were stored at −85ºC to −60ºC until submitted frozen to the Frozen Tissue Bank.

Isolated RNA was utilized for analysis using either the Rat S1500+ v1.2 TempO-Seq or Mouse S1500+ v1.2 TempO-Seq (BioSpyder, Carlsbad, CA) platform with a minimum of 500 mapped read counts/transcript and approximately 1.5 million counts/sample. Work instructions developed by Battelle using the BioSpyder User Guide[16] were followed, including the optimized overnight annealing procedure, outlined as follows. Two microliters of each diluted RNA sample (50 ng/µL) was hybridized with the S1500+ surrogate detector oligo pool mix (2 µL per sample) in a 384-well plate using the following thermocycler settings: 70°C for 10 minutes, followed by a gradual decrease to 45°C over 50 minutes, held at 45°C for 16 hours, and ending with a decrease to 25°C. The plates were held at 25°C for no longer than 8 hours. After annealing, the annealed RNA was transferred to 96-well plates for nuclease digestion (24 µL nuclease mix addition followed by 90 minutes at 37°C), followed by ligation (24 µL ligation mix addition followed by 60 minutes at 37°C) and heat denaturation (at 80°C for 15 minutes). For amplification, polymerase chain reaction (PCR) Pre-Mix and Primers were transferred from the BioSpyder S1500+ surrogate kit 96-well plates into a 384-well plate. Ten microliters of each ligated product were then added to the 384-well plate, sealed, and centrifuged briefly before 30 cycles of amplification.

All steps during the TempO-Seq S1500+ surrogate assay were performed using a QuantStudio 6 Flex System. The amplification step produced well-specific barcoded primer pairs that allowed identification of each well after being combined into a single sequencing library. Five microliters of amplified libraries were pooled together and purified using NucleoSpin gel and a PCR clean-up kit (Macherey-Nagel Inc., Allentown, PA). Once cleaned, the library concentration was determined by quantitative PCR using a KAPA Library Quantification Kit (Roche Sequencing, Indianapolis, IN), and the library was diluted to a final concentration of 400 pM. A PhiX control library (Illumina, San Diego, CA) was spiked into the final library as a system control. The final library was loaded onto an Illumina next generation sequencing cartridge, NovaSeq 6000 S1 Reagent Kit v1.5 (100 cycles) (Illumina, San Diego, CA), along with the BioSpyder-provided custom sequencing primer. Processing of sequencing data was conducted using Illumina's BCL2FASTQ software employing default parameter settings.

Sequence Data Processing

FASTQ files of TempO-Seq reads were aligned to the probe sequences from the target platform using Bowtie version 1.3.1[17] with the following parameters: -v 3 -k 1 -m 1 --best --strata. This configuration allows up to three mismatches and reports the single best alignment. After alignment, the total sequenced reads, the percentage of reads aligning to the platform manifest, the alignment rate, and the percentage of expressed probes (≥5 reads per probe) were calculated for each sample.

Sequencing Quality Checks and Outlier Removal

Each sample was evaluated for quality using the following metrics: sequencing depth, alignment to the platform manifest, number of aligned reads, % of probes with at least five reads, average per base quality, and per base N content. Samples were flagged for values below the following thresholds for the QC metrics: sequencing depth <300 K, total alignment rate <40%, unique alignment rate <30%, number of aligned reads <300 K, or percentage of probes with at least five reads <50%. FastQC was run on all samples to ensure adequate per base quality and per base N content, where N represents bases that could not be identified. Nine samples were flagged after applying the per-sample QC metrics. In addition, principal component, hierarchical cluster, and inter-replicate correlation analyses were used collectively to identify outlier samples. These analyses confirmed the nine flagged samples as outliers, identified additional outliers, and discovered a set of mouse kidney samples with potential tissue contamination. A total of 2 rat and 21 mouse samples were removed before downstream analysis, with 198 rat and 164 mouse samples available for downstream analysis.

The processing of samples from the study of 1,2-DCB was conducted in parallel with one other chemical (1,4-dichlorobenzene) that was studied under a similar protocol, therefore allowing for a more powerful collective assessment of the data. Specifically, during RNA isolation and extraction, the samples from both studies were distributed over twelve 96-well plates (i.e., three plates per species per chemical). Prior to amplification and library generation, the samples were randomized over five 384-well plates (i.e., one plate per tissue). The sample layout on the plate avoided the edge wells to preclude edge-well effects, which can affect downstream sequencing results. The final sample counts used for benchmark dose (BMD) analysis of the transcriptomics data are shown in Table 1.

Table 1. Final Sample Counts for Benchmark Dose Analysis of the Transcriptomics Data

	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm
Rats							
Heart	10	5	5	5	5	5	5
Kidney	9	5	5	5	5	5	5
Liver	10	5	5	5	5	5	5
Lung	10	5	5	5	5	5	4
Ovary	10	5	5	5	5	5	5
Mice							
Heart	10	5	5	5	5	7	NA
Kidney	5	4	4	3	2	6	NA
Liver	9	2	4	5	5	6	NA
Lung	10	4	5	5	5	6	NA
Ovary	10	5	5	5	5	7	NA

NA = not applicable.

Data Normalization

The aligned read counts for attenuated probes were properly readjusted to calculate unattenuated equivalent counts using the attenuation factors provided in the platform manifest. To account for between-sample sequencing depth variation, unattenuated read counts were normalized at the probe level by applying reads per million normalization. A pseudo-read-count of 1.0 was added to each normalized expression value, and then the values were log2-transformed to complete the normalization. Principal component-based visualizations of the final expression data set used from modeling are available in Appendix D.

Data Analysis

Statistical Analysis of Body Weights, Organ Weights, and Clinical Pathology

Two approaches were employed to assess the significance of pairwise comparisons between exposed and 0 ppm groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Williams[18; 19] and Dunnett.[20] Clinical pathology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[21] and Dunn.[22] The Jonckheere test[23] was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams or Shirley test) was more appropriate for pairwise comparisons than a test that assumes no monotonic dose response (Dunnett or Dunn test). Trend-sensitive tests were used when the Jonckheere test was significant at $p \leq 0.01$.

Prior to analysis, values identified by the outlier test of Dixon and Massey[24] were examined by NIEHS staff. Values from animals suspected of illness from causes other than experimental exposure and values that the laboratory indicated as inadequate because of measurement problems were eliminated from the analysis.

A no-observed-effect level (NOEL) was identified as the highest exposure concentration not showing a significant ($p \leq 0.05$) pairwise difference relative to the 0 ppm group. A lowest-observed-effect level (LOEL) was identified as the lowest exposure concentration demonstrating a significant ($p \leq 0.05$) pairwise difference relative to the 0 ppm group. Throughout the results section for apical endpoints, interpretation of BMDs is made in relationship to NOEL and LOEL values for specific endpoints, as defined here, and are not meant to reflect an overall study NOEL or LOEL.

Benchmark Dose Analysis of Body Weights, Organ Weights, and Clinical Pathology

Data files for apical endpoints, including clinical pathology, organ weights, and body weights, were created using nontransformed individual animal data. With the exception of body weights and body weight gain, to be included in the data file, an endpoint had to show a significant trend and at least one significant pairwise response. The expression data files were then loaded into BMDExpress 3.2.0119 using a "generic" platform annotation. BMD modeling was conducted with ToxicR Laplace model averaging, applying a benchmark response (BMR) of 1 standard deviation. Constant variance was assumed in the BMD modeling. Results from the BMD analysis were subsequently subjected to a defined category analysis in which modeled responses were excluded if they did not meet the following criteria: $R^2 > 0.6$, BMD/benchmark dose lower confidence limits (BMD_L) < 10, and BMD < highest dose. Some endpoints that met the initial statistical criteria for inclusion (i.e., significant trend and at least one significant pairwise response) did not yield a BMD result because no viable model was obtained.

Benchmark Dose Analysis of Transcriptomics Data

The BMD analysis of the transcriptomic data was performed in a manner consistent with the guidance provided in the National Toxicology Program best practices for genomic dose-response modeling as reviewed by an independent panel of experts in October 2017. These recommendations are described in the 2018 publication *National Toxicology Program Approach to Genomic Dose-Response Modeling*.[25]

Dose-response modeling of transcriptional effects was carried out using BMDExpress 3.20.0095, a robust, interactive, and user-friendly update of BMDExpress software[26] that can be downloaded at no cost (https://github.com/auerbachs/BMDExpress-3/releases). The initial mapped read counts from the TempO-Seq data from each well were counts per million normalized. The values were log2 transformed and imported into BMDExpress. The platform selection in BMDExpress was S1500_Plus_Rat for rats (date: October 31, 2024) and S1500_Plus_Mouse for mice (date: October 31, 2024). Before importing the data into BMDExpress, all detection oligos (DO) with "0" count values in any sample were excluded.

In BMDExpress, the data underwent a twofold prefiltering process. First, a Williams trend test[18; 19] was performed with nominal p value < 0.05 with 10,000 permutations. The DOs that passed the Williams trend test were then subject to the Curve Fit Prefilter for which the Hill, Power, Linear, Exponential 3, and Exponential 5 models were selected. A BMR factor of 2 standard deviations was used and the variance setting was constant.

The BMD analysis on the transcripts that passed the Curve Fit Prefilter was conducted using the ToxicR MAP/Laplace Bayesian MA fitting approach, implemented in BMDExpress. All continuous models (Hill, Power, Exponential 3, Exponential 5) were utilized. The parameters were set as follows: BMR type as relative deviation; BMR factor at 25%; variance as constant; and a Step Function Threshold at 0.5.

Gene set analysis (a.k.a. Functional Classification) was carried out using Gene Ontology biological process (GO BP) gene sets. For the GO analysis, the following settings were selected: (1) remove BMD > highest dose from category descriptive statistics; (2) remove BMD with R^2 < the cutoff of 0.6; (3) remove DOs with step function lower than first dose; (4) minimum number of genes in gene set of 40; (5) maximum number of genes in gene set of 500; (5) under DO to gene conversion, identify conflicting probe sets with correlation cutoff for conflicting probe sets of 0.5. Individual gene functional classification was conducted similarly to the GO gene set analysis, except that the maximum and minimum gene set size requirements were omitted.

Active GO BP terms were identified using criteria requiring at least three genes and being at least 5% populated. BMD, BMD_L, and benchmark dose upper confidence limit (BMD_U) values at the 5% level (i.e., 5th percentile) were reported as potency metrics for the active GO BP gene sets. BMD, BMD_L, and BMD_U values for the individual gene analyses reflected the average of DOs that met the fit criteria and corresponded to that gene. The exact BMD modeling pipeline parameters used to analyze the data presented in this report were evaluated using the data from Gwinn et. al.[27] and compared to the EPA Transcriptomic Assessment Product analysis pipeline.[28] The results of this analysis can be found in Appendix G under G.5.1 Analysis Pipeline Evaluation.

Empirical False Discovery Rate Determination for Genomic Dose-response Modeling

Synthetic null data were generated using the probe-filtered (i.e., “no 0”) 0 ppm data from each tissue from the 1,2-DCB rat and mouse studies. The synthetic null data were generated using the Synthetic and Null Data Generator (SaNDGen; https://rstudio.niehs.nih.gov/sandgen/), which employed the normal distribution method previously described for generating synthetic data.[29] In short, for each set of tissue/species, 0 ppm data were used to generate a distribution for each probe. This distribution was resampled to generate 1,000 values for each probe. These values were distributed into synthetic samples, which were then organized into 20 different experiments paralleling the distribution of samples in the experimental study (i.e., 10 samples in the 0 ppm group and 5 for each exposed group). Each of the 20 synthetic null experiments was processed through BMDExpress using the identical parameters used to analyze the experimental data. The resultant data were then used to determine the empirical false discovery rates, which are reported as percentages of possible genes and GO BPs. The results of the empirical false discovery rate

analysis are available in Appendix D. The associated bm2 analysis files that are the basis of the empirical false discovery rate can be found in Appendix G.

Data Accessibility

Primary and analyzed data used in this study are available to the public at https://doi.org/10.22427/NIEHS-DATA-NIEHS-12.[30]

Results

Apical Endpoint Analysis

Animal Condition, Body Weights, and Organ Weights

Rats

All internal concentration assessment and core rats exposed to 1,2-dichlorobenzene (1,2-DCB) survived to the end of the study. In the 500 ppm group, all core animals began exhibiting a ruffled coat on study day 1, with one rat also noted with mild ataxia on study days 0 and 2 and being lethargic on study day 1 (Appendix G). There was a negative trend and significant decrease in terminal body weight in the 500 ppm group compared to the 0 ppm group (Table 2). The benchmark dose (benchmark dose lower confidence limit)—BMD (BMD_L)—for decreased terminal body weight was 227.1 (116.4) ppm (Table 3). The BMDs for body weights were reviewed by a subject matter expert for anomalous modeling results (i.e., when the traditional statistics are notably different from the calculated BMD values).

At study termination, relative left and right kidney weights showed significant pairwise increases at ≥100 ppm with positive trends; the BMDs (BMD_Ls) for increased relative left and right kidney weights were 101.4 (40.9) and 93.1 (43.2) ppm, respectively (Table 3). A significant increase in absolute and relative liver weights occurred in the ≥250 and ≥100 ppm groups, respectively; both endpoints had positive trends. The BMDs (BMD_Ls) for increased absolute and relative liver weights were 114.0 (41.8) and 57.9 (29.9) ppm, respectively. The BMDs for all organ weights were reviewed by a subject matter expert for anomalous modeling results (i.e., when the traditional statistics are notably different from the calculated BMD values). Significant trend and pairwise comparisons were not observed in absolute left or right kidney weights or absolute or relative heart or lung weights (Appendix G).

Mice

In the internal concentration assessment mice, there was one accidental death in the 1 ppm group on study day 1 and two mice in the 250 ppm group were found dead, one each on study days 3 and 4. All core mice exposed to 1,2-DCB survived to the end of the study with the exception of one mouse in the 250 ppm group that presented as lethargic, had shallow breathing, and was euthanized moribund on study day 4 (Appendix G). There was a significant decrease in terminal body weight, although a significant trend was not found (Table 2).

At study termination, relative right kidney weight exhibited a positive trend and was significantly increased in the 250 ppm group with a BMD (BMD_L) of 161.5 (82.3) ppm (Table 3). There was also a significant increase in absolute and relative liver weights in the ≥100 ppm groups with positive trends. The BMDs (BMD_Ls) for increased absolute and relative liver weights were 57.4 (36.4) and 60.5 (37.7) ppm, respectively. Significant trend and pairwise comparisons were not observed in absolute right kidney weight or absolute or relative left kidney, heart, or lung weights (Appendix G).

Table 2. Summary of Body Weights and Body Weight Gain of Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Endpoint[a,b]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm	BMD_{1Std} (ppm)	BMD_{L1Std} (ppm)
Rats									
n	10	5	5	5	5	5	5	NA	NA
Body Weight									
Study Day									
0	200.1 ± 3.6	203.2 ± 3.2	211.4 ± 2.9	206.5 ± 4.2	196.2 ± 2.2	198.9 ± 4.2	207.9 ± 4.7	ND	ND
4	205.4 ± 3.7*	204.6 ± 4.3	207.6 ± 3.1	207.8 ± 4.8	197.9 ± 2.1	199.7 ± 4.9	187.0 ± 6.0*	298.0	137.9
5	204.2 ± 3.3**	206.9 ± 4.0	207.5 ± 3.3	207.8 ± 4.8	196.6 ± 3.4	200.2 ± 4.5	179.9 ± 3.6**	227.1	116.5
Body Weight Gain									
Study Day Interval									
0–5	4.1 ± 0.9**	3.7 ± 0.9	−3.9 ± 2.3	1.3 ± 2.0	0.4 ± 2.7	1.3 ± 1.1	−28.0 ± 1.7**	273.4	215.6
Mice									
n	10	5	5	5	5	8	NA	NA	NA
Body Weight									
Study Day									
0	21.1 ± 0.3	21.4 ± 0.4	22.2 ± 0.3	21.6 ± 0.2	21.0 ± 0.6	21.2 ± 0.4	NA	ND	ND
2	20.4 ± 0.3**	21.4 ± 0.5	21.7 ± 0.4	20.7 ± 0.3	20.0 ± 0.8	17.6 ± 0.4**	NA	107.8	62.4
3	20.4 ± 0.3**	21.4 ± 0.6	21.7 ± 0.5	20.5 ± 0.4	20.4 ± 0.7	17.6 ± 0.4**	NA	118.5	69.5
4	20.5 ± 0.4**	21.0 ± 0.6	21.4 ± 0.5	20.5 ± 0.4	20.8 ± 0.7	17.5 ± 0.5**	NA	133.3	82.8
5	20.3 ± 0.4	20.8 ± 0.5	21.3 ± 0.7	20.5 ± 0.5	21.0 ± 0.7	18.5 ± 0.4*[c]	NA	ND	ND
Body Weight Gain									
Study Day Interval									
0–5	−0.8 ± 0.2*	−0.7 ± 0.3	−0.9 ± 0.5	−1.1 ± 0.5	0.0 ± 0.2	−2.9 ± 0.3**[c]	NA	159.8	115.4

Statistical significance for an exposed group indicates a significant pairwise test compared to the 0 ppm group. Statistical significance for the 0 ppm group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.

BMD_{1Std} = benchmark dose corresponding to a benchmark response set to 1 standard deviation from the mean; BMD_{L1Std} = benchmark dose lower confidence limit corresponding to a benchmark response set to 1 standard deviation from the mean; NA = not applicable; ND = not determined.

[a]Data are displayed as mean ± standard error of the mean; body weight data are presented in grams.

[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

[c]n = 7. One animal was euthanized moribund on study day 4.

Table 3. Summary of Select Organ Weights of Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Endpoint[a,b,c]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm	BMD_{1Std} (ppm)	BMD_{L1Std} (ppm)
Rats									
n	10	5	5	5	5	5	5	NA	NA
Terminal Body Wt. (g)	204.2 ± 3.3**	206.9 ± 4.0	207.5 ± 3.3	207.8 ± 4.8	196.6 ± 3.4	200.2 ± 4.5	179.9 ± 3.6**	227.1	116.4
Left Kidney									
Relative (mg/g)[d]	3.12 ± 0.03**	3.25 ± 0.07	3.23 ± 0.13	3.15 ± 0.04	3.39 ± 0.09*	3.45 ± 0.05**	3.69 ± 0.11**	101.4	40.9
Right Kidney									
Relative (mg/g)	3.20 ± 0.05**	3.24 ± 0.06	3.32 ± 0.08	3.15 ± 0.10	3.49 ± 0.11**	3.56 ± 0.06**	3.84 ± 0.11**	93.1	43.2
Liver									
Absolute (g)	7.37 ± 0.18**	7.67 ± 0.23	7.81 ± 0.18	7.93 ± 0.31	7.93 ± 0.25	9.08 ± 0.43**	9.68 ± 0.47**	114.0	41.8
Relative (mg/g)	36.08 ± 0.46**	37.06 ± 0.61	37.65 ± 0.72	38.12 ± 0.94	40.29 ± 0.77**	45.29 ± 1.22**	53.80 ± 2.04**	57.9	29.9
Mice									
n	10	5	5	5	5	7	NA	NA	NA
Terminal Body Wt. (g)	20.3 ± 0.4	20.8 ± 0.5	21.3 ± 0.7	20.5 ± 0.5	21.0 ± 0.7	18.5 ± 0.4*	NA	ND	ND
Right Kidney									
Relative (mg/g)	6.49 ± 0.13*[e]	6.36 ± 0.13	6.21 ± 0.25	6.62 ± 0.20	6.38 ± 0.09	7.17 ± 0.17*	NA	161.5	82.3
Liver									
Absolute (g)	0.94 ± 0.05**	0.96 ± 0.03	0.90 ± 0.03	0.92 ± 0.04	1.16 ± 0.05**	1.41 ± 0.04**	NA	57.4	36.4
Relative (mg/g)	46.05 ± 1.74**	46.51 ± 1.52	42.45 ± 1.25	44.73 ± 0.75	55.36 ± 1.40**	76.01 ± 2.05**	NA	60.5	37.7

Statistical significance for an exposed group indicates a significant pairwise test compared to the 0 ppm group. Statistical significance for the 0 ppm group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
BMD_{1Std} = benchmark dose corresponding to a benchmark response set to 1 standard deviation from the mean; BMD_{L1Std} = benchmark dose lower confidence limit corresponding to a benchmark response set to 1 standard deviation from the mean; NA = not applicable; ND = not determined.
[a]Descriptions of organ weight endpoints and changes are provided in Appendix F.
[b]Data are displayed as mean ± standard error of the mean.
[c]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[d]Relative organ weights (organ weight-to-body weight ratios) are given as mg organ weight/g body weight.
[e]n = 9. One value was excluded as an outlier.

Clinical Pathology

Rats

At study termination, there were no hematology parameters that exhibited significant trend and pairwise comparisons (Appendix G).

The albumin/globulin (A/G) ratio had a positive trend with a significant increase in the 500 ppm group (Table 4); the increase in the ratio was mainly driven by a mild (nonsignificant) decrease in globulin concentrations (Appendix G). The BMD (BMD_L) for increased A/G ratio was 182.3 (99.4) ppm. There was a positive trend in cholesterol concentration and a significant increase in the 500 ppm group; the BMD (BMD_L) was 238.4 (147.3) ppm. Glucose concentration had a negative trend with a significant decrease in the 500 ppm group; the BMD (BMD_L) was 360.3 (183.9) ppm. Bile salt/acid concentration had a positive trend and significant increases in the 250 and 500 ppm groups; the BMD (BMD_L) was 143.8 (45.9) ppm.

When measured on clinical chemistry analyzers using standard reagents, normal rat baseline total and direct bilirubin blood concentrations are usually below the lower limit of quantification (LOQ); the LOQ for total bilirubin and direct bilirubin were 0.15 and 0.08 mg/dL, respectively. In this study, all the total and direct bilirubin values for the 0–250 ppm groups were below LOQ (Appendix G) and therefore no statistical analysis was performed on these parameters. However, in the 500 ppm group, almost all the total and direct bilirubin individual animal values were above the LOQ, ranging between 0.16–0.23 mg/dL (n = 3) and 0.11–0.19 mg/dL, (n = 5), respectively.

Mice

Because of serum volume limitations (i.e., quantity not sufficient), some pairwise statistical comparisons across different exposed groups and involving different parameters (Appendix G) were underpowered and may not accurately reflect the existence of "no effect."

Cholesterol concentration and alanine aminotransferase (ALT) activity had positive trends with significant increases in the 250 ppm group; the BMD (BMD_L) for ALT was 157.8 (108.9) ppm (Table 4). A BMD (BMD_L) for increased cholesterol was not determined because no viable model was available. Alkaline phosphatase activity had a negative trend with significant decreases in the 100 and 250 ppm groups with a BMD (BMD_L) of 33.4 (10.9) ppm. Sorbitol dehydrogenase activity had a positive trend, with significant increases in the 100 and 250 ppm groups with a BMD (BMD_L) of 93.0 (56.8) ppm.

Similar to rats, normal mouse baseline total and direct bilirubin blood concentrations are usually below the LOQ; the LOQ for total bilirubin and direct bilirubin were 0.15 and 0.08 mg/dL, respectively. In this study, all the total and direct bilirubin values for the 0–100 ppm groups were below LOQ (Appendix G) and therefore no statistical analysis was performed on these parameters. However, in the 250 ppm group, some of the total and direct bilirubin individual animal values were above the LOQ, with one total bilirubin value of 0.18 mg/dL and direct bilirubin values ranging between 0.09 and 0.1 mg/dL (n = 4).

Table 4. Summary of Select Clinical Chemistry Data for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Endpoint[a,b,c]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm	BMD_{1Std} (ppm)	BMD_{L1Std} (ppm)
Rats									
A/G Ratio	3.02 ± 0.11** (10)	3.12 ± 0.07 (5)	2.96 ± 0.14 (5)	2.78 ± 0.19 (5)	3.06 ± 0.12 (5)	3.39 ± 0.11 (5)	3.69 ± 0.11** (5)	182.3	99.4
Cholesterol (mg/dL)	87.7 ± 2.8** (10)	89.0 ± 2.8 (5)	82.6 ± 0.7 (5)	93.8 ± 7.5 (5)	97.0 ± 3.2 (5)	94.8 ± 5.4 (5)	134.8 ± 8.1** (5)	238.4	147.3
Glucose (mg/dL)	135.0 ± 6.7** (10)	144.6 ± 6.1 (5)	129.8 ± 1.8 (5)	135.0 ± 6.4 (5)	127.8 ± 2.5 (5)	130.2 ± 15.6 (5)	104.8 ± 2.4** (5)	360.3	183.9
Bile Salts/Acids (µmol/L)	6.0 ± 1.3** (10)	4.6 ± 0.8 (5)	9.3 ± 3.0 (4)	13.6 ± 3.8 (5)	15.2 ± 5.5 (5)	23.0 ± 5.6** (5)	42.4 ± 12.6** (5)	143.8	45.9
Mice									
Cholesterol (mg/dL)	115.8 ± 4.9** (5)	126.5 ± 15.5 (2)	129.0 ± 13.0 (2)	133.0 (1)	141.0 (1)	194.3 ± 10.3** (4)	NA	NVM	NVM
Alanine Aminotransferase (IU/L)	58.9 ± 11.8** (8)	102.8 ± 45.6 (4)	40.0 ± 2.0 (2)	78.3 ± 12.2 (3)	84.2 ± 17.3 (5)	248.6 ± 34.2** (7)	NA	157.8	108.9
Alkaline Phosphatase (IU/L)	191.5 ± 8.1** (8)	234.8 ± 9.7 (4)	159.5 ± 26.5 (2)	175.7 ± 11.9 (3)	129.2 ± 5.3** (5)	109.4 ± 3.1** (7)	NA	33.4	10.9
Sorbitol Dehydrogenase (IU/L)	25.6 ± 1.1** (7)	28.0 ± 0.1 (2)	28.9 ± 4.4 (2)	22.0 ± 5.5 (3)	40.9 ± 6.7** (4)	108.6 ± 7.0** (7)	NA	93.0	56.8

Statistical significance for an exposed group indicates a significant pairwise test compared to the 0 ppm group. Statistical significance for the 0 ppm group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

BMD_{1Std} = benchmark dose corresponding to a benchmark response set to 1 standard deviation from the mean; BMD_{L1Std} = benchmark dose lower confidence limit corresponding to a benchmark response set to 1 standard deviation from the mean; NA = not applicable; A/G ratio = albumin/globulin ratio; NVM = nonviable model.

[a]Data are displayed as mean ± standard error of the mean (number of animals).

[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests. Data with sample size of one were included in the trend test but excluded from the pairwise test.

[c]Clinical chemistry data not reported were removed as outliers or were due to preanalytical or analytical conditions or errors, including but not limited to below linearity, short sample, quantity not sufficient, or extreme hemolysis.

Apical Endpoint Benchmark Dose Summary

A summary of the calculated BMDs for each toxicological endpoint, by species, is provided in Table 5. The endpoint-specific lowest-observed-effect level (LOEL) and no-observed-effect level (NOEL) are included and could be informative for endpoints that lack a calculated BMD either because no viable model was available or because the estimated BMD was below the lower limit of extrapolation (<0.333 ppm).

Table 5. BMD, BMD_L, LOEL, and NOEL Summary for Apical Endpoints, Sorted by BMD or LOEL from Low to High, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Endpoint	BMD_{1Std} (ppm)	BMD_{L1Std} (ppm)	LOEL (ppm)[a]	NOEL (ppm)	Direction of Change
Rats					
Relative Liver Weight	57.9	29.9	100	30	UP
Relative Right Kidney Weight	93.1	43.2	100	30	UP
Relative Left Kidney Weight	101.4	40.9	100	30	UP
Absolute Liver Weight	114.0	41.8	250	100	UP
Bile Salts/Acids	143.8	45.9	250	100	UP
A/G Ratio	182.3	99.4	500	250	UP
Terminal Body Weight	227.1	116.4	500	250	DOWN
Cholesterol	238.4	147.3	500	250	UP
Body Weight Gain	273.4	215.6	500	250	DOWN
Body Weight on Study Day 4	298.0	137.9	500	250	DOWN
Glucose	360.3	183.9	500	250	DOWN
Mice					
Alkaline Phosphatase	33.4	10.9	100	30	DOWN
Absolute Liver Weight	57.4	36.4	100	30	UP
Relative Liver Weight	60.5	37.7	100	30	UP
Sorbitol Dehydrogenase	93.0	56.8	100	30	UP
Body Weight on Study Day 2	107.8	62.4	250	100	DOWN
Body Weight on Study Day 3	118.5	69.5	250	100	DOWN
Body Weight on Study Day 4	133.3	82.8	250	100	DOWN
Alanine Aminotransferase	157.8	108.9	250	100	UP
Body Weight Gain	159.8	115.4	250	100	DOWN
Relative Right Kidney Weight	161.5	82.3	250	100	UP
Cholesterol	NVM	NVM	**250**	100	UP

BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; LOEL = lowest-observed-effect level; NOEL = no-observed-effect level; BMD_{1Std} = benchmark dose corresponding to a benchmark response set to 1 standard deviation from the mean; BMD_{L1Std} = benchmark dose lower confidence limit corresponding to a benchmark response set to 1 standard deviation from the mean; A/G ratio = albumin/globulin ratio; NVM = no viable model.

[a]Values in bold text indicate the LOEL of endpoints for which a BMD could not be calculated.

Gene Set Benchmark Dose Analysis

Chemical-induced alterations in heart, kidney, liver, lung, and ovary gene transcript expression were examined to determine those gene sets most sensitive to 1,2-DCB exposure. To that end, BMD analysis of transcripts and gene sets (Gene Ontology [GO] biological process) was conducted to determine the potency of the chemical to elicit gene expression changes in the heart, kidney, liver, lung, and ovary. This analysis used transcript-level BMD data to assess an aggregate score of gene set potency (5th percentile of transcript BMDs) and enrichment.

The "active" gene sets with the lowest 5th percentile BMD values are shown for the heart (Table 6), kidney (Table 7), liver (Table 8), lung (Table 9), and ovary (Table 10), with a summary of the top gene set per tissue based on lowest BMD 5th percentile provided in Table 11. No gene sets had estimated 5th percentile BMD values <0.333 ppm. The gene sets shown in Table 6 to Table 10 should be interpreted with caution from the standpoint of the underlying biological mechanism and any relationship to toxicity or toxic agents referenced in the GO term definitions. The data primarily should be considered as a metric of potency for chemical-induced transcriptional changes (i.e., a concerted biological change) that could serve as a surrogate of estimated biological potency and, by extension, toxicological potency when more definitive toxicological data are unavailable.

Heart

Rats

The most sensitive GO biological processes for which a BMD value could be reliably calculated were cellular response to hydrogen peroxide (GO:0070301), cell cycle phase transition (GO:0044770), and mitotic cell cycle phase transition (GO:0044772) with 5th percentile BMDs (BMD_{LS}) of 148.7 (58.2), 161.5 (81.6), and 161.5 (81.6) ppm, respectively (Table 6).

Mice

The most sensitive GO biological processes for which a BMD value could be reliably calculated were cellular modified amino acid metabolic process (GO:0006575) and hemopoiesis (GO:0030097) with 5th percentile BMDs (BMD_{LS}) of 4.4 (1.7) and 58.8 (17.9) ppm, respectively (Table 6). The full list of affected gene sets in the heart of female rats and mice can be found in Appendix G.

Kidney

Rats

The most sensitive GO biological processes for which a BMD value could be reliably calculated were embryonic organ development (GO:0048568) and monocarboxylic acid biosynthetic process (GO:0072330), and regulation of cold-induced thermogenesis (GO:0120161) with 5th percentile BMDs (BMD_{LS}) of 114.0 (73.9), 151.9 (109.0), and 151.9 (109.0) ppm, respectively (Table 7).

Mice

The most sensitive GO biological processes for which a BMD value could be reliably calculated were organic hydroxy compound transport (GO:0015850) and unsaturated fatty acid metabolic process (GO:0033559) with 5th percentile BMDs (BMD_{LS}) of 2.3 (0.6) and 2.6 (1.2) ppm, respectively (Table 7). The full list of affected gene sets in the kidney of female rats and mice can be found in Appendix G.

Liver

Rats

The most sensitive GO biological processes for which a BMD value could be reliably calculated were xenobiotic metabolic process (GO:0006805), detoxification (GO:0098754), and cellular detoxification (GO:1990748) with 5th percentile BMDs (BMD_{LS}) of 29.8 (25.3), 33.8 (18.2), and 33.8 (18.2) ppm, respectively (Table 8).

Mice

The most sensitive GO biological processes for which a BMD value could be reliably calculated were positive regulation of lipid biosynthetic process (GO:0046889) and positive regulation of lipid metabolic process (GO:0045834) with 5th percentile BMDs (BMD_{LS}) of 1.5 (0.5) and 2.1 (0.7) ppm, respectively (Table 8). The full list of affected gene sets in the liver of female rats and mice can be found in Appendix G.

Lung

Rats

The most sensitive GO biological processes for which a BMD value could be reliably calculated were detoxification (GO:0098754), cellular oxidant detoxification (GO:0098869), and cellular detoxification (GO:1990748), all three with a 5th percentile BMD (BMD_{L}) of 175.9 (109.3) ppm (Table 9).

Mice

The most sensitive GO biological processes for which a BMD value could be reliably calculated were unsaturated fatty acid metabolic process (GO:0033559), cholesterol metabolic process (GO:0008203), sterol metabolic process (GO:0016125), and secondary alcohol metabolic process (GO:1902652) with 5th percentile BMDs (BMD_{LS}) of 2.1 (0.7), 2.4 (0.7), 2.4 (0.7), and 2.4 (0.7) ppm, respectively (Table 9). The full list of affected gene sets in the lung of female rats and mice can be found in Appendix G.

Ovary

Rats

The most sensitive GO biological processes for which a BMD value could be reliably calculated were kidney development (GO:0001822), gonad development (GO:0008406), and male gonad development (GO:0008584), all three with a 5th percentile BMD (BMD_{L}) of 156.7 (50.8) ppm (Table 10).

Mice

The most sensitive GO biological processes for which a BMD value could be reliably calculated were hemopoiesis (GO:0030097) and establishment of protein localization to organelle (GO:0072594) with 5th percentile BMDs (BMD_{LS}) of 108.0 (35.9) and 110.3 (57.1) ppm, respectively (Table 10). The full list of affected gene sets in the ovary of female rats and mice can be found in Appendix G.

Table 6. Top 10 Heart Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Genes with Changed Direction Up/Down
Rats			
GO:0070301 cellular response to hydrogen peroxide	5/57 (8.8%)	148.7 (58.2–382.1)	4/1
GO:0044770 cell cycle phase transition	3/58 (5.2%)	161.5 (81.6–247.4)	0/3
GO:0044772 mitotic cell cycle phase transition	3/52 (5.8%)	161.5 (81.6–247.4)	0/3
GO:0048872 homeostasis of number of cells	5/58 (8.6%)	171.1 (90.5–395.6)	3/2
GO:0001568: blood vessel development	5/45 (11.1%)	174.1 (96.0–271.1)	1/4
GO:0001936 regulation of endothelial cell proliferation	6/48 (12.5%)	176.6 (107.9–257.6)	3/3
GO:0010594 regulation of endothelial cell migration	6/58 (10.3%)	176.6 (107.9–257.6)	4/2
GO:0010634 positive regulation of epithelial cell migration	4/51 (7.8%)	176.6 (107.9–257.6)	2/2
GO:0071229 cellular response to acid chemical	5/54 (9.3%)	179.7 (66.7–285.1)	1/4
GO:0071230 cellular response to amino acid stimulus	5/50 (10.0%)	179.7 (66.7–285.1)	1/4
Mice			
GO:0006575 cellular modified amino acid metabolic process	3/59 (5.1%)	4.4 (1.7–16.7)	3/0
GO:0030097 hemopoiesis	5/56 (8.9%)	58.8 (17.9–116.1)	2/3
GO:0002687 positive regulation of leukocyte migration	4/50 (8.0%)	69.1 (38.2–113.2)	4/0
GO:0030595 leukocyte chemotaxis	3/49 (6.1%)	69.1 (38.2–113.2)	3/0
GO:0043087 regulation of GTPase activity	3/59 (5.1%)	69.1 (38.2–113.2)	3/0
GO:0043547 positive regulation of GTPase activity	3/42 (7.1%)	69.1 (38.2–113.2)	3/0
GO:0097529 myeloid leukocyte migration	3/51 (5.9%)	69.1 (38.2–113.2)	3/0
GO:0007178 transmembrane receptor protein serine/threonine kinase signaling pathway	4/52 (7.7%)	87.5 (41.0–425.7)	3/1
GO:0009617 response to bacterium	6/114 (5.3%)	101.1 (52.8–140.7)	3/3
GO:0002685 regulation of leukocyte migration	5/69 (7.3%)	101.1 (54.7–142.2)	5/0

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology.

[a]Active genes and GO biological process definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

Table 7. Top 10 Kidney Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Genes with Changed Direction Up/Down
Rats			
GO:0048568 embryonic organ development	3/47 (6.4%)	114.0 (73.9–NC)	0/3
GO:0072330 monocarboxylic acid biosynthetic process	6/56 (10.7%)	151.9 (109.0–183.8)	3/3
GO:0120161 regulation of cold-induced thermogenesis	9/55 (16.4%)	151.9 (109.0–183.8)	3/6
GO:0098754 detoxification	12/58 (20.7%)	163.7 (105.1–421.6)	9/3
GO:1990748 cellular detoxification	10/51 (19.6%)	163.7 (105.1–421.6)	7/3
GO:0015718 monocarboxylic acid transport	8/64 (12.5%)	165.8 (52.8–289.8)	3/5
GO:0006805 xenobiotic metabolic process	11/59 (18.6%)	169.1 (112.1–177.2)	10/1
GO:0009062 fatty acid catabolic process	14/41 (34.2%)	169.1 (112.1–177.2)	12/2
GO:0072329 monocarboxylic acid catabolic process	15/49 (30.6%)	169.1 (112.1–177.2)	13/2
GO:0098869 cellular oxidant detoxification	8/43 (18.6%)	171.4 (84.7–903.1)	6/2
Mice			
GO:0015850 organic hydroxy compound transport	6/58 (10.3%)	2.3 (0.6–219.7)	4/2
GO:0033559 unsaturated fatty acid metabolic process	7/40 (17.5%)	2.6 (1.2–54.7)	5/2
GO:0055088 lipid homeostasis	5/52 (9.6%)	2.7 (0.8–13.0)	4/1
GO:0006869 lipid transport	11/77 (14.3%)	3.0 (0.8–9.9)	5/6
GO:0044242 cellular lipid catabolic process	7/44 (15.9%)	3.0 (0.8–401.3)	3/4
GO:1905952 regulation of lipid localization	4/52 (7.7%)	3.0 (0.8–401.3)	1/3
GO:0015718 monocarboxylic acid transport	8/52 (15.4%)	3.0 (0.9–401.3)	2/6
GO:0031589 cell-substrate adhesion	3/40 (7.5%)	3.1 (0.9–792.8)	0/3
GO:0031669 cellular response to nutrient levels	5/62 (8.1%)	3.3 (1.0–408.4)	1/4
GO:0090277 positive regulation of peptide hormone secretion	3/40 (7.5%)	3.5 (1.2–24.1)	1/2

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology; NC = nonconvergent.

[a]Active genes and GO biological process definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

Table 8. Top 10 Liver Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Genes with Changed Direction Up/Down
Rats			
GO:0006805 xenobiotic metabolic process	31/59 (52.5%)	29.8 (25.3–89.7)	22/9
GO:0098754 detoxification	30/58 (51.7%)	33.8 (18.2–99.6)	21/9
GO:1990748 cellular detoxification	23/51 (45.1%)	33.8 (18.2–99.6)	14/9
GO:0048872 homeostasis of number of cells	20/58 (34.5%)	34.9 (10.5–93.0)	20/0
GO:0006690 icosanoid metabolic process	20/50 (40.0%)	35.9 (19.9–101.2)	12/8
GO:0033559 unsaturated fatty acid metabolic process	24/54 (44.4%)	35.9 (19.9–101.2)	13/11
GO:1901136 carbohydrate derivative catabolic process	16/49 (32.7%)	36.3 (9.5–438.0)	14/2
GO:0120254 olefinic compound metabolic process	28/64 (43.8%)	37.4 (9.5–455.2)	15/13
GO:0044770 cell cycle phase transition	26/58 (44.8%)	38.7 (14.3–79.1)	26/0
GO:0098869 cellular oxidant detoxification	22/43 (51.2%)	40.1 (11.7–95.6)	13/9
Mice			
GO:0046889 positive regulation of lipid biosynthetic process	15/45 (33.3%)	1.5 (0.5–5.5)	6/9
GO:0045834 positive regulation of lipid metabolic process	21/61 (34.4%)	2.1 (0.7–5.0)	9/12
GO:0002708 positive regulation of lymphocyte mediated immunity	6/40 (15.0%)	2.2 (0.6–6.3)	1/5
GO:0006805 xenobiotic metabolic process	25/56 (44.6%)	2.2 (0.3–88.7)	17/8
GO:0048511 rhythmic process	25/89 (28.1%)	2.9 (0.9–13.7)	17/8
GO:0097191 extrinsic apoptotic signaling pathway	15/56 (26.8%)	2.9 (0.9–13.7)	12/3
GO:0019218 regulation of steroid metabolic process	17/46 (37.0%)	3.5 (0.9–4,078.8)	10/7
GO:0002705 positive regulation of leukocyte mediated immunity	7/47 (14.9%)	3.7 (0.9–4,079.6)	2/5
GO:0002706 regulation of lymphocyte mediated immunity	8/59 (13.6%)	3.7 (0.9–4,079.6)	3/5
GO:0120254 olefinic compound metabolic process	26/51 (51%)	3.7 (0.8–4,080.3)	14/12

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology.

[a]Active genes and GO biological process definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

Table 9. Top 10 Lung Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Genes with Changed Direction Up/Down
Rats			
GO:0098754 detoxification	11/58 (19.0%)	175.9 (109.3–224.0)	7/4
GO:0098869 cellular oxidant detoxification	10/43 (23.3%)	175.9 (109.3–224.0)	6/4
GO:1990748 cellular detoxification	10/51 (19.6%)	175.9 (109.3–224.0)	6/4
GO:0019362 pyridine nucleotide metabolic process	5/40 (12.5%)	181.2 (46.6–348.4)	5/0
GO:0046496 nicotinamide nucleotide metabolic process	5/40 (12.5%)	181.2 (46.6–348.4)	5/0
GO:0072524 pyridine-containing compound metabolic process	5/41 (12.2%)	181.2 (37.4–352.1)	5/0
GO:0001676 long-chain fatty acid metabolic process	5/55 (9.1%)	193.6 (60.7–298.8)	5/0
GO:0006690 icosanoid metabolic process	5/50 (10.0%)	193.6 (60.7–298.8)	5/0
GO:0006805 xenobiotic metabolic process	6/59 (10.2%)	193.6 (60.7–298.8)	5/1
GO:0033559 unsaturated fatty acid metabolic process	5/54 (9.3%)	193.6 (60.7–298.8)	5/0
Mice			
GO:0033559 unsaturated fatty acid metabolic process	8/40 (20.0%)	2.1 (0.7–4.8)	8/0
GO:0008203 cholesterol metabolic process	8/48 (16.7%)	2.4 (0.7–5.8)	8/0
GO:0016125 sterol metabolic process	8/49 (16.3%)	2.4 (0.7–5.8)	8/0
GO:1902652 secondary alcohol metabolic process	10/53 (18.9%)	2.4 (0.7–5.8)	10/0
GO:0002708 positive regulation of lymphocyte mediated immunity	4/40 (10.0%)	2.8 (0.9–843.5)	3/1
GO:0120254 olefinic compound metabolic process	11/51 (21.6%)	3.5 (0.7–53.9)	11/0
GO:0006690 icosanoid metabolic process	8/43 (18.6%)	3.7 (0.9–11.6)	8/0
GO:0006805 xenobiotic metabolic process	14/56 (25.0%)	3.8 (1.0–10.4)	13/1
GO:0006066 alcohol metabolic process	22/90 (24.4%)	5.0 (0.9–60.7)	22/0
GO:0015850 organic hydroxy compound transport	7/58 (12.1%)	5.1 (1.3–26.0)	7/0

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology.

[a]Active genes and GO biological process definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

Table 10. Top 10 Ovary Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Genes with Changed Direction Up/Down
Rats			
GO:0001822 kidney development	4/57 (7.0%)	156.7 (50.8–280.4)	4/0
GO:0008406 gonad development	3/51 (5.9%)	156.7 (50.8–280.4)	2/1
GO:0008584 male gonad development	3/46 (6.5%)	156.7 (50.8–280.4)	2/1
GO:0045582 positive regulation of T cell differentiation	3/40 (7.5%)	160.7 (51.6–289.6)	3/0
GO:0016055 Wnt signaling pathway	4/41 (9.8%)	162.6 (54.1–417.6)	4/0
GO:0030217 T cell differentiation	3/48 (6.3%)	162.6 (54.1–417.6)	3/0
GO:0030856 regulation of epithelial cell differentiation	3/53 (5.7%)	163.7 (49.2–293.8)	3/0
GO:0006694 steroid biosynthetic process	3/54 (5.6%)	175.7 (70.6–293.3)	1/2
GO:0008203 cholesterol metabolic process	3/54 (5.6%)	175.7 (70.6–293.3)	1/2
GO:0016125 sterol metabolic process	3/56 (5.4%)	175.7 (70.6–293.3)	1/2
Mice			
GO:0030097 hemopoiesis	3/56 (5.4%)	108.0 (35.9–NC)	2/1
GO:0072594 establishment of protein localization to organelle	5/77 (6.5%)	110.3 (57.1–152.8)	3/2
GO:0048167 regulation of synaptic plasticity	4/75 (5.3%)	119.5 (80.8–248.7)	1/3
GO:0050777 negative regulation of immune response	3/53 (5.7%)	125.7 (60.1–425.4)	3/0
GO:0006898 receptor-mediated endocytosis	3/48 (6.3%)	136.8 (83.0–271.7)	3/0
GO:0043409 negative regulation of MAPK cascade	3/47 (6.4%)	136.8 (83.0–271.7)	3/0
GO:0045580 regulation of T cell differentiation	3/60 (5.0%)	149.2 (81.9–684.2)	2/1
GO:0002761 regulation of myeloid leukocyte differentiation	3/50 (6.0%)	153.6 (37.5–NC)	2/1
GO:0016050 vesicle organization	3/54 (5.6%)	155.0 (87.7–288.7)	1/2
GO:0051235 maintenance of location	3/46 (6.5%)	155.5 (74.0–NC)	2/1

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology; NC = nonconvergent.

[a]Active genes and GO biological process definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

Gene Set Benchmark Dose Summary

A summary of the most potent gene set per tissue is provided in Table 11. In both rats and mice exposed to 1,2-DCB, the liver had the gene set with the lowest BMD 5th percentile value, corresponding to xenobiotic metabolic process (GO:0006805) and positive regulation of lipid biosynthetic process (GO:0046889), respectively. The BMDs (BMD_{LS}) were 29.8 (25.3) and 1.5 (0.5) ppm, respectively.

Table 11. Most Potent Gene Ontology Biological Process Gene Set per Tissue, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Tissue	Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Genes with Changed Direction Up/Down
Rats				
Liver	**GO:0006805** xenobiotic metabolic process	31/59 (52.5%)	29.8[c] (25.3−89.7)	22/9
Kidney	**GO:0048568** embryonic organ development	3/47 (6.4%)	114.0 (73.9−NC)	0/3
Heart	**GO:0070301** cellular response to hydrogen peroxide	5/57 (8.8%)	148.7 (58.2−382.1)	4/1
Ovary	**GO:0001822** kidney development	4/57 (7.0%)	156.7 (50.8−280.4)	4/0
Lung	**GO:0098754** detoxification	11/58 (19.0%)	175.9 (109.3−224.0)	7/4
Mice				
Liver	**GO:0046889** positive regulation of lipid biosynthetic process	15/45 (33.3%)	1.5[c] (0.5−5.5)	6/9
Lung	**GO:0033559** unsaturated fatty acid metabolic process	8/40 (20.0%)	2.1 (0.7−4.8)	8/0
Kidney	**GO:0015850** organic hydroxy compound transport	6/58 (10.3%)	2.3 (0.6−219.7)	4/2
Heart	**GO:0006575** cellular modified amino acid metabolic process	3/59 (5.1%)	4.4 (1.7−16.7)	3/0
Ovary	**GO:0030097** hemopoiesis	3/56 (5.4%)	108.0 (35.9−NC)	2/1

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology; NC = nonconvergent.

[a]Active genes and GO biological process definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

[c]Transcriptional point of departure determined based on the lowest Gene Ontology biological process BMD_{rd25} 5th percentile value.

Gene Benchmark Dose Analysis

The top 10 genes ranked by BMD potency are shown in the heart (Table 12), kidney (Table 13), liver (Table 14), lung (Table 15), and ovary (Table 16). No genes had estimated median BMD values <0.333 ppm with the exception of one lung gene in mice. As with the GO analysis, the biological or toxicological significance of the changes in gene expression shown in Table 12 to Table 16 should be interpreted with caution. The data primarily should be considered as a metric of potency for chemical-induced transcriptional changes that could serve as a conservative surrogate of estimated biological potency, and by extension toxicological potency, when more definitive toxicological data are unavailable.

Heart

Rats

All 10 of the most sensitive heart genes were downregulated. These genes were *Prc1* (protein regulator of cytokinesis 1), *Lcn2* (lipocalin 2), *Loc103689966* (MARCKS-related protein-like), *Marcksl1* (MARCKS-like 1), *Loc102549061*/*H2bc3* (H2B clustered histone 3), *Plk4* (polo-like kinase 4), *Apln* (apelin), *Ccnb2* (cyclin B2), *Ccnb2-ps2* (cyclin B2, pseudogene 2), and *Pcbd1* (pterin-4 alpha-carbinolamine dehydratase 1) with BMDs (BMD_{LS}s) of 3.0 (0.7), 9.4 (2.0), 16.0 (4.8), 16.0 (4.8), 23.0 (3.2), 93.9 (16.1), 95.9 (12.4), 102.8 (40.3), 102.8 (40.3), and 105.6 (32.0) ppm, respectively.

Mice

Half of the top 10 most sensitive heart genes were upregulated and the other half were downregulated. The upregulated genes with a calculated BMD were *Hlf* (hepatic leukemia factor), *Tcap* (titin-cap), *Mthfd1l* (methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like), *Bhlhe40* (basic helix-loop-helix family, member e40), and *Gnmt* (glycine N-methyltransferase) with BMD (BMD_{LS}) of 1.9 (0.9), 2.5 (1.1), 2.6 (1.1), 3.9 (1.6), and 3.9 (1.8) ppm, respectively. The downregulated genes with a calculated BMD were *Rsad2* (radical S-adenosyl methionine domain containing 2), *Prc1* (protein regulator of cytokinesis 1), *Nfil3* (nuclear factor, interleukin 3, regulated), *Ifi44* (interferon-induced protein 44), and *Cldn5* (claudin 5) with BMDs (BMD_{LS}) of 1.3 (0.5), 1.8 (0.8), 2.5 (1.2), 3.1 (1.2), and 4.4 (1.7) ppm, respectively.

Table 12. Top 10 Heart Genes Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Rats			
Prc1	3.0 (0.7–11.5)	1.9	DOWN
Lcn2	9.4 (2.0–68.3)	1.5	DOWN
Loc103689966	16.0 (4.8–46.6)	1.8	DOWN
Marcksl1	16.0 (4.8–46.6)	1.8	DOWN
Loc102549061/H2bc3	23.0 (3.2–134.3)	1.8	DOWN
Plk4	93.9 (16.1–257.2)	1.7	DOWN

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Apln	95.9 (12.4–646.3)	1.5	DOWN
Ccnb2	102.8 (40.3–179.2)	2.3	DOWN
Ccnb2-ps2	102.8 (40.3–179.2)	2.3	DOWN
Pcbd1	105.6 (32.0–234.3)	1.5	DOWN
Mice			
Rsad2	1.3 (0.5–803.9)	1.7	DOWN
Prc1	1.8 (0.8–7.9)	2.3	DOWN
Hlf	1.9 (0.9–4.4)	1.9	UP
Tcap	2.5 (1.1–5.8)	2.0	UP
Nfil3	2.5 (1.2–5.2)	1.8	DOWN
Mthfd1l	2.6 (1.1–5.8)	2.2	UP
Ifi44	3.1 (1.2–7.9)	1.8	DOWN
Bhlhe40	3.9 (1.6–7.7)	2.0	UP
Gnmt	3.9 (1.8–9.4)	1.5	UP
Cldn5	4.4 (1.7–8.9)	1.8	DOWN

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response.
[a]Gene definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Kidney

Rats

The most sensitive upregulated genes with a calculated BMD were *Slc51a* (solute carrier family 51 member A), *Acot12* (acyl-CoA thioesterase 12), *Gstp1* (glutathione S-transferase pi 1), and *Ugt2b37* (UDP-glucuronosyltransferase 2 family, member 37) with BMDs (BMD_{L}s) of 36.3 (8.9), 50.5 (14.6), 109.9 (66.6), and 120.6 (29.3) ppm, respectively. The most sensitive genes exhibiting a decrease in expression were *Ak2* (adenylate kinase 2), *Slc6a8* (solute carrier family 6 member 8), *Atf4* (activating transcription factor 4), *Slc5a6* (solute carrier family 5 member 6), *Ipo13* (importin 13), and *Fgf13* (fibroblast growth factor 13) with BMDs (BMD_{L}s) of 55.5 (10.9), 64.0 (12.6), 83.3 (22.9), 120.9 (20.6), 140.9 (44.7), and 142.6 (57.8) ppm, respectively.

Mice

The most sensitive upregulated genes with a calculated BMD were *Dbp* (D site albumin promoter binding protein), *Slc51a* (solute carrier family 51, alpha subunit), *Tsku* (tsukushi, small leucine rich proteoglycan), *Cyp2a5* (cytochrome P450, family 2, subfamily a, polypeptide 5), *Hlf* (hepatic leukemia factor), and *Tk1* (thymidine kinase 1) with BMDs (BMD_{L}s) of 1.0 (0.3), 1.0 (0.2), 1.6 (0.5), 2.2 (0.9), 2.2 (1.2), and 2.4 (0.7) ppm, respectively. The most sensitive downregulated genes with a calculated BMD were *Cdk2* (cyclin dependent kinase 2), *Ndrg1* (N-

myc downstream regulated gene 1), *Cdkn1a* (cyclin dependent kinase inhibitor 1A), and *Slfn4* (schlafen 4) with BMDs (BMD_{L}s) of 0.9 (0.2), 1.2 (0.8), 1.7 (0.5), and 2.0 (0.5) ppm, respectively.

Table 13. Top 10 Kidney Genes Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Rats			
Slc51a	36.3 (8.9–107.4)	4.3	UP
Acot12	50.5 (14.6–364.9)	1.5	UP
Ak2	55.5 (10.9–369.9)	1.5	DOWN
Slc6a8	64.0 (12.6–553.1)	1.4	DOWN
Atf4	83.3 (22.9–NC)	1.3	DOWN
Gstp1	109.9 (66.6–115.1)	4.0	UP
Ugt2b37	120.6 (29.3–243.9)	2.1	UP
Slc5a6	120.9 (20.6–266.0)	2.0	DOWN
Ipo13	140.9 (44.7–585.6)	1.5	DOWN
Fgf13	142.6 (57.8–251.8)	2.2	DOWN
Mice			
Cdk2	0.9 (0.2–21.5)	1.7	DOWN
Dbp	1.0 (0.3–3.1)	5.9	UP
Slc51a	1.0 (0.2–4.6)	2.6	UP
Ndrg1	1.2 (0.8–4.6)	2.2	DOWN
Tsku	1.6 (0.5–429.6)	2.3	UP
Cdkn1a	1.7 (0.5–5.2)	2.6	DOWN
Slfn4	2.0 (0.5–9.3)	1.7	DOWN
Cyp2a5	2.2 (0.9–5.5)	1.9	UP
Hlf	2.2 (1.2–4.0)	2.0	UP
Tk1	2.4 (0.7–3,488.7)	1.7	UP

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; NC = nonconvergent.
[a]Gene definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Liver

Rats

The most sensitive upregulated genes with a calculated BMD were *Aldh1a1* (aldehyde dehydrogenase 1 family, member A1), *Unc93b1* (unc-93 homolog B1, TLR signaling regulator), *Ephx1* (epoxide hydrolase 1), *Slc37a4* (solute carrier family 37 member 4), *Gsta2* (glutathione S-transferase alpha 2), *Gsta5* (glutathione S-transferase alpha 5), *Ccnb2* (cyclin B2), *Ccnb2-ps2* (cyclin B2, pseudogene 2), and *Ces1f* (carboxylesterase 1F) with BMD (BMD_{L}s) of 16.7 (15.9),

16.9 (1.9), 24.0 (9.9), 28.5 (9.6), 29.8 (25.3), 29.8 (25.3), 31.8 (9.7), 31.8 (9.7), and 32.6 (4.7) ppm, respectively. One gene, *Sult1c3* (sulfotransferase family 1C member 3), was downregulated with a BMD (BMD_L) of 16.4 (3.3) ppm.

Mice

Half of the top 10 most sensitive liver genes were upregulated while the other half were downregulated. The upregulated genes with a calculated BMD were *Dbp* (D site albumin promoter binding protein), *Cyp2d9* (cytochrome P450, family 2, subfamily d, polypeptide 9), *Cyp7a1* (cytochrome P450, family 7, subfamily a, polypeptide 1), *Hlf* (hepatic leukemia factor), and *Por* (cytochrome p450 oxidoreductase) with BMD (BMD_{LS}) of 0.7 (0.2), 1.0 (0.2), 1.2 (0.3), 1.4 (0.6), and 1.8 (0.7) ppm, respectively. The genes exhibiting a decrease in expression were *Il1b* (interleukin 1 beta), *Creld2* (cysteine-rich with EGF-like domains 2), *Chka* (choline kinase alpha), *Nfil3* (nuclear factor, interleukin 3, regulated), and *Cyp3a44* (cytochrome P450, family 3, subfamily a, polypeptide 44) with BMDs (BMD_{LS}) of 0.8 (0.1), 1.4 (0.4), 1.4 (0.4), 1.4 (0.6), and 1.7 (0.2) ppm, respectively.

Table 14. Top 10 Liver Genes Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Rats			
Sult1c3	16.4 (3.3–58.9)	3.1	DOWN
Aldh1a1	16.7 (15.9–19.5)	10.1	UP
Unc93b1	16.9 (1.9–97.7)	1.6	UP
Ephx1	24.0 (9.9–93.0)	11.4	UP
Slc37a4	28.5 (9.6–67.2)	1.5	UP
Gsta2	29.8 (25.3–89.7)	6.1	UP
Gsta5	29.8 (25.3–89.7)	6.1	UP
Ccnb2	31.8 (9.7–76.6)	2.2	UP
Ccnb2-ps2	31.8 (9.7–76.6)	2.2	UP
Ces1f	32.6 (4.7–797.6)	1.4	UP
Mice			
Dbp	0.7 (0.2–1.9)	8.1	UP
Il1b	0.8 (0.1–8.6)	2.1	DOWN
Cyp2d9	1.0 (0.2–255.2)	2.1	UP
Cyp7a1	1.2 (0.3–6.3)	3.6	UP
Creld2	1.4 (0.4–4.6)	2.3	DOWN
Hlf	1.4 (0.6–3.3)	2.9	UP
Chka	1.4 (0.4–3.8)	2.4	DOWN
Nfil3	1.4 (0.6–4.0)	2.1	DOWN
Cyp3a44	1.7 (0.2–170.3)	2.3	DOWN
Por	1.8 (0.7–4.6)	1.8	UP

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response.

[a]Gene definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Lung

Rats

The most sensitive upregulated genes with a calculated BMD were *Akr1b8*/*Akr1b15* (aldo-keto reductase family 1 member B15), *Fetub* (fetuin B), *Nqo1* (NAD(P)H quinone dehydrogenase 1), *Lpar2* (lysophosphatidic acid receptor 2), *Gstp1* (glutathione S-transferase pi 1), *Dbp* (D-box binding PAR bZIP transcription factor), *Srxn1* (sulfiredoxin 1), *Me1* (malic enzyme 1), and *Aldoc* (aldolase, fructose-bisphosphate C) with BMD (BMD_Ls) of 118.9 (36.3), 130.0 (123.9), 149.7 (60.4), 167.5 (64.1), 172.0 (99.1), 174.8 (76.5), 179.8 (119.4), 181.2 (46.6), and 181.2 (28.3) ppm, respectively. One gene, *Jchain* (joining chain of multimeric IgA and IgM), was downregulated with a BMD (BMD_L) of 184.1 (49.3) ppm.

Mice

The most sensitive lung gene in mice, exhibiting an increase in expression, was *Nqo1* (NAD(P)H dehydrogenase, quinone 1) with an estimated median BMD <0.333 ppm. The most sensitive upregulated genes with a calculated BMD were *Il4* (interleukin 4), *Ces1g* (carboxylesterase 1G), *Dbp* (D site albumin promoter binding protein), *Hlf* (hepatic leukemia factor), *Ifit3* (interferon-induced protein with tetratricopeptide repeats 3), *Tspan4* (tetraspanin 4), *Chil3* (chitinase-like 3), and *Cyp2a4* (cytochrome P450, family 2, subfamily a, polypeptide 4) with BMDs (BMD_Ls) of 0.6 (0.2), 0.6 (0.2), 1.3 (0.7), 1.5 (0.8), 1.5 (0.6), 1.7 (0.7), 1.7 (0.2), and 1.9 (0.6) ppm, respectively. One gene, *Adm* (adrenomedullin), was downregulated with a BMD (BMD_L) of 1.7 (0.5) ppm.

Table 15. Top 10 Lung Genes Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Rats			
Akr1b8*/*Akr1b15	118.9 (36.3–218.3)	2.8	UP
Fetub	130.0 (123.9–205.5)	1.8	UP
Nqo1	149.7 (60.4–240.0)	2.9	UP
Lpar2	167.5 (64.1–1,083.9)	1.4	UP
Gstp1	172.0 (99.1–204.4)	2.7	UP
Dbp	174.8 (76.5–268.1)	8.1	UP
Srxn1	179.8 (119.4–243.7)	3.8	UP
Me1	181.2 (46.6–348.4)	1.6	UP
Aldoc	181.2 (28.3–355.9)	1.7	UP
Jchain	184.1 (49.3–866.9)	2.1	DOWN
Mice			
Nqo1	<0.333[b] (NR)	9.1	UP
Il4	0.6 (0.2–345.6)	2.1	UP
Ces1g	0.6 (0.2–1.7)	8.9	UP

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Dbp	1.3 (0.7–2.2)	7.5	UP
Hlf	1.5 (0.8–2.9)	3.0	UP
Ifit3	1.5 (0.6–5.5)	1.6	UP
Adm	1.7 (0.5–5.1)	2.2	DOWN
Tspan4	1.7 (0.7–4.7)	2.2	UP
Chil3	1.7 (0.2–274.5)	1.5	UP
Cyp2a4	1.9 (0.6–4.2)	5.4	UP

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; NR = the BMD_{Lrd25}–BMD_{Urd25} range is not reportable because the BMD_{rd25} median is below the lower limit of extrapolation (<1/3 of the lowest nonzero exposure concentration tested).
[a]Gene definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.
[b]<0.333 = a best-fit model was identified and a BMD_{rd25} was estimated that was <1/3 of the lowest nonzero exposure concentration tested.

Ovary

Rats

The most sensitive upregulated genes with a calculated BMD were *Gria2* (glutamate ionotropic receptor AMPA type subunit 2), *Lef1* (lymphoid enhancer binding factor 1), *Dbp* (D-box binding PAR bZIP transcription factor), *Elovl5* (ELOVL fatty acid elongase 5), *Pxmp2* (peroxisomal membrane protein 2), *Ace* (angiotensin I converting enzyme), and *Maob* (monoamine oxidase B) with BMD (BMD_{LS}) of 109.6 (25.0), 112.3 (21.0), 118.3 (23.1), 139.3 (22.0), 141.6 (35.9), 152.6 (50.1), and 157.3 (28.0) ppm, respectively. The most sensitive genes exhibiting a decrease in expression were *Ddit4* (DNA-damage-inducible transcript 4), *Hmgcs1* (3-hydroxy-3-methylglutaryl-CoA synthase 1), and *Svs5* (seminal vesicle secretory protein 5) with BMD (BMD_{LS}) of 102.9 (27.9), 122.8 (15.7), and 123.9 (15.5) ppm, respectively.

Mice

The most sensitive upregulated genes with a calculated BMD were *Tmem182* (transmembrane protein 182), *Hlf* (hepatic leukemia factor), *Dbp* (D site albumin promoter binding protein), and *Rgs2* (regulator of G-protein signaling 2) with BMDs (BMD_{LS}) of 0.9 (0.2), 2.2 (1.1), 2.3 (1.0), and 2.6 (0.6) ppm, respectively. The most sensitive genes exhibiting a decrease in expression were *Syn1* (synapsin I), *Pcolce* (procollagen C-endopeptidase enhancer protein), *Hsd3b1* (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1), *Adh1* (alcohol dehydrogenase 1 (class I)), *Ecm1* (extracellular matrix protein 1), and *Pecam1* (platelet/endothelial cell adhesion molecule 1) with BMDs (BMD_{LS}) of 0.6 (0.2), 1.9 (0.5), 2.1 (0.6), 2.4 (1.0), 2.5 (0.7), and 3.5 (1.0) ppm, respectively.

Table 16. Top 10 Ovary Genes Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Gene Symbol[a]	BMD_{rd25} (BMD_{Lrd25}–BMD_{Urd25}) in ppm	Maximum Fold Change	Direction of Expression Change
Rats			
Ddit4	102.9 (27.9–243.8)	2.0	DOWN
Gria2	109.6 (25.0–243.9)	2.2	UP
Lef1	112.3 (21.0–NC)	1.4	UP
Dbp	118.3 (23.1–172.4)	2.6	UP
Hmgcs1	122.8 (15.7–263.5)	3.1	DOWN
Svs5	123.9 (15.5–244.1)	6.5	DOWN
Elovl5	139.3 (22.0–469.0)	1.6	UP
Pxmp2	141.6 (35.9–260.6)	1.8	UP
Ace	152.6 (50.1–271.2)	1.7	UP
Maob	157.3 (28.0–280.7)	2.0	UP
Mice			
Syn1	0.6 (0.2–3.3)	2.6	DOWN
Tmem182	0.9 (0.2–3.9)	2.8	UP
Pcolce	1.9 (0.5–1,026.5)	1.5	DOWN
Hsd3b1	2.1 (0.6–10.4)	1.6	DOWN
Hlf	2.2 (1.1–5.0)	2.1	UP
Dbp	2.3 (1.0–5.4)	2.2	UP
Adh1	2.4 (1.0–5.5)	3.0	DOWN
Ecm1	2.5 (0.7–1,117.8)	1.6	DOWN
Rgs2	2.6 (0.6–519.9)	1.5	UP
Pecam1	3.5 (1.0–379.9)	1.5	DOWN

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; NC = nonconvergent.
[a]Gene definitions are available in Appendix E and in the Chemical Effects in Biological Systems (CEBS) data repository: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Theoretical Inhaled Dose

To provide information relevant for route-to-route extrapolation, to allow for comparison between two dichlorobenzene isomers, and to normalize data for comparison of internal dose between species, a theoretical inhaled mg/kg/day dose was calculated for each species and exposure group. Information relevant to the calculations is provided below, with the calculated theoretical inhaled doses shown in Table 17.

- Estimates of minute volume (Mv) were based on EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*[31] as

well as *Recommendations for and Documentation of Biological Values for Use in Risk Assessment*.[32]

- Study day 0 body weights (BW) were used in the calculations due to the short duration of the study and minimal effects on body weight.
- Target exposure concentrations, rather than actual exposure concentrations, were used for the calculations due to the high degree of congruency.

The inhaled dose over a 6-hour exposure period was calculated as:

Dose (mg/kg/day) = C [(mg/m^3)/1,000)] × Mv (L/min) × 360 min/day exposure ÷ BW (kg)

where

C (mg/m^3) = 0.0409 × C (ppm) × molecular weight of test article

Mv (L/min) is estimated as LnMv = b0 + b1 × Ln(BW) where b0 and b1 are −0.578 and 0.821 for rats and 0.326 and 1.050 for mice, respectively.[31; 32]

Table 17. Theoretical Inhaled Daily Dose (mg/kg/day) of 1,2-Dichlorobenzene Following Six-hour Whole-Body Inhalation Exposure

	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm
Rat	0	1.62	16.11	48.42	162.13	403.23	808.75
Mouse	0	2.48	24.74	74.20	247.18	618.16	NA

Within each exposure group and species, calculations were performed for the core and internal concentration assessment animals separately, using group mean body weight data. The data presented here and used for internal dose assessment normalization (mg/kg/day) are a mean of the values calculated by the core and internal concentration assessment animals.
NA = not applicable.

Internal Concentration Assessment

1,2-DCB concentrations were quantified in blood, lung, and liver on study day 4 (following the last exposure) and study day 5 (approximately 18 hours following the last exposure) using a validated analytical method (Appendix B). Data are reported before and after normalizing to the exposure concentration (ppm) and to the estimated theoretical inhaled dose (mg/kg/day).

Rats

On study day 4, blood 1,2-DCB concentration increased with increasing exposure concentration (Table 18). The increase was proportional to the exposure concentration up to 100 ppm as evidenced by the dose-normalized values (4.46–6.86 [ng/mL]/[mg/kg/day]). At 250 and 500 ppm, blood concentrations increased more than proportionally to the exposure concentration with normalized values of 48.4 and 122 (ng/mL)/(mg/kg/day), respectively, demonstrating saturation of metabolism and/or clearance processes.

On study day 4, 1,2-DCB liver and lung concentrations, in general, were similar to blood (except for 250 ppm lung where concentration was lower than blood and 250 and 500 ppm liver where concentration was higher than blood), suggesting low tissue distribution and/or retention. As observed with blood, tissue concentrations, in general, increased proportionally to exposure

concentration up to 100 ppm as evidenced by the normalized values (ng/g)/(mg/kg/day) (liver, 4.82–9.54; lung, 3.91–11.2). At 250 and 500 ppm, tissue concentrations increased more than proportionally to the exposure concentration with normalized values (ng/g)/(mg/kg/day), respectively, of 89.4 and 264 for the liver and 18.4 and 118 for the lung.

On study day 5, blood, liver, and lung concentrations were much lower than on study day 4 with concentrations falling below the limit of detection (LOD) at 1 ppm (all matrices) and 10 ppm (lung only) demonstrating rapid elimination of 1,2-DCB. As observed for study day 4, concentrations in general were similar between blood and tissues.

Mice

On study day 4, blood 1,2-DCB concentration increased with increasing exposure concentration (Table 19). The increase was proportional to the exposure concentration up to 100 ppm as evidenced by the dose-normalized values (3.41–4.47 [ng/mL]/[mg/kg/day]). At 250 ppm, blood concentrations increased more than proportionally to the exposure concentration with a normalized value of 90.3 (ng/mL)/(mg/kg/day).

On study day 4, 1,2-DCB liver and lung concentrations were similar to blood, suggesting low tissue distribution and/or retention. As observed with the blood, lung and liver concentrations, in general, increased proportionally to exposure concentration up to 100 ppm as evidenced by the normalized values (ng/g)/(mg/kg/day) (liver, 3.63–5.21; lung, 1.57–5.23). At 250 ppm, tissue concentrations increased more than proportionally to the exposure concentration, with dose-normalized values of 150 and 93.3 (ng/g)/(mg/kg/day) for liver and lung, respectively.

On study day 5, blood, liver, and lung concentrations were much lower than on study day 4 with concentrations falling below the LOD at 1 ppm and 10 ppm in all matrices demonstrating rapid elimination of 1,2-DCB. As observed for study day 4, concentrations in general were similar between blood and tissues.

In general, when normalized to the theoretical inhaled dose, there were no apparent species differences in blood and tissue concentrations of 1,2-DCB at study days 4 or 5.

Table 18. Summary of Blood, Lung, and Liver Concentration Data for Female Rats Exposed to 1,2-Dichlorobenzene for Five Days

Endpoint[a,b]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm
Study Day 4							
n	3	3	3	3	3	3	3
Blood							
Blood concentration (ng/mL)	BD[c]	11.1 ± 0.742	80.6 ± 11.9	216 ± 9.07	816 ± 18.4	19,500 ± 700	98,300 ± 16,700
Normalized blood concentration [(ng/mL)/(mg/kg/day)][d,e]	–[f]	6.86 ± 0.458	5.01 ± 0.740	4.46 ± 0.187	5.03 ± 0.113	48.4 ± 1.74	122 ± 20.6
Normalized blood concentration [(ng/mL)/ppm][e]	–	11.1 ± 0.742	8.06 ± 1.19	7.20 ± 0.302	8.16 ± 0.184	78.0 ± 2.80	197 ± 33.3
Liver							
Liver concentration (ng/g)	BD	15.4 ± 1.82	77.7 ± 18.3	237 ± 17.5	881 ± 57.0	36,000 ± 4,720	213,000 ± 45,200
Normalized liver concentration [(ng/g)/(mg/kg/day)][d,e]	–	9.54 ± 1.12	4.82 ± 1.14	4.89 ± 0.361	5.43 ± 0.352	89.4 ± 11.7	264 ± 55.9
Normalized liver concentration [(ng/g)/ppm][e]	–	15.4 ± 1.82	7.77 ± 1.83	7.90 ± 0.582	8.81 ± 0.570	144 ± 18.9	427 ± 90.5
Lung							
Lung concentration (ng/g)	BD	18.1 ± 4.45	63.0 ± 20.0	543 ± 187	1,120 ± 306	7,410 ± 1,770	95,400 ± 54,300
Normalized lung concentration [(ng/g)/(mg/kg/day)][d,e]	–	11.2 ± 2.75	3.91 ± 1.24	11.2 ± 3.87	6.92 ± 1.89	18.4 ± 4.39	118 ± 67.1
Normalized lung concentration [(ng/g)/ppm][e]	–	18.1 ± 4.45	6.30 ± 2.00	18.1 ± 6.24	11.2 ± 3.06	29.6 ± 7.08	191 ± 109
Study Day 5							
n	3	3	3	3	3	3	3
Blood							
Blood concentration (ng/mL)	BD	BD	0.491 ± 0.104	1.25 ± 0.379	6.80 ± 0.586	41.1 ± 4.05	434 ± 155
Normalized blood concentration [(ng/mL)/(mg/kg/day)]	–	BD	0.0305 ± 0.00647	0.0258 ± 0.00782	0.0419 ± 0.00361	0.102 ± 0.0101	0.537 ± 0.192
Normalized blood concentration [(ng/mL)/ppm]	–	BD	0.0491 ± 0.0104	0.0416 ± 0.0126	0.0680 ± 0.00586	0.164 ± 0.0162	0.868 ± 0.310

Endpoint[a,b]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm	500 ppm
Liver							
Liver concentration (ng/g)	BD	BD	1.10 ± 0.519	1.65 ± 0.124	6.92 ± 0.698	24.3 ± 2.11	156 ± 65.8
Normalized liver concentration [(ng/g)/(mg/kg/day)]	–	BD	0.0680 ± 0.0322	0.0340 ± 0.00257	0.0427 ± 0.00431	0.0603 ± 0.00523	0.193 ± 0.0813
Normalized liver concentration [(ng/g)/ppm]	–	BD	0.110 ± 0.0519	0.0549 ± 0.00415	0.0692 ± 0.00698	0.0972 ± 0.00843	0.313 ± 0.132
Lung							
Lung concentration (ng/g)	BD	BD	BD	1.32 ± 0.692	6.57 ± 2.24	24.2 ± 7.02	231 ± 114
Normalized lung concentration [(ng/g)/(mg/kg/day)]	–	BD	BD	0.0272 ± 0.0143	0.0405 ± 0.0138	0.0599 ± 0.0174	0.286 ± 0.141
Normalized lung concentration [(ng/g)/ppm]	–	BD	BD	0.0439 ± 0.0231	0.0657 ± 0.0224	0.0967 ± 0.0281	0.462 ± 0.227

BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data are presented as mean ± standard error.
[b]If over 20% of the animals in a group were above the LOD, one-half of the LOD was substituted for values below the LOD. LOD for blood = 0.157 ng/mL; LOD for liver = 0.471 ng/g; LOD for lung = 0.471 ng/g.
[c]When the 0 ppm group did not have over 20% of its values above the LOD, no mean or standard error was calculated, and no statistical analysis was performed.
[d]Theoretical inhaled doses estimated for the 1, 10, 30, 100, 250, and 500 ppm groups are 1.62, 16.11, 48.42, 162.13, 403.23, and 808.75 mg/kg/day, respectively.
[e]Normalized concentrations were calculated by dividing the measured concentration in either ng/mL for blood or ng/g for tissues by either the theoretical inhaled dose (mg/kg/day) or nominal exposure concentration (ppm). No statistical analysis was performed on normalized endpoints.
[f]Not applicable.

Table 19. Summary of Blood, Lung, and Liver Concentration Data for Female Mice Exposed to 1,2-Dichlorobenzene for Five Days

Endpoint[a,b]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm
Study Day 4						
n	3	2	3	3	3	3
Blood						
Blood concentration (ng/mL)	BD[c]	8.99 ± 1.62	111 ± 10.8	320 ± 44.2	842 ± 108	55,800 ± 20,700
Normalized blood concentration [(ng/mL)/(mg/kg/day)][d,e]	–[f]	3.63 ± 0.652	4.47 ± 0.436	4.31 ± 0.596	3.41 ± 0.438	90.3 ± 33.5
Normalized blood concentration [(ng/mL)/ppm][e]	–	8.99 ± 1.62	11.1 ± 1.08	10.7 ± 1.47	8.42 ± 1.08	223 ± 82.7
Liver						
Liver concentration (ng/g)	BD	12.9 ± 0.80	89.8 ± 11.4	347 ± 78.0	1,220 ± 181	92,900 ± 13,600
Normalized liver concentration [(ng/g)/(mg/kg/day)][d,e]	–	5.21 ± 0.323	3.63 ± 0.461	4.68 ± 1.05	4.93 ± 0.733	150 ± 21.9
Normalized liver concentration [(ng/g)/ppm][e]	–	12.9 ± 0.80	8.98 ± 1.14	11.6 ± 2.60	12.2 ± 1.81	372 ± 54.2
Lung						
Lung concentration (ng/g)	BD	7.86 ± 0.12	129 ± 41.3	194 ± 70.3	388 ± 73.7	57,700 ± 9,400
Normalized lung concentration [(ng/g)/(mg/kg/day)][d,e]	–	3.18 ± 0.0485	5.23 ± 1.67	2.62 ± 0.948	1.57 ± 0.298	93.3 ± 15.2
Normalized lung concentration [(ng/g)/ppm][e]	–	7.86 ± 0.12	12.9 ± 4.13	6.48 ± 2.34	3.88 ± 0.737	231 ± 37.6
Study Day 5						
n	3	3	3	3[g]	3	3[h]
Blood						
Blood concentration (ng/mL)	BD	BD	BD	2.78 ± 2.71	1.00 ± 0.875	5.97 ± 2.12
Normalized blood concentration [(ng/mL)/(mg/kg/day)]	–	BD	BD	0.0375 ± 0.0365	0.00405 ± 0.00354	0.00966 ± 0.00343
Normalized blood concentration [(ng/mL)/ppm]	–	BD	BD	0.0928 ± 0.0902	0.0100 ± 0.00875	0.0239 ± 0.00848

Endpoint[a,b]	0 ppm	1 ppm	10 ppm	30 ppm	100 ppm	250 ppm
Liver						
Liver concentration (ng/g)	BD	BD	BD	0.749 ± 0.351	1.99 ± 0.313	30.4 ± 8.45
Normalized liver concentration [(ng/g)/(mg/kg/day)]	–	BD	BD	0.0101 ± 0.00473	0.00804 ± 0.00127	0.0491 ± 0.0137
Normalized liver concentration [(ng/g)/ppm]	–	BD	BD	0.0250 ± 0.0117	0.0199 ± 0.00313	0.121 ± 0.0338
Lung						
Lung concentration (ng/g)	BD	BD	BD	0.497 ± 0.133	1.16 ± 0.326	7.50 ± 1.68
Normalized lung concentration [(ng/g)/(mg/kg/day)]	–	BD	BD	0.00669 ± 0.00179	0.00471 ± 0.00132	0.0121 ± 0.00271
Normalized lung concentration [(ng/g)/ppm]	–	BD	BD	0.0166 ± 0.00443	0.0116 ± 0.00326	0.0300 ± 0.0067

BD = below detection; group did not have more than 20% of its values above the limit of detection (LOD).
[a]Data are presented as mean ± standard error.
[b]If over 20% of the animals in a group were above the LOD, one-half of the LOD was substituted for values below the LOD. LOD for blood = 0.157 ng/mL; LOD for liver = 0.471 ng/g; LOD for lung = 0.471 ng/g.
[c]When the 0 ppm group did not have over 20% of its values above the LOD, no mean or standard error was calculated, and no statistical analysis was performed.
[d]Theoretical inhaled doses estimated for the 1, 10, 30, 100, and 250 ppm groups are 2.48, 24.74, 74.20, 247.18, and 618.16 mg/kg/day, respectively.
[e]Normalized concentrations were calculated by dividing the measured concentration in either ng/mL for blood or ng/g for tissues by either the theoretical inhaled dose (mg/kg/day) or nominal exposure concentration (ppm). No statistical analysis was performed on normalized endpoints.
[f]Not applicable.
[g]n = 2 for blood. No samples were received for one animal.
[h]n = 2 for liver and lung. No samples were received for one animal.

Summary

1,2-Dichlorobenzene (1,2-DCB) is a chlorinated aromatic hydrocarbon that is widely used in industrial applications, including as a solvent for waxes, resins, and paints, and it serves as an ingredient in the production of disinfectants and deodorants. These studies used a transcriptomic approach and standard toxicological endpoints to estimate the in vivo biological potency of 1,2-DCB. The data from these studies are intended to support risk assessment and establishment of acceptable exposure levels of 1,2-DCB in environmental and occupational settings.

In rats exposed to 1,2-DCB, the most sensitive apical endpoint was an increase in relative liver weight with a calculated benchmark dose (benchmark dose lower confidence limit)—BMD (BMD_L)—of 57.9 (29.9) ppm. Increases in relative right and left kidney weights were the next most sensitive apical endpoint changes observed with BMDs (BMD_Ls) of 93.1 (43.2) and 101.4 (40.9) ppm, respectively. In mice exposed to 1,2-DCB, the most sensitive apical endpoint was a decrease in alkaline phosphatase activity with a BMD (BMD_L) of 33.4 (10.9) ppm. The next most sensitive apical endpoints observed were increases in absolute and relative liver weights with BMDs (BMD_Ls) of 57.4 (36.4) and 60.5 (37.7) ppm, respectively.

In the heart, transcriptional changes at the gene set level were calculated to occur at a BMD (BMD_L) as low as 148.7 (58.2) ppm in rats, corresponding to cellular response to hydrogen peroxide (GO:0070301), and as low as 4.4 (1.7) ppm in mice, corresponding to cellular modified amino acid metabolic process (GO:0006575). The most sensitive heart genes for which a reliable BMD could be determined were *Prc1,* with a BMD (BMD_L) of 3.0 (0.7) ppm, in rats and *Rsad2*, with a BMD (BMD_L) of 1.3 (0.5) ppm, in mice.

In the kidney, transcriptional changes at the gene set level were calculated to occur at a BMD (BMD_L) as low as 114.0 (73.9) ppm in rats, corresponding to embryonic organ development (GO:0048568), and as low as 2.3 (0.6) ppm in mice, corresponding to organic hydroxy compound transport (GO:0015850). The most sensitive kidney gene in rats for which a reliable BMD could be determined was *Slc51a* with a BMD (BMD_L) of 36.3 (8.9) ppm. The most sensitive kidney gene in mice for which a reliable BMD could be determined was *Cdk2* with a BMD (BMD_L) of 0.9 (0.2) ppm.

In the liver, transcriptional changes at the gene set level were calculated to occur at a BMD (BMD_L) as low as 29.8 (25.3) ppm in rats, corresponding to xenobiotic metabolic process (GO:0006805), and as low as 1.5 (0.5) ppm in mice, corresponding to positive regulation of lipid biosynthetic process (GO:0046889). The most sensitive liver genes for which a reliable BMD could be determined were *Sult1c3* in rats and *Dbp* in mice with BMDs (BMD_Ls) of 16.4 (3.3) and 0.7 (0.2) ppm, respectively.

In the lung, transcriptional changes at the gene set level were calculated to occur at a BMD (BMD_L) as low as 175.9 (109.3) ppm in rats, corresponding to detoxification (GO:0098754), cellular oxidant detoxification (GO:0098869), and cellular detoxification (GO:1990748), and as low as 2.1 (0.7) ppm in mice, corresponding to unsaturated fatty acid metabolic process (GO:0033559). One lung gene in mice had a BMD estimate below the lower limit of extrapolation (<0.333 ppm). The most sensitive lung genes for which a reliable BMD could be determined were *Akr1b8*/*Akr1b15*, with a BMD (BMD_L) of 118.9 (36.3) ppm, in rats and *Il4* and *Ces1g* in mice, both with a BMD (BMD_L) of 0.6 (0.2) ppm.

In the ovary, transcriptional changes at the gene set level were calculated to occur at a BMD (BMD_L) as low as 156.7 (50.8) ppm in rats, corresponding to kidney development (GO:0001822), gonad development (GO:0008406), and male gonad development (GO:0008584), and as low as 108.0 (35.9) ppm in mice, corresponding to hemopoiesis (GO:0030097). The most sensitive ovary genes for which a reliable BMD could be determined were *Ddit4* in rats and *Syn1* in mice with BMDs (BMD_Ls) of 102.9 (27.9) and 0.6 (0.2) ppm, respectively.

Under the conditions of this short-term transcriptomic study in female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6D2F1/Crl mice, the most sensitive point of departure with a reliable estimate in rats was a transcriptional change in a heart gene, *Prc1*, with a BMD (BMD_L) of 3.0 (0.7) ppm. Transcriptional changes at the gene set level provided potency estimates slightly higher than *Prc1* and apical endpoints provided potency estimates higher than *Prc1*. In mice, the most sensitive point of departure with a reliable estimate was a transcriptional change in two lung genes, *Il4* and *Ces1g*, and an ovary gene, *Syn1*, all three with a BMD (BMD_L) of 0.6 (0.2) ppm. Transcriptional changes at the gene set level provided potency estimates slightly higher than *Il4*, *Ces1g*, and *Syn1* and apical endpoints provided potency estimates higher than *Il4*, *Ces1g*, and *Syn1*.

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Appendix A. Chemical Characterization and Generation of Chamber Concentrations

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A.1. Procurement and Characterization of 1,2-Dichlorobenzene

1,2-dichlorobenzene (1,2-DCB) was obtained from Oakwood Products, Inc. (Estill, SC) in a single lot (216310R29M). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH). Reports on analyses performed in support of the 1,2-DCB studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The identity and purity of lot 216310R29M, a clear, colorless liquid at room temperature, was evaluated using gas chromatography (GC) with mass spectrometry (MS) detection (Table A-1, System A). The MS spectrum (Figure A-1) was consistent with the National Institute of Standards and Technology library spectrum for 1,2-DCB (Figure A-2) and a certified reference material of 1,2-DCB (lot LRAC7973, Sigma Aldrich), and no impurity peaks were present at ≥0.1%. The overall purity of the test article was estimated at approximately 100%.

Bulk 1,2-DCB was stored in the original shipping container at room temperature. Reanalysis of the bulk chemical was performed by the analytical chemistry laboratory within 30 days of study termination and no degradation was detected (Table A-1, System A).

A.2. Vapor Generation and Exposure System

A diagram of the generation and distribution system is shown in Figure A-3. 1,2-DCB was pumped from a stainless-steel reservoir and fed into a heated (approximately 250°F) glass vaporizer column containing 6 mm glass beads and wrapped around the full length by heat tape. A waste collection flask was connected to the bottom of the column for collection of residual 1,2-DCB not completely vaporized within the vaporizer column.

Preheated (approximately 250°F) nitrogen entered the column from below, vaporized 1,2-DCB, and carried the vapor from the generator cabinet located in the control room to the distribution manifold located in the exposure room through a heated (approximately 200°F) Teflon® transport line. The mixture of nitrogen and 1,2-DCB was diluted with heated (approximately 175°F) air before it entered the distribution manifold. The pressure in the distribution manifold was kept fixed, which ensured constant flow through the manifold and into all chambers as the flow of vapor to each chamber was adjusted. Concentration in the manifold was determined by the chemical pump rate, dilution air flow rate, nitrogen flow rate, and special modifications to the distribution manifold. Specifically, to achieve the 1 ppm exposure concentration, siphon/heated dilution lines were used to reduce the main manifold concentration approximately 30-fold before delivering the vapor to the 1 ppm chamber. Additionally, to ensure appropriate build up and decay times, heated entrainment air was added to the 10 and 30 ppm delivery lines.

Individual heated (approximately 200°F) Teflon delivery lines carried the vapor from the exposure valves in the distribution cabinet to the chamber inlets. The exposure valves diverted vapor delivery to the manifold exhaust until the generation system was stable, and exposures were ready to proceed. 1,2-DCB vapor delivery rates to each chamber were controlled by precision metering valves at the manifold. When the exposure started, the exposure valves actuated and directed the vapor into the chamber inlet where it was diluted with conditioned air to achieve the desired exposure concentration.

Conditioned air was defined as the mix of air derived from each exposure chamber's wet and dry air duct supplies. The temperature of the resultant mixture of air was adjusted by passage over a temperature-controlled radiator after sequential treatment with Purafil®, charcoal, and high-efficiency particulate air (HEPA) filters. Air for the ducts was either passed over desiccant lowering the dew point (dry duct) or injected with clean steam raising the dew point (wet duct). Approximately 25% excess capacity of vapor was available for adjustments to chamber concentrations, as necessary. The excess capacity was emptied into the exhaust line from the manifold through a manually controlled adjustable flowmeter valve set to maintain constant pressure within the manifold. An alarm system automatically alerted the operator when any temperature controller detected a temperature outside of operating range. In addition, a variable power transformer limited power to each heater, thereby guarding against a temperature controller failure that could lead to excessive heating.

The exposure system consisted of seven exposure chambers with target test article concentrations of 0 (control group), 1, 10, 30, 100, 250, and 500 ppm. The inhalation exposure chamber (Lab Products, Inc., Seaford, DE) was designed so that uniform vapor concentrations could be maintained throughout the chamber with catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (Model 3022A; TSI, Inc., Shoreview, MN) was used in the exposure chambers, both with and without animals, to ensure vapor (not aerosol) was produced. Particle counts of fewer than 200 particles/cm^3 are typical of an exposure atmosphere when no generation is occurring. Particle counts above this level, especially if the counts increase with exposure concentration and are above the level during the off-exposure period, suggest a contribution to the aerosol concentration from the generation system. No particle counts above the minimum resolvable level were detected.

A.3. Vapor Concentration Monitoring

Exposure chamber and room concentrations of 1,2-DCB were monitored using an online gas chromatograph (GC) equipped with a flame ionization detector (FID) (Table A-1, System B). All chambers were sampled at approximately twice per hour during exposure through Teflon tubing connected to each exposure chamber's relative-humidity sampling lines at a location close to the GC/FID. The samples flowed into a 16-port Hastelloy®-C stream-select valve that directed a continuous stream of sampled atmosphere to a 6-port Hastelloy-C gas-sampling valve with a 1 mL sample loop. Valves were mounted in a dedicated valve oven located on top of the GC oven. The valve oven temperature was maintained at approximately 175°C. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and GC allowed for digital measurement of sample flow (approximately 1 L/min).

The online GCs were checked for suitability before the start of each exposure day and after every ninth sample throughout the exposure period using a standard 1,2-DCB vapor supplied by a standard generator (KIN-TEK Analytical, Inc., La Marque, TX). The online GC/FID at the testing facility was calibrated by quantitative determination of 1,2-DCB in exposure chamber samples on sorbent tubes (ORBO-101 and ORBO-100; graphitized carbon black; Supelco; Bellefonte, PA) and analyzed using an offline GC/FID at the analytical chemistry laboratory (Table A-1, System C). Prior to the study, stability of 1,2-DCB on sorbent tubes was confirmed for up to 7 days when stored at ambient (extracted) or refrigerated (either extracted or

unextracted) temperatures. Known volumes of atmosphere from each inhalation exposure chamber were collected using a calibrated critical-orifice controlled sampler at a constant flow rate. The sorbent samples were extracted with acetone containing propylbenzene internal standard (IS) and transferred to autosampler vials for analysis on the offline GC/FID. Online GC/FID calibration was established by correlating the chamber concentrations determined from analysis of the sorbent gas-sampling tubes against the online monitor peak area determined at the time of sampling.

Summaries of the chamber vapor concentrations are given in Table A-2. The mean 1,2-DCB concentrations were within 3% of target values for all rat chambers and within 2% of target values for all mouse chambers. The concentrations were stable, with concentration relative standard deviation (RSD) within 4% for all the chambers throughout the studies. The mean 1,2-DCB concentrations in the 0 ppm chamber and the exposure room were below the limit of detection (0.04 ppm) for both studies.

A.4. Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined prior to (without animals) and during (with animals) the studies. The time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) were estimated from the concentration versus time curves.

The theoretical prediction of T_{90} is based on a 1.7 m^3 chamber mixing volume and a 15 ft^3/min flow rate is approximately 9.2 minutes. Estimated T_{90} ranged from 8 to 10 minutes for all chambers without animals. A value of 12 minutes was used for the study. Estimated T_{10} ranged from 10 to 13 minutes without animals. Buildup and decay of vapor concentrations were measured for the lowest (1 ppm) and highest (500 ppm for rat; 250 ppm for mouse) exposure concentration chambers during the studies. For the rat study, T_{90} ranged from 8 to 11 minutes and T_{10} ranged from 11 to 12 minutes. For the mouse study, T_{90} ranged from 8 to 11 minutes and T_{10} was 13 minutes for both chambers. A T_{90} value of 12 minutes was used for the studies.

Prior to the studies, the persistence of 1,2-DCB was monitored in the 250 and 500 ppm chambers after exposure without animals present. Although T_{10} values were acceptable, the concentration of 1,2-DCB never reached 0 ppm in the ≥10 ppm chambers and required ≥144 minutes to reach 1% of the starting concentration (T_1) without animals present. During the studies and with animals present, T_1 was 79 (500 ppm rat chamber) and 192 minutes (250 ppm mouse chamber). The reason for the prolonged T_1 values is unknown, but the prolonged T_1 values were considered to have no effect on study findings given the low levels compared to the target chamber concentrations.

Concentration uniformity was evaluated in all exposure chambers without animals present. Vapor was sampled from 12 chamber positions, one in the front and one in the back, for each of the six possible animal cage positions per chamber and measured using the online monitor (Table A-1, System B). Uniformity measurements were all within the acceptable criterion of <5% of the RSD. Uniformity measurements were made during the studies from the lowest (1 ppm) and highest (500 ppm for rat; 250 ppm for mouse) exposure concentration chambers. All measurements met acceptable criteria and were comparable to the results prior to study start.

To measure stability and purity of 1,2-DCB in the generation and delivery system, samples of the test atmosphere were collected from the distribution line, generator reservoir, and the highest, lowest, and control exposure concentration chambers for both studies at the beginning and end of the exposure day and extracted with methanol. In addition, analysis was performed on a second set of samples collected from the same locations and exposure times and extracted in a second solvent (acetone) to demonstrate that any impurities in 1,2-DCB (if present) were not obscured by the primary solvent (methanol). Exposure atmosphere samples were collected on ORBO 101 (500 ppm) and ORBO 100 (1 ppm and control) CarboTrap B sorbent tubes. All sample collections included an SKC, Inc. (Eighty Four, PA) silica gel sorbent tube arranged in series with the ORBO primary tube. Samples were shipped on ice to the analytical chemistry laboratory for analysis (Table A-1, System C). No impurity peaks were present in any samples. Peaks were not observed in the silica gel samples, demonstrating 100% capture of the inhalation exposure atmosphere onto the ORBO 101 and ORBO 100 sorbent media. Stability and purity of 1,2-DCB was maintained throughout the exposure system.

To demonstrate acceptable stability and composition of the test article in the inhalation exposure system while animals were present in the exposure chambers, samples for stability analysis were collected by the testing facility from the distribution line, the highest and the lowest exposure concentration chambers, and the vapor generator reservoir during the rat study. Additionally, the exposure system reservoir was sampled during the mouse study. The purity of 1,2-DCB in the exposure system with animals present in the exposure chambers was approximately 100%. Impurities with peak areas >0.1% of the total peak area were not present.

Table A-1. Gas Chromatography Systems Used in the Five-day Inhalation Studies of 1,2-Dichlorobenzene

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry with electron impact ionization	Restek Rtx-1 (30 m × 0.25 mm ID, 1.0 µm film thickness)	Helium at 1 mL/min	80°C for 2 minutes, then 10°C/min to 250°C, held for 15 minutes
System B			
Flame ionization (250°C)	Restek Rtx-5 (15 m × 0.53 mm ID, 1.5 µm film thickness)	Nitrogen at 7.9 psi	Isothermal at 110°C
System C			
Flame ionization (250°C)	Agilent DB-5a (30 m × 0.32 mm ID, 1.5 µm film thickness)	Helium at 1 mL/min	80°C for 2 minutes, then 5°C/min to 150°C, then 20°C/min to 250°C, held for 4 minutes

ID = internal diameter.

Table A-2. Summary of Chamber Concentrations in the Five-day Inhalation Study of 1,2-Dichlorobenzene

Exposure Date	Target Concentration (ppm)	Total Number of Readings	Determined Concentration (ppm)[a]	Percent of Target ± RSD	Acceptable Samples (%)[b]
Rat Chambers					
December 6–10, 2024	0 (Room)	83	ND	NA	98
	0	82	ND	NA	99
	1	120	0.974 ± 0.034	97 ± 4	96
	10	78	9.82 ± 0.28	98 ± 3	100
	30	79	29.5 ± 0.4	98 ± 1	100
	100	79	99.9 ± 3.0	100 ± 3	99
	250	79	243 ± 5	97 ± 2	100
	500	117	497 ± 8	99 ± 2	100
Mouse Chambers					
December 15–19, 2024	0 (Room)	78	ND	NA	97
	0	77	ND	NA	100
	1	134	0.988 ± 0.040	99 ± 4	96
	10	77	9.82 ± 0.18	98 ± 2	100
	30	77	29.7 ± 0.5	99 ± 2	100
	100	78	99.9 ± 1.9	100 ± 2	100
	250	122	248 ± 7	99 ± 3	98

RSD = relative standard deviation; ND = not detectable; NA = not applicable.
[a]Data shown as mean of readings ± standard deviation.
[b]Acceptable range: Target concentration ± 10%; except for room and 0 ppm chamber: <limit of detection (0.04 ppm).

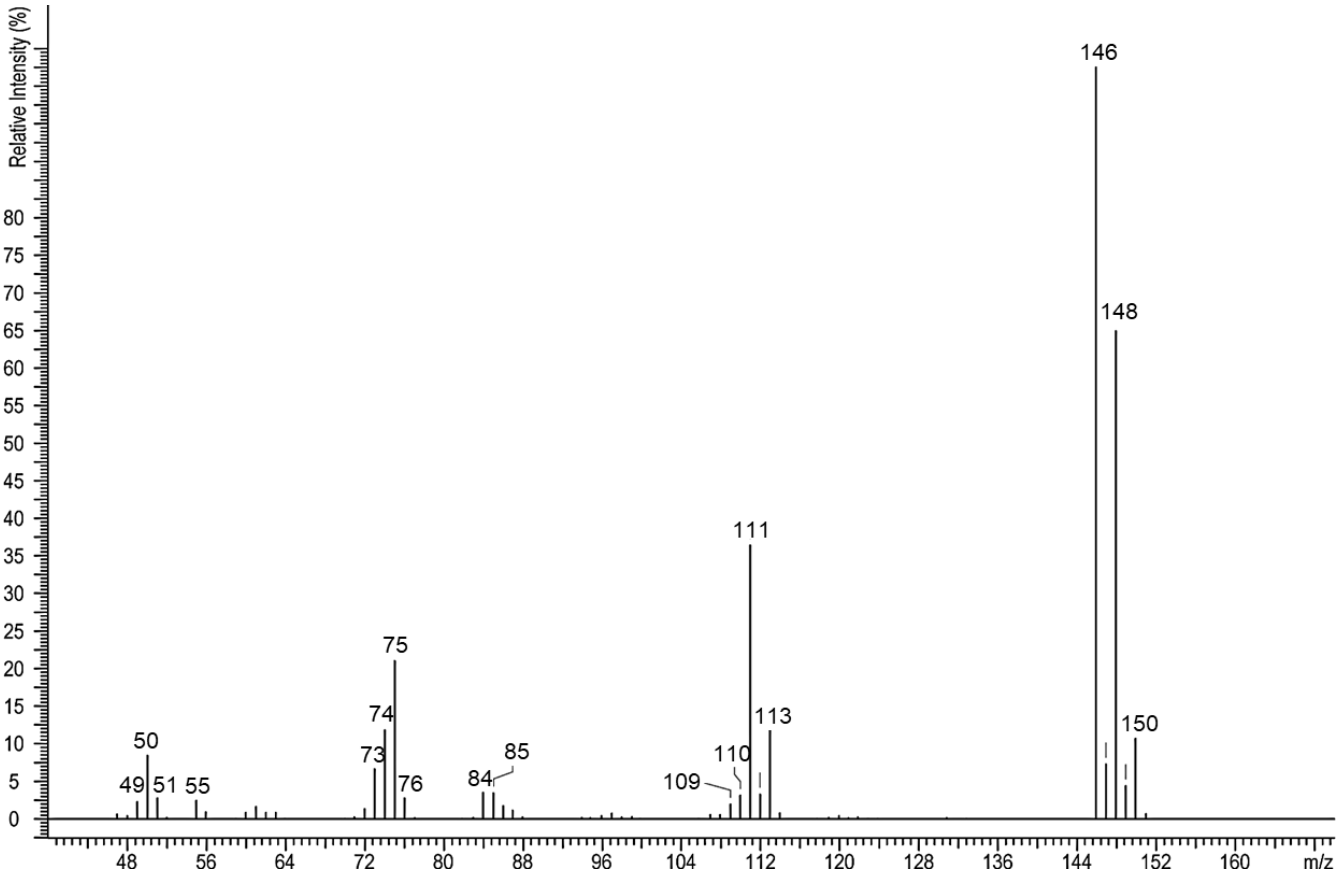

Figure A-1. Mass Spectrum of 1,2-Dichlorobenzene

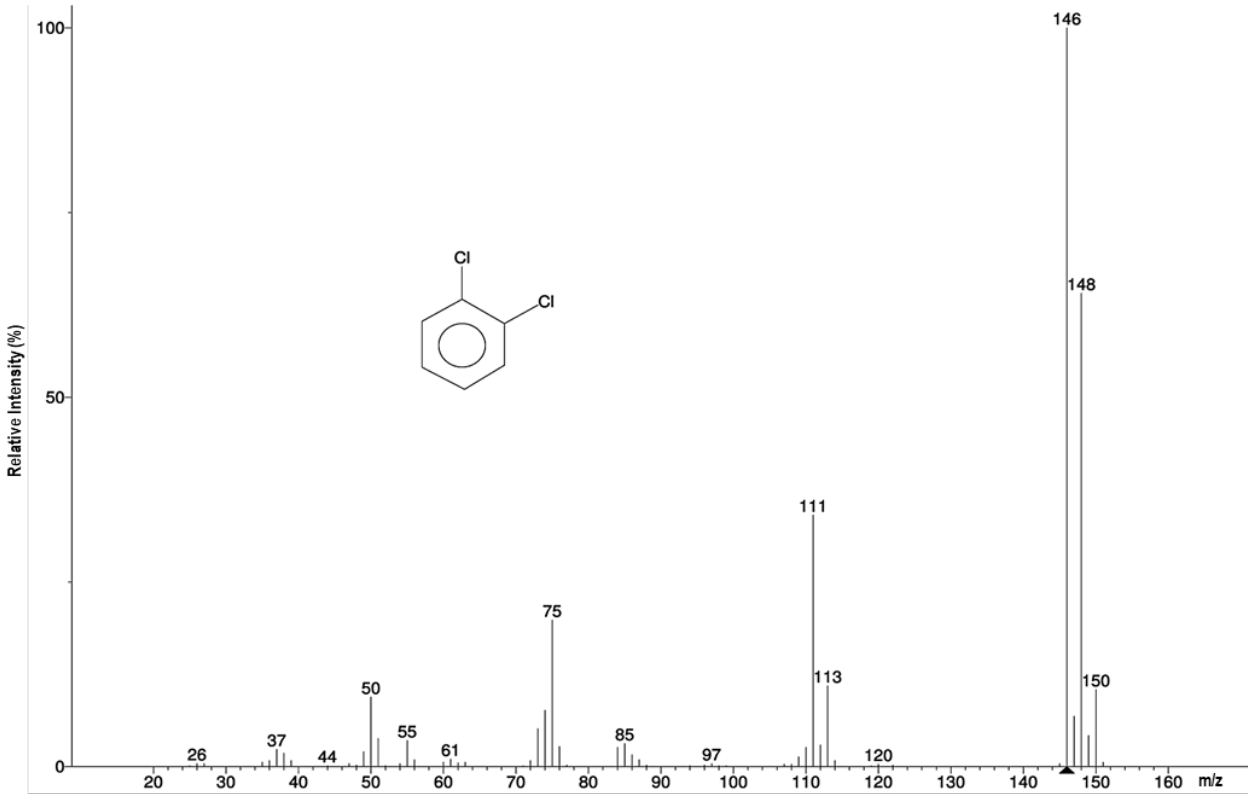

Figure A-2. Library Reference Spectrum of 1,2-Dichlorobenzene

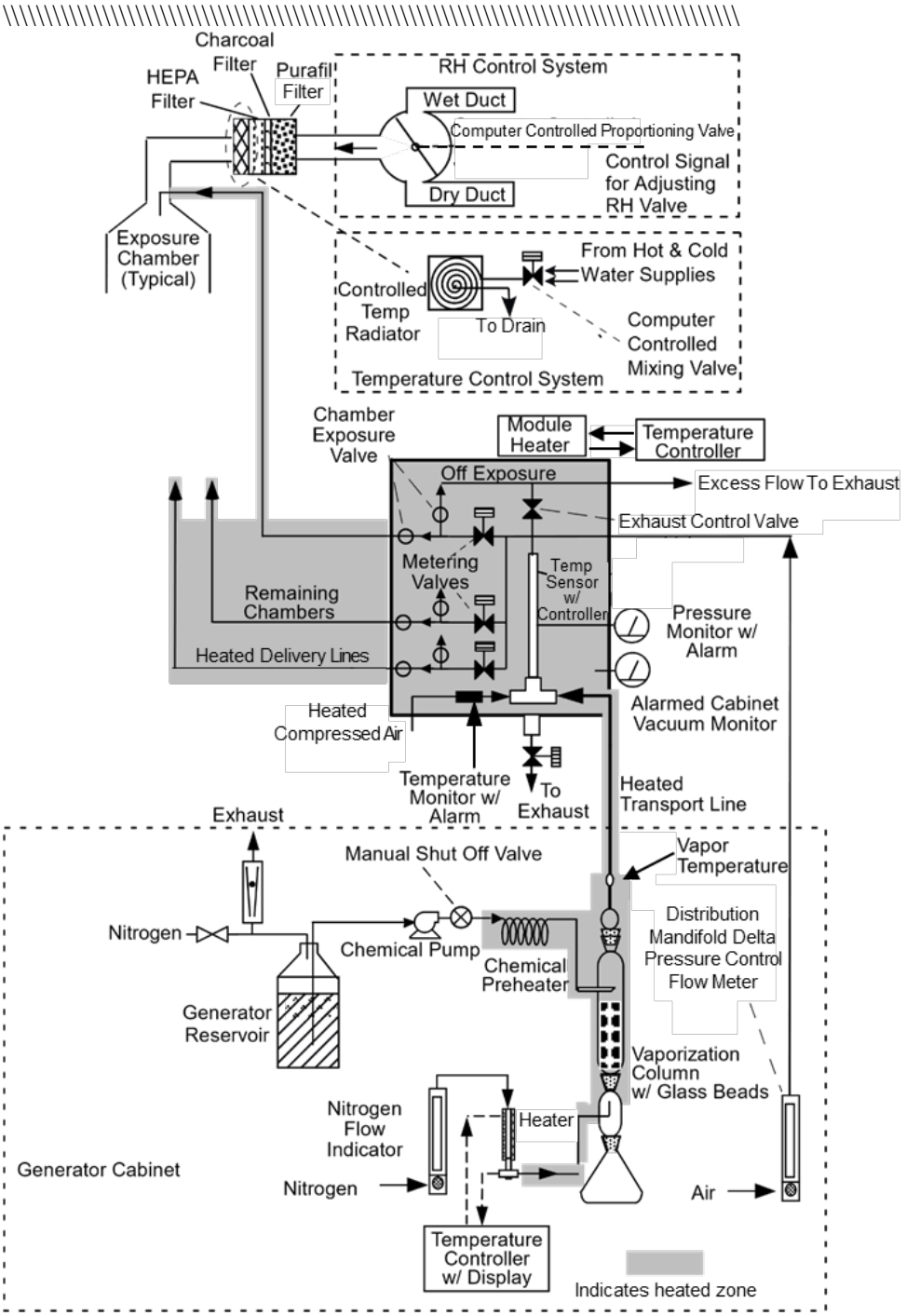

Figure A-3. Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of 1,2-Dichlorobenzene

Appendix B. Internal Concentration Assessment

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Tables

B.1. Validation of Analytical Method

A method utilizing headspace solid-phase microextraction-gas chromatography mass spectrometry (HS-SPME-GC/MS; Table B-1) was adapted from the literature[33; 34] and validated for simultaneous quantitation of 1,2-dichlorobenzene (1,2-DCB) and 1,4-dichlorobenzene (1,4-DCB) in blood, liver, and lung.

Experiments were designed to demonstrate the linearity, sensitivity, selectivity, intra- and interday accuracy and precision, and reproducibility. Accuracy and precision were evaluated as percent relative error (% RE) and percent relative standard deviation (% RSD), respectively. The lower limit of quantitation (LLOQ) was the lowest calibration standard that could be accurately quantitated for six replicates within 20% RE and reproducible within 20% RSD. The limit of detection (LOD) was defined as 3 times the standard deviation of the LLOQ response, expressed as concentration. The ability to quantitate analyte concentrations above the validated range was also evaluated by preparing samples in respective matrices with concentrations above the upper limit of the validated range and diluting into the validated range using various approaches. The validated method was used to demonstrate the performance of the method to quantify analytes in study matrices (Hsd:Sprague Dawley® SD® [HSD] rat, B6D2F1/Crl mouse and B6C3F1 mouse blood, liver, and lung). HSD rat, B6D2F1/Crl mouse, and B6C3F1 mouse blood, liver, and lung were obtained from various sources, including BioIVT (Westbury, NY), Southern Research (Birmingham, AL), Battelle (Columbus, OH), and/or AmplifyBio (West Jefferson, OH). The stability of analytes in blood and tissues during sample storage to cover the study sample storage duration and under the conditions of the sample analysis was also assessed.

The method was validated in male HSD rat blood over an analyte concentration range of 0.5 to 250 ng/mL using seven concentrations. Quality control (QC) standards were prepared at 1, 25, and 200 ng/mL. 1,2- and 1,4-DCB solvent standards containing both analytes were prepared in acetone. $^{13}C_6$ 1,2-DCB (added as a mixture of $^{13}C_6$ 1,2-DCB and $^{13}C_6$ 1,4-DCB; Cambridge Isotope Laboratories, Inc., Tewksbury, MA) was used as the internal standard (IS). The working IS solution was prepared as 250 ng/mL of $^{13}C_6$ 1,4-DCB and $^{13}C_6$ 1,2-DCB in acetone.

Blood calibration standards and QC samples were prepared by aliquoting 20 µL analyte spiking solution in acetone into 180 µL HSD rat blood. Then, 100 µL of analyte-spiked blood was transferred into a 2 mL headspace vial, and 25 µL IS solution (250 ng/mL) in acetone was added prior to immediately capping the vials. Method (with IS) and matrix blanks (without IS) were prepared similarly except analyte spiking solution was not added to method and matrix blanks. Liver and lung QC samples were prepared by homogenizing 100 mg tissue in 170 µL phosphate buffered saline (PBS) with one 5 mm stainless-steel bead in the presence of 75 µL IS (or acetone for blanks without IS) and appropriate 30 µL analyte standard spiking solution (or acetone for blanks) using the TissueLyser at 25 Hz for 60 seconds 2 to 3 times until complete homogenization was observed. The final tissue dilution was 1:3. One hundred twenty-five microliters of the processed homogenate was transferred into individual 2 mL headspace vials and immediately capped. Stability of analytes during sample analysis at ambient temperature and during sample storage in ultracold temperature was assessed by preparing QC samples as described above with IS present and comparing to freshly prepared QC samples on day 0. The final IS concentration in all calibration standards, blanks, and QC samples was 50 ng/mL. All

samples were analyzed using the HS-SPME-GC/MS method (Table B-1) and quantified as described in section B.3.

Validation data for 1,2-DCB are shown in Table B-2. Calibration curves were linear ($r^2 \geq 0.99$) over the range (0.5 to 250 ng/mL) with an intra- and interday accuracy and precision of ≤ ±8.4% RE and ≤15.0% RSD, respectively. The intra- and interday accuracy and precision for QC standards were ≤ ±14.4% RE and ≤12.8% RSD, respectively. Carryover was assessed by comparing responses from three air blanks that were run immediately following the highest calibration standard of 250 ng/mL and met the acceptance criteria of <30% of the average response of the lowest standard (0.5 ng/mL). Selectivity was assessed by analyzing six replicates of blanks from two different lots of blood, with and without IS. Interference was minimal; the responses were 0.6%–7.4% of the lowest calibration concentration response (0.5 ng/mL). With each analytical batch, two air blanks were also analyzed to assess carryover. The air blanks were empty, 2 mL screw-top vials that did not go through sample processing. There was no appreciable carryover.

Dilution verification was conducted using different dilution schemes (i.e., using blood or blood diluted with acetone with or without IS) to determine if concentrations above the validated range could be quantified after diluting into the validated range. Because the blood sample collection during the study included the addition of IS, the data for blood prepared with IS and then diluted with blood containing IS are reported (Table B-3). In blood, 1,2-DCB concentrations up to 100,000 ng/mL can be quantified with good precision (≤0.5% RSD). The RE was ≤ ±7.1% for up to 20,000 ng/mL, demonstrating that 1,2-DCB can be quantified with good accuracy up to 20,000 ng/mL after dilution. Estimated RE was higher, approximately ±34%, for concentrations >20,000 ng/mL.

The validated method was used to assess the performance in secondary (study) matrices with QC samples prepared as described above. The estimated RSD in all secondary matrices was ≤6.6% (Table B-4). Estimated RE in B6D2F1/Crl mouse blood was ≤ ±11.2%, whereas the RE in all other tissues was 23.0%–33.0%. Overall data showed that the method is acceptable for quantitation of 1,2-DCB in secondary matrices, with the caveat that there is higher variability in some matrices. Dilution verification was also conducted for rat liver QC samples prepared at 150,000 ng/g and diluted using blood after homogenization (Table B-4). The accuracy of the dilutions was 15.3% and precision was 9.4%, demonstrating that liver samples could be diluted into the validated range for analysis. Because lung and liver homogenates behaved similarly, this assessment was not conducted for lung.

Analyte stability in blood and tissues was evaluated after storing QC samples under analysis conditions for 21 days or at –70°C for 100 or 149 days and then analyzing with freshly prepared samples on day 0 (Table B-5). When samples were prepared with IS present, 1,2-DCB showed acceptable stability in blood for up to 21 days at ambient temperature and for up to 149 days when frozen. 1,2-DCB was stable in liver at –70°C for up to 100 days. Stability of 1,2-DCB in lung was not evaluated and was expected to be similar to the liver.

B.2. Study Sample Collection

Blood, lung, and liver samples were collected from the internal concentration assessment animals immediately following the last exposure on study day 4 (once chamber concentrations were at or

below the regulatory limit of 12 ppm without additional health and safety considerations). Blood, lung, and liver samples were also collected from core animals designated for internal concentration assessment on study day 5 (approximately 18 hours following the last exposure).

B.2.1. Blood

On study day 4, blood was collected from the internal concentration assessment animals via cardiac puncture for all surviving rats and mice (up to 3/exposure group) while animals were anesthetized with CO_2/O_2 (70%/30%). On study day 5, blood samples from designated core animals (3/exposure group) were taken via retro orbital plexus (rats) or retro orbital sinus (mice) while animals were anesthetized with CO_2/O_2 (70%/30%). All blood samples were collected within a 2-hour window. Blood was collected into tubes containing tripotassium ethylenediaminetetraacetic acid (K_3 EDTA) and three aliquots of 100 µL were transferred into headspace vials and kept on wet ice. To aliquoted blood, IS (25 µL of 250 ng/mL of combined $^{13}C_6$ 1,4-DCB and $^{13}C_6$ 1,2-DCB in acetone) was added and kept on wet ice until transferred to frozen storage (−85°C to −60°C). The final IS concentration in samples was 50 ng/mL.

All samples were frozen within 1 hour of collection. QC samples were also prepared at the analytical chemistry laboratory to track the impacts of storage and shipping, if any, by spiking 0.1 mL blank blood aliquots with 25 ng/mL 1,2- and 1,4-DCB. At the testing facility, these blood aliquots were spiked with IS and processed similarly to study samples.

B.2.2. Lung and Liver

After blood collection, animals were euthanized by exsanguination and lung and liver tissues were collected (following organ weight measurements for core animals designated for internal concentration assessment) from each animal within 1 hour of each other. Up to three aliquots of approximately 100 mg (rats) or 50 mg (mice) (exact weights were recorded) lung and liver tissue were collected from each animal and flash frozen. Samples were stored frozen (−85°C to −60°C) until analyzed.

B.2.3. Study Sample Preparation

One blood aliquot from each animal was directly analyzed for 1,2-DCB using the validated method using conditions given in Table B-1. Calibration standards, QC samples (1 and 100 ng/mL), matrix blanks, method blanks, and tracking QC samples prepared in HSD rat blood were run with each analytical batch. Blood samples with 1,2-DCB concentrations above the validated method were reanalyzed after diluting into the validated range using blood diluent with IS present at the same concentration as study samples such that the final IS concentration in the sample was 50 ng/mL.

Rat lung and liver study samples were homogenized as described above under section B.1. Mouse liver and lung study samples were prepared by homogenizing 50 mg of tissue with 100 µL of PBS and 37.5 µL IS as described for rat but with one 3 mm stainless-steel bead. The final IS concentration in tissues was 50 ng/mL. After homogenization, samples were centrifuged at approximately 20,800 rcf for approximately 10 minutes. One hundred twenty-five microliters of the supernatant was transferred to individual 2 mL headspace vials, immediately sealed, and analyzed by HS-SPME-GC/MS (Table B-1). Lung and liver samples with concentrations above

the validated method were reanalyzed after diluting with HSD rat blood without IS into the validated range and IS at a final concentration of 50 ng/mL was added following dilution.

B.3. Instrumentation and Quantitation

All samples were analyzed using the HS-SPME-GC/MS method (Table B-1) and quantified using blood calibration curves. The peak area response ratio (analyte/IS) was calculated in each injection for 1,2-DCB. A linear regression equation with 1/x weighting was calculated relating the peak area response ratio (y) to the nominal concentration (x) in blood calibration standards for 1,2-DCB.

The concentration of each calibration standard, QC standard, and blank was calculated in nanograms per milliliter using its individual response ratio and the regression equation. Tissue concentration was converted to ng/g using the tissue weight and homogenization parameters (1 part tissue to 2 parts PBS/acetone).

Data from the study samples were considered valid if they were bracketed by valid QC sets. In general, each sample set, method blank, and control was bracketed by two QC sets, which consisted of a calibration blank and two concentrations of calibration standards. A QC set passed when the measured concentration for QC standards was ≤15% of its nominal value for at least 67% of all QC standards and at least 50% of the QC standards at each concentration level (high or low) having determined concentrations ≤15% of the nominal value. All QC standard sets met these acceptance criteria.

Twenty-nine tracking blood QC standards were analyzed with the study samples. Estimated REs for the tracking QC standards ranged from 8% to 23.6%, demonstrating that there was no appreciable analyte loss during sample collection and storage.

Table B-1. Analytical System and Parameters Used in the Internal Concentration Assessment of 1,2-Dichlorobenzene

Instrument and Parameter	System A
System	Agilent GC 7890B plus Agilent MS 5977B
Sampling	
Headspace Autosampler	PAL RSI 85 Autosampler (CTC Analytics AG, Zwingen, Switzerland)
Solid Phase Microextraction	Topaz inlet liner (0.75 mm × 6.35 mm × 78.5 mm) and 100% polydimethylsiloxane fiber
Sample Incubation Temperature	40°C
Sample Incubation Time	10 minutes
Fiber Conditioning Temperature	300°C
Pre-desorption Conditioning Time	5 minutes
Sampling Time	15 minutes
Sample Vial Penetration Speed	20 mm/sec
Inlet Penetration Speed	100 mm/sec
Sample Desorption Time	2 minutes
Post-desorption Conditioning Time	15 minutes

Instrument and Parameter	System A
Column and Program	
Column	Restek Rtx-5 (30 m × 0.25 mm ID, 0.25 µm film thickness)
Carrier Gas	Helium at 1 mL/min
Oven Temperature Program	80°C for 2 minutes, then 5°C/min to 100°C, then 25°C/min to 250°C, 5-minute hold
Inlet Temperature	250°C
Inlet Purge Flow, Time	50.0 mL/min at 0.2 minutes
GC Cycle Time	22 minutes
Solvent Delay	3.5 to 4 minutes
Retention Time	~5.3 minutes
Auxiliary Temperature	250°C
MS Detector	
Source Temperature	230°C
Quadrupole Temperature	150°C
Ionization Mode	Electron Ionization Positive (EI+)
Acquisition Mode	SIM for 30 minutes m/z 146 (1,2-DCB quantitation ion) m/z 111(1,2-DCB confirmation ion) m/z 152 ($^{13}C_6$ 1,2-DCB quantitation ion) m/z 117 ($^{13}C_6$ 1,2-DCB confirmation ion)

GC = gas chromatography; MS = mass spectrometry; ID = internal diameter; EI = electron ionization; SIM = single ion monitoring.

Table B-2. Method Validation Data for 1,2-Dichlorobenzene in Rat Blood

Parameter	Male Sprague Dawley Rat
Matrix Concentration Range (ng/mL)	0.5–250[a]
Sensitivity	
LLOQ (ng/mL)	0.5
LOD (ng/mL)	0.157
Coefficient of determination (r^2)	>0.99
Accuracy (% RE)	
Calibration standards intraday	≤ ±8.4
Calibration standards interday	≤ ±3.2
QC standards intraday[b]	≤ ±14.4
QC standards interday[b]	≤ ±3.2
Precision (% RSD)	
Calibration standards intraday	≤15.0
Calibration standards interday	≤10.3
QC standards intraday[b]	≤6.9
QC standards interday[b]	≤12.8

LLOQ = lower limit of quantitation; LOD = limit of detection; RE = relative error; QC = quality control; RSD = relative standard deviation.
[a]Range validated with seven matrix calibration standards.
[b]Quality control standards prepared at 1, 25, and 200 ng/mL. n = 4 for intraday and n = 12 for interday except for 1 ng/mL where n = 1.

Table B-3. Method Dilution Verification Data for 1,2-Dichlorobenzene in Male Sprague Dawley Rat Blood

Parameter[a]	9,900 ng/mL[b]	19,600 ng/mL[c]	47,600 ng/mL[d]	90,900 ng/mL[e]
Accuracy (% RE)	7.1	5.1	34.7	–33.9
Precision (% RSD)	0.5	0.5	0.4	0.1

RE = relative error; RSD = relative standard deviation.
[a]All values are a result of three replicate quality control standards.
[b]50-fold dilution with blood containing internal standard (IS).
[c]100-fold dilution with blood containing IS.
[d]250-fold dilution with blood containing IS.
[e]500-fold dilution with blood containing IS.

Table B-4. Secondary Matrix Evaluation Data for 1,2-Dichlorobenzene

Matrix	Accuracy (%RE)	Precision (%RSD)
Harlan Sprague Dawley Rat Blood[a]	≤ ±23.0	≤5.4
Harlan Sprague Dawley Rat Liver[b]	25.8	2.8
Harlan Sprague Dawley Rat Liver, Dilution Verification[c]	15.3	9.4
Harlan Sprague Dawley Rat Lung[b]	25.3	3.3
B6C3F1 Mouse Blood[d]	≤ ±33.0	≤6.6
B6C3F1 Mouse Liver[b]	24.7	2.6
B6C3F1 Mouse Lung[b]	26.0	3.0
B6D2F1 Mouse Blood[e]	≤ ±11.2	≤1.5

RE = relative error; RSD = relative standard deviation.
[a]Results from one analysis of six 1 ng/mL quality control (QC) standards and one analysis of three 1 ng/mL and three 25 ng/mL QC standards.
[b]Results from one analysis of six 3.0 ng/g QC standards (1 part tissue:2 parts water).
[c]Results from one analysis of four 150,000 ng/g samples diluted with blood at a 250-fold dilution.
[d]Results from two analyses of six 1 ng/mL QC standards.
[e]Results from one analysis of three 1 ng/mL and one analysis of three 25 ng/mL QC standards.

Table B-5. Storage Stability of 1,2-Dichlorobenzene in Sample Matrices

Matrix	Environment	Percent of Day 0
Sprague Dawley Rat Blood[a]	Ambient, light	80.5 to 102.1
Sprague Dawley Rat Blood[b]	–70°C, dark	74.3 to 79.3
Harlan Sprague Dawley Rat Liver[c]	–70°C, dark	98.7 to 108.3

[a]Four replicate quality control (QC) standards at three concentrations (1, 25, and 200 ng/mL); processed with internal standard (IS), stored at ambient temperature in the light for 21 days, and injected with freshly processed matrix calibration standards.
[b]Four replicate QC standards at three concentrations (1, 25, and 200 ng/mL); processed with IS, stored at –70°C for 149 days, and injected with freshly processed matrix calibration standards.
[c]Four replicate QC standards at three concentrations (3, 75, and 600 ng/g); stored at –70°C for 100 days and injected with freshly processed matrix calibration standards.

Appendix C. Animal Identifiers

Tables

Table C-1. Animal Numbers and FASTQ Data File Names for Female Rats Exposed to 1,2-Dichlorobenzene for Five Days

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
1	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F1HE_S78_R1_001.fastq.gz
1	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F1KI_S78_R1_001.fastq.gz[a]
1	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F1LI_S86_R1_001.fastq.gz
1	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F1LU_S86_R1_001.fastq.gz
1	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F1OV_S12_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F2HE_S109_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F2KI_S109_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F2LI_S77_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F2LU_S77_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F2OV_S3_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F3HE_S91_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F3KI_S91_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F3LI_S99_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F3LU_S99_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F3OV_S25_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F4HE_S88_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F4KI_S88_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F4LI_S96_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F4LU_S96_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F4OV_S22_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F5HE_S98_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F5KI_S98_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F5LI_S106_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F5LU_S106_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F5OV_S32_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F6HE_S99_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F6KI_S99_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F6LI_S107_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F6LU_S107_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F6OV_S33_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F7HE_S86_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F7KI_S86_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F7LI_S94_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F7LU_S94_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F7OV_S20_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F8HE_S110_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F8KI_S110_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F8LI_S78_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F8LU_S78_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
8	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F8OV_S4_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F9HE_S93_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F9KI_S93_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F9LI_S101_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F9LU_S101_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F9OV_S27_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Heart	2DR1F10HE_S92_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Kidney	2DR1F10KI_S92_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Liver	2DR1F10LI_S100_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Lung	2DR1F10LU_S100_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Ovary	2DR1F10OV_S26_R1_001.fastq.gz
11	ICA Animals	Vehicle Control	0	Yes	None	NA
12	ICA Animals	Vehicle Control	0	Yes	None	NA
13	ICA Animals	Vehicle Control	0	Yes	None	NA
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DR2F101HE_S100_R1_001.fastq.gz
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DR2F101KI_S100_R1_001.fastq.gz
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DR2F101LI_S108_R1_001.fastq.gz
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DR2F101LU_S108_R1_001.fastq.gz
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DR2F101OV_S34_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DR2F102HE_S112_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DR2F102KI_S112_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DR2F102LI_S80_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DR2F102LU_S80_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DR2F102OV_S6_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DR2F103HE_S104_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DR2F103KI_S104_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DR2F103LI_S112_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DR2F103LU_S112_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DR2F103OV_S38_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DR2F104HE_S79_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DR2F104KI_S79_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DR2F104LI_S87_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DR2F104LU_S87_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DR2F104OV_S13_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DR2F105HE_S73_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DR2F105KI_S73_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DR2F105LI_S81_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DR2F105LU_S81_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DR2F105OV_S7_R1_001.fastq.gz
106	ICA Animals	1,2-Dichlorobenzene	1	Yes	None	NA
107	ICA Animals	1,2-Dichlorobenzene	1	Yes	None	NA

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
108	ICA Animals	1,2-Dichlorobenzene	1	Yes	None	NA
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DR3F201HE_S89_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DR3F201KI_S89_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DR3F201LI_S97_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DR3F201LU_S97_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DR3F201OV_S23_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DR3F202HE_S83_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DR3F202KI_S83_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DR3F202LI_S91_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DR3F202LU_S91_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DR3F202OV_S17_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DR3F203HE_S94_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DR3F203KI_S94_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DR3F203LI_S102_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DR3F203LU_S102_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DR3F203OV_S28_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DR3F204HE_S111_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DR3F204KI_S111_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DR3F204LI_S79_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DR3F204LU_S79_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DR3F204OV_S5_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DR3F205HE_S90_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DR3F205KI_S90_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DR3F205LI_S98_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DR3F205LU_S98_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DR3F205OV_S24_R1_001.fastq.gz
206	ICA Animals	1,2-Dichlorobenzene	10	Yes	None	NA
207	ICA Animals	1,2-Dichlorobenzene	10	Yes	None	NA
208	ICA Animals	1,2-Dichlorobenzene	10	Yes	None	NA
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DR4F301HE_S95_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DR4F301KI_S95_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DR4F301LI_S103_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DR4F301LU_S103_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DR4F301OV_S29_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DR4F302HE_S74_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DR4F302KI_S74_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DR4F302LI_S82_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DR4F302LU_S82_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DR4F302OV_S8_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DR4F303HE_S103_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DR4F303KI_S103_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DR4F303LI_S111_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DR4F303LU_S111_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DR4F303OV_S37_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DR4F304HE_S76_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DR4F304KI_S76_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DR4F304LI_S84_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DR4F304LU_S84_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DR4F304OV_S10_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DR4F305HE_S81_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DR4F305KI_S81_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DR4F305LI_S89_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DR4F305LU_S89_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DR4F305OV_S15_R1_001.fastq.gz
306	ICA Animals	1,2-Dichlorobenzene	30	Yes	None	NA
307	ICA Animals	1,2-Dichlorobenzene	30	Yes	None	NA
308	ICA Animals	1,2-Dichlorobenzene	30	Yes	None	NA
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DR5F401HE_S106_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DR5F401KI_S106_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DR5F401LI_S114_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DR5F401LU_S114_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DR5F401OV_S40_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DR5F402HE_S108_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DR5F402KI_S108_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DR5F402LI_S76_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DR5F402LU_S76_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DR5F402OV_S2_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DR5F403HE_S97_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DR5F403KI_S97_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DR5F403LI_S105_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DR5F403LU_S105_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DR5F403OV_S31_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DR5F404HE_S77_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DR5F404KI_S77_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DR5F404LI_S85_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DR5F404LU_S85_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DR5F404OV_S11_R1_001.fastq.gz
405	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DR5F405HE_S87_R1_001.fastq.gz
405	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DR5F405KI_S87_R1_001.fastq.gz
405	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DR5F405LI_S95_R1_001.fastq.gz
405	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DR5F405LU_S95_R1_001.fastq.gz
405	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DR5F405OV_S21_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
406	ICA Animals	1,2-Dichlorobenzene	100	Yes	None	NA
407	ICA Animals	1,2-Dichlorobenzene	100	Yes	None	NA
408	ICA Animals	1,2-Dichlorobenzene	100	Yes	None	NA
501	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DR6F501HE_S105_R1_001.fastq.gz
501	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DR6F501KI_S105_R1_001.fastq.gz
501	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DR6F501LI_S113_R1_001.fastq.gz
501	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DR6F501LU_S113_R1_001.fastq.gz
501	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DR6F501OV_S39_R1_001.fastq.gz
502	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DR6F502HE_S107_R1_001.fastq.gz
502	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DR6F502KI_S107_R1_001.fastq.gz
502	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DR6F502LI_S115_R1_001.fastq.gz
502	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DR6F502LU_S115_R1_001.fastq.gz
502	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DR6F502OV_S41_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DR6F503HE_S75_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DR6F503KI_S75_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DR6F503LI_S83_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DR6F503LU_S83_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DR6F503OV_S9_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DR6F504HE_S101_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DR6F504KI_S101_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DR6F504LI_S109_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DR6F504LU_S109_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DR6F504OV_S35_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DR6F505HE_S96_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DR6F505KI_S96_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DR6F505LI_S104_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DR6F505LU_S104_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DR6F505OV_S30_R1_001.fastq.gz
506	ICA Animals	1,2-Dichlorobenzene	250	Yes	None	NA
507	ICA Animals	1,2-Dichlorobenzene	250	Yes	None	NA
508	ICA Animals	1,2-Dichlorobenzene	250	Yes	None	NA
601	Core Animals	1,2-Dichlorobenzene	500	Yes	Heart	2DR7F601HE_S84_R1_001.fastq.gz
601	Core Animals	1,2-Dichlorobenzene	500	Yes	Kidney	2DR7F601KI_S84_R1_001.fastq.gz
601	Core Animals	1,2-Dichlorobenzene	500	Yes	Liver	2DR7F601LI_S92_R1_001.fastq.gz
601	Core Animals	1,2-Dichlorobenzene	500	Yes	Lung	2DR7F601LU_S92_R1_001.fastq.gz[a]
601	Core Animals	1,2-Dichlorobenzene	500	Yes	Ovary	2DR7F601OV_S18_R1_001.fastq.gz
602	Core Animals	1,2-Dichlorobenzene	500	Yes	Heart	2DR7F602HE_S80_R1_001.fastq.gz
602	Core Animals	1,2-Dichlorobenzene	500	Yes	Kidney	2DR7F602KI_S80_R1_001.fastq.gz
602	Core Animals	1,2-Dichlorobenzene	500	Yes	Liver	2DR7F602LI_S88_R1_001.fastq.gz
602	Core Animals	1,2-Dichlorobenzene	500	Yes	Lung	2DR7F602LU_S88_R1_001.fastq.gz
602	Core Animals	1,2-Dichlorobenzene	500	Yes	Ovary	2DR7F602OV_S14_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
603	Core Animals	1,2-Dichlorobenzene	500	Yes	Heart	2DR7F603HE_S82_R1_001.fastq.gz
603	Core Animals	1,2-Dichlorobenzene	500	Yes	Kidney	2DR7F603KI_S82_R1_001.fastq.gz
603	Core Animals	1,2-Dichlorobenzene	500	Yes	Liver	2DR7F603LI_S90_R1_001.fastq.gz
603	Core Animals	1,2-Dichlorobenzene	500	Yes	Lung	2DR7F603LU_S90_R1_001.fastq.gz
603	Core Animals	1,2-Dichlorobenzene	500	Yes	Ovary	2DR7F603OV_S16_R1_001.fastq.gz
604	Core Animals	1,2-Dichlorobenzene	500	Yes	Heart	2DR7F604HE_S102_R1_001.fastq.gz
604	Core Animals	1,2-Dichlorobenzene	500	Yes	Kidney	2DR7F604KI_S102_R1_001.fastq.gz
604	Core Animals	1,2-Dichlorobenzene	500	Yes	Liver	2DR7F604LI_S110_R1_001.fastq.gz
604	Core Animals	1,2-Dichlorobenzene	500	Yes	Lung	2DR7F604LU_S110_R1_001.fastq.gz
604	Core Animals	1,2-Dichlorobenzene	500	Yes	Ovary	2DR7F604OV_S36_R1_001.fastq.gz
605	Core Animals	1,2-Dichlorobenzene	500	Yes	Heart	2DR7F605HE_S85_R1_001.fastq.gz
605	Core Animals	1,2-Dichlorobenzene	500	Yes	Kidney	2DR7F605KI_S85_R1_001.fastq.gz
605	Core Animals	1,2-Dichlorobenzene	500	Yes	Liver	2DR7F605LI_S93_R1_001.fastq.gz
605	Core Animals	1,2-Dichlorobenzene	500	Yes	Lung	2DR7F605LU_S93_R1_001.fastq.gz
605	Core Animals	1,2-Dichlorobenzene	500	Yes	Ovary	2DR7F605OV_S19_R1_001.fastq.gz
606	ICA Animals	1,2-Dichlorobenzene	500	Yes	None	NA
607	ICA Animals	1,2-Dichlorobenzene	500	Yes	None	NA
608	ICA Animals	1,2-Dichlorobenzene	500	Yes	None	NA

ICA = internal concentration assessment; NA = no transcriptomics data collected for selected animal.
[a]Removed due to quality control fail.

Table C-2. Animal Numbers and FASTQ Data File Names for Female Mice Exposed to 1,2-Dichlorobenzene for Five Days

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
1	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F1HE_S6_R1_001.fastq.gz
1	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F1KI_S6_R1_001.fastq.gz[a]
1	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F1LI_S11_R1_001.fastq.gz
1	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F1LU_S11_R1_001.fastq.gz
1	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F1OV_S93_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F2HE_S35_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F2KI_S35_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F2LI_S3_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F2LU_S3_R1_001.fastq.gz
2	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F2OV_S85_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F3HE_S14_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F3KI_S14_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F3LI_S19_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F3LU_S19_R1_001.fastq.gz
3	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F3OV_S101_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F4HE_S16_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
4	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F4KI_S16_R1_001.fastq.gz[a]
4	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F4LI_S21_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F4LU_S21_R1_001.fastq.gz
4	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F4OV_S103_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F5HE_S26_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F5KI_S26_R1_001.fastq.gz[b]
5	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F5LI_S31_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F5LU_S31_R1_001.fastq.gz
5	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F5OV_S113_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F6HE_S27_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F6KI_S27_R1_001.fastq.gz[a]
6	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F6LI_S32_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F6LU_S32_R1_001.fastq.gz
6	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F6OV_S114_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F7HE_S9_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F7KI_S9_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F7LI_S14_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F7LU_S14_R1_001.fastq.gz
7	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F7OV_S96_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F8HE_S32_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F8KI_S32_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F8LI_S37_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F8LU_S37_R1_001.fastq.gz
8	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F8OV_S119_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F9HE_S21_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F9KI_S21_R1_001.fastq.gz[a]
9	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F9LI_S26_R1_001.fastq.gz[c]
9	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F9LU_S26_R1_001.fastq.gz
9	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F9OV_S108_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Heart	2DM1F10HE_S15_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Kidney	2DM1F10KI_S15_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Liver	2DM1F10LI_S20_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Lung	2DM1F10LU_S20_R1_001.fastq.gz
10	Core Animals	Vehicle Control	0	Yes	Ovary	2DM1F10OV_S102_R1_001.fastq.gz
11	ICA Animals	Vehicle Control	0	Yes	None	NA
12	ICA Animals	Vehicle Control	0	Yes	None	NA
13	ICA Animals	Vehicle Control	0	Yes	None	NA
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DM2F101HE_S23_R1_001.fastq.gz
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DM2F101KI_S23_R1_001.fastq.gz
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DM2F101LI_S28_R1_001.fastq.gz[c]
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DM2F101LU_S28_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
101	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DM2F101OV_S110_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DM2F102HE_S25_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DM2F102KI_S25_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DM2F102LI_S30_R1_001.fastq.gz[c]
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DM2F102LU_S30_R1_001.fastq.gz
102	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DM2F102OV_S112_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DM2F103HE_S31_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DM2F103KI_S31_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DM2F103LI_S36_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DM2F103LU_S36_R1_001.fastq.gz
103	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DM2F103OV_S118_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DM2F104HE_S7_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DM2F104KI_S7_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DM2F104LI_S12_R1_001.fastq.gz
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DM2F104LU_S12_R1_001.fastq.gz[b]
104	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DM2F104OV_S94_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Heart	2DM2F105HE_S1_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Kidney	2DM2F105KI_S1_R1_001.fastq.gz[c]
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Liver	2DM2F105LI_S6_R1_001.fastq.gz[b]
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Lung	2DM2F105LU_S6_R1_001.fastq.gz
105	Core Animals	1,2-Dichlorobenzene	1	Yes	Ovary	2DM2F105OV_S88_R1_001.fastq.gz
106	ICA Animals	1,2-Dichlorobenzene	1	No	None	NA
107	ICA Animals	1,2-Dichlorobenzene	1	Yes	None	NA
108	ICA Animals	1,2-Dichlorobenzene	1	Yes	None	NA
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DM3F201HE_S17_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DM3F201KI_S17_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DM3F201LI_S22_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DM3F201LU_S22_R1_001.fastq.gz
201	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DM3F201OV_S104_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DM3F202HE_S11_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DM3F202KI_S11_R1_001.fastq.gz[a]
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DM3F202LI_S16_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DM3F202LU_S16_R1_001.fastq.gz
202	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DM3F202OV_S98_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DM3F203HE_S22_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DM3F203KI_S22_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DM3F203LI_S27_R1_001.fastq.gz[c]
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DM3F203LU_S27_R1_001.fastq.gz
203	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DM3F203OV_S109_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DM3F204HE_S33_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DM3F204KI_S33_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DM3F204LI_S38_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DM3F204LU_S38_R1_001.fastq.gz
204	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DM3F204OV_S120_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Heart	2DM3F205HE_S13_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Kidney	2DM3F205KI_S13_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Liver	2DM3F205LI_S18_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Lung	2DM3F205LU_S18_R1_001.fastq.gz
205	Core Animals	1,2-Dichlorobenzene	10	Yes	Ovary	2DM3F205OV_S100_R1_001.fastq.gz
206	ICA Animals	1,2-Dichlorobenzene	10	Yes	None	NA
207	ICA Animals	1,2-Dichlorobenzene	10	Yes	None	NA
208	ICA Animals	1,2-Dichlorobenzene	10	Yes	None	NA
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DM4F301HE_S18_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DM4F301KI_S18_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DM4F301LI_S23_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DM4F301LU_S23_R1_001.fastq.gz
301	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DM4F301OV_S105_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DM4F302HE_S2_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DM4F302KI_S2_R1_001.fastq.gz[b]
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DM4F302LI_S7_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DM4F302LU_S7_R1_001.fastq.gz
302	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DM4F302OV_S89_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DM4F303HE_S30_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DM4F303KI_S30_R1_001.fastq.gz[a]
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DM4F303LI_S35_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DM4F303LU_S35_R1_001.fastq.gz
303	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DM4F303OV_S117_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DM4F304HE_S36_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DM4F304KI_S36_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DM4F304LI_S4_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DM4F304LU_S4_R1_001.fastq.gz
304	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DM4F304OV_S86_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Heart	2DM4F305HE_S4_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Kidney	2DM4F305KI_S4_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Liver	2DM4F305LI_S9_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Lung	2DM4F305LU_S9_R1_001.fastq.gz
305	Core Animals	1,2-Dichlorobenzene	30	Yes	Ovary	2DM4F305OV_S91_R1_001.fastq.gz
306	ICA Animals	1,2-Dichlorobenzene	30	Yes	None	NA
307	ICA Animals	1,2-Dichlorobenzene	30	Yes	None	NA
308	ICA Animals	1,2-Dichlorobenzene	30	Yes	None	NA
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DM5F401HE_S29_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DM5F401KI_S29_R1_001.fastq.gz[a]

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DM5F401LI_S34_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DM5F401LU_S34_R1_001.fastq.gz
401	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DM5F401OV_S116_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DM5F402HE_S34_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DM5F402KI_S34_R1_001.fastq.gz[a]
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DM5F402LI_S2_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DM5F402LU_S2_R1_001.fastq.gz
402	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DM5F402OV_S84_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DM5F403HE_S20_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DM5F403KI_S20_R1_001.fastq.gz[b]
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DM5F403LI_S25_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DM5F403LU_S25_R1_001.fastq.gz
403	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DM5F403OV_S107_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Heart	2DM5F404HE_S37_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Kidney	2DM5F404KI_S37_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Liver	2DM5F404LI_S5_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DM5F404LU_S5_R1_001.fastq.gz
404	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DM5F404OV_S87_R1_001.fastq.gz
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405	Core Animals	1,2-Dichlorobenzene	100	Yes	Lung	2DM5F405LU_S15_R1_001.fastq.gz
405	Core Animals	1,2-Dichlorobenzene	100	Yes	Ovary	2DM5F405OV_S97_R1_001.fastq.gz
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407	ICA Animals	1,2-Dichlorobenzene	100	Yes	None	NA
408	ICA Animals	1,2-Dichlorobenzene	100	Yes	None	NA
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501	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F501KI_S28_R1_001.fastq.gz[a]
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501	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F501LU_S33_R1_001.fastq.gz
501	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F501OV_S115_R1_001.fastq.gz
502	Core Animals	1,2-Dichlorobenzene	250	No	Heart	NA
502	Core Animals	1,2-Dichlorobenzene	250	No	Kidney	NA
502	Core Animals	1,2-Dichlorobenzene	250	No	Liver	NA
502	Core Animals	1,2-Dichlorobenzene	250	No	Lung	NA
502	Core Animals	1,2-Dichlorobenzene	250	No	Ovary	NA
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DM6F503HE_S3_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F503KI_S3_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DM6F503LI_S8_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F503LU_S8_R1_001.fastq.gz
503	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F503OV_S90_R1_001.fastq.gz

Animal Number	Selection	Group	Exposure Concentration (ppm)	Survived to Study Termination	Tissue	FASTQ File ID
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504	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F504KI_S24_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DM6F504LI_S29_R1_001.fastq.gz[c]
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F504LU_S29_R1_001.fastq.gz
504	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F504OV_S111_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DM6F505HE_S19_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F505KI_S19_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DM6F505LI_S24_R1_001.fastq.gz
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F505LU_S24_R1_001.fastq.gz[c]
505	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F505OV_S106_R1_001.fastq.gz
506R	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DM6F506RHE_S12_R1_001.fastq.gz
506R	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F506RKI_S12_R1_001.fastq.gz
506R	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DM6F506RLI_S17_R1_001.fastq.gz
506R	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F506RLU_S17_R1_001.fastq.gz
506R	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F506ROV_S99_R1_001.fastq.gz
507	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DM6F507HE_S8_R1_001.fastq.gz
507	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F507KI_S8_R1_001.fastq.gz
507	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DM6F507LI_S13_R1_001.fastq.gz
507	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F507LU_S13_R1_001.fastq.gz
507	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F507OV_S95_R1_001.fastq.gz
508	Core Animals	1,2-Dichlorobenzene	250	Yes	Heart	2DM6F508HE_S5_R1_001.fastq.gz
508	Core Animals	1,2-Dichlorobenzene	250	Yes	Kidney	2DM6F508KI_S5_R1_001.fastq.gz
508	Core Animals	1,2-Dichlorobenzene	250	Yes	Liver	2DM6F508LI_S10_R1_001.fastq.gz
508	Core Animals	1,2-Dichlorobenzene	250	Yes	Lung	2DM6F508LU_S10_R1_001.fastq.gz
508	Core Animals	1,2-Dichlorobenzene	250	Yes	Ovary	2DM6F508OV_S92_R1_001.fastq.gz
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510	ICA Animals	1,2-Dichlorobenzene	250	Yes	None	NA
511	ICA Animals	1,2-Dichlorobenzene	250	No	None	NA
512	ICA Animals	1,2-Dichlorobenzene	250	No	None	NA
513	ICA Animals	1,2-Dichlorobenzene	250	Yes	None	NA

ICA = internal concentration assessment; NA = no transcriptomics data collected for selected animal.
[a]Removed due to potential tissue contamination.
[b]Removed due to principal component analysis/inter-replicate correlation analysis outlier.
[c]Removed due to quality control fail.

Appendix D. Transcriptomic Quality Control and Empirical False Discovery Rate

Table of Contents

Tables

Figures

D.1. Gene Expression Quality Control

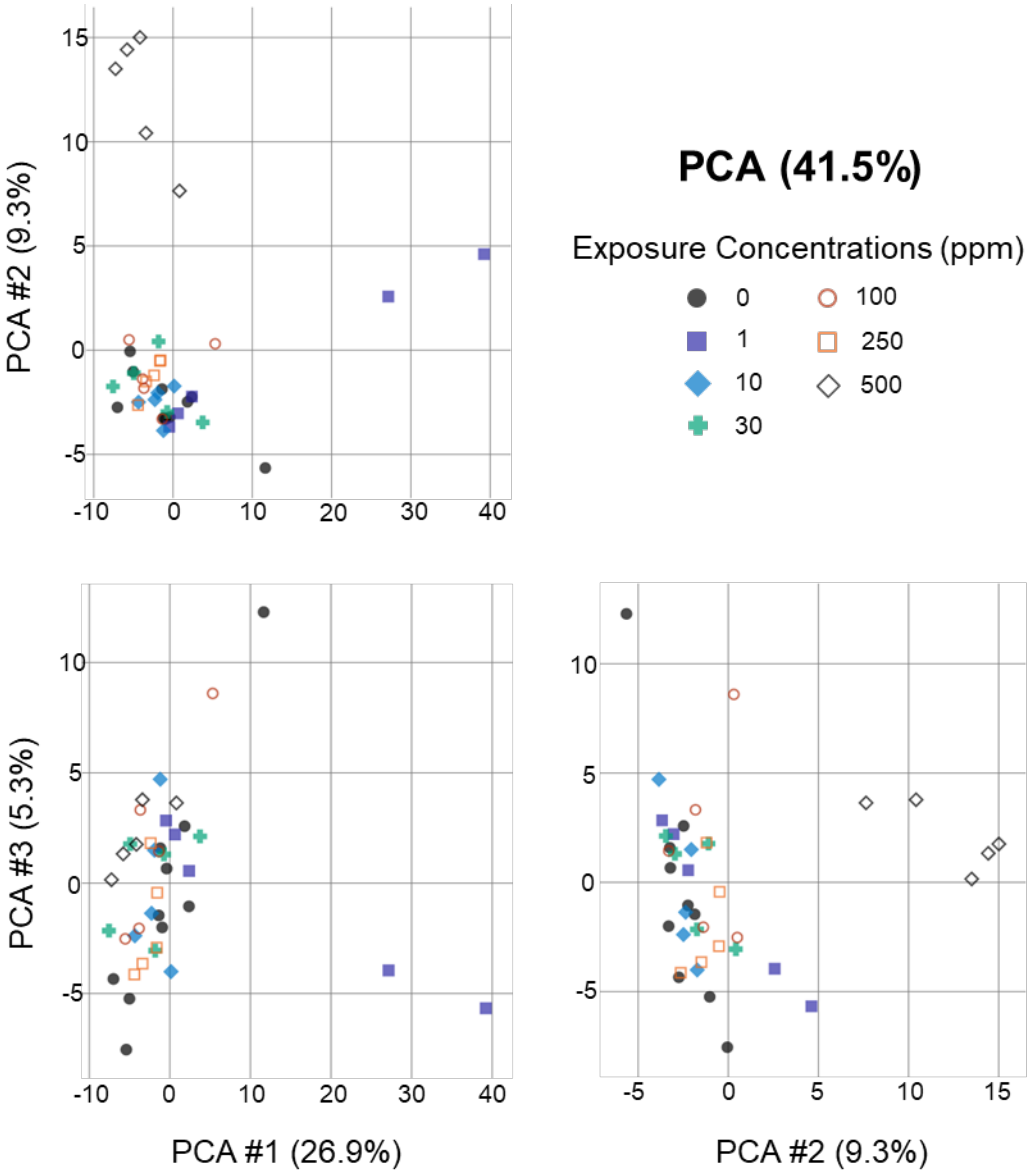

Figure D-1. A Principal Component Analysis of the Normalized Data from the Heart of Female Rats

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

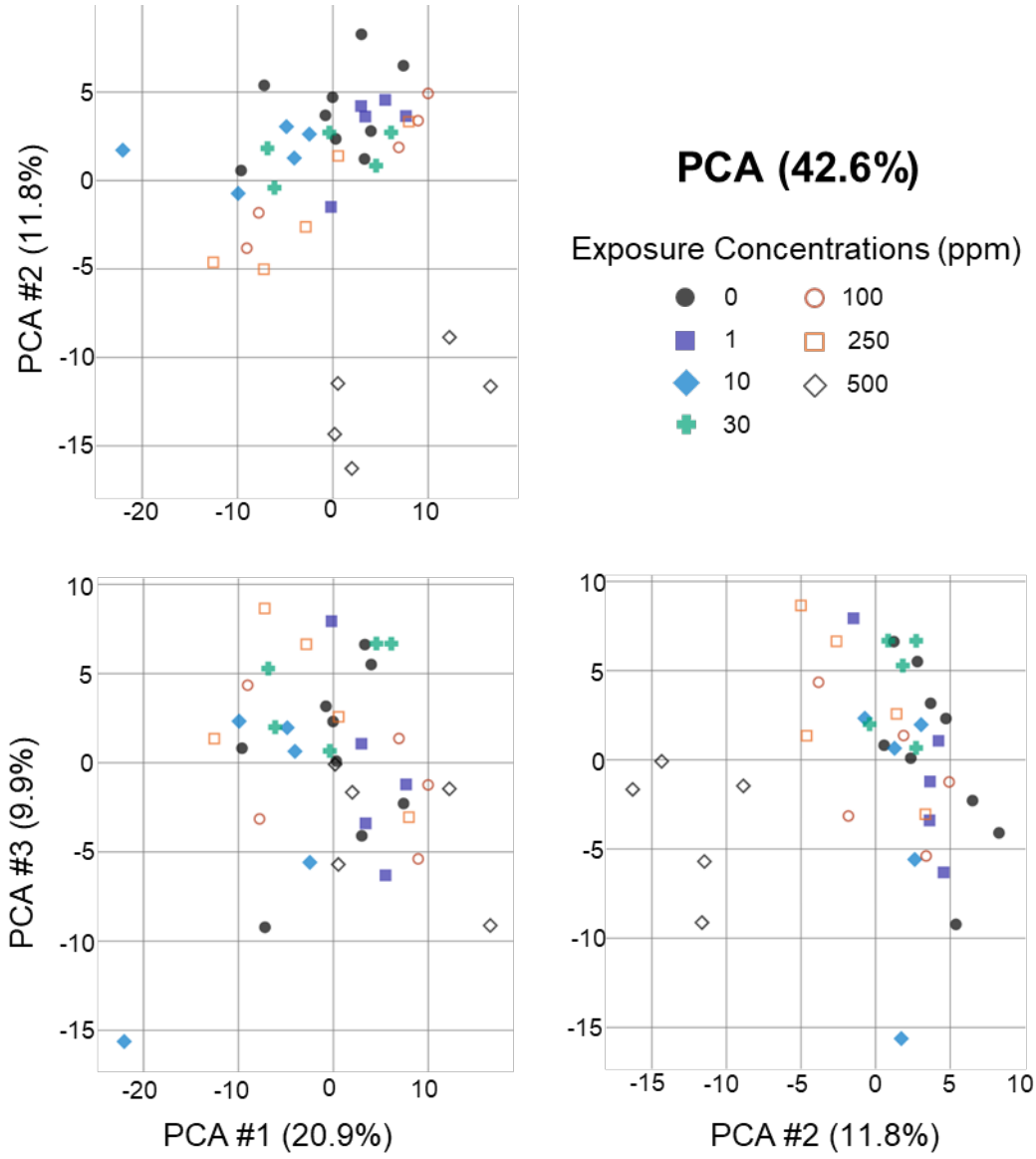

Figure D-2. A Principal Component Analysis of the Normalized Data from the Kidney of Female Rats

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

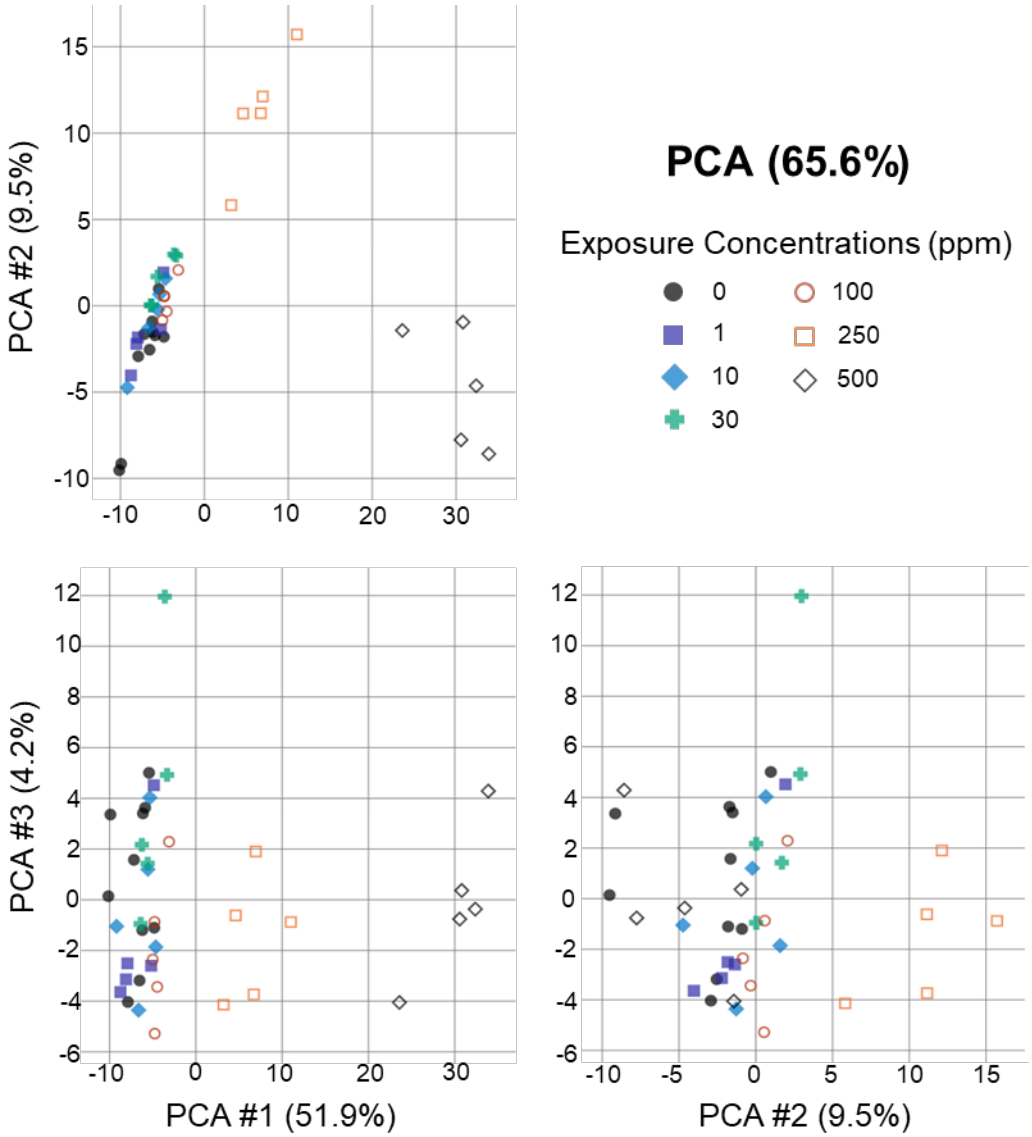

Figure D-3. A Principal Component Analysis of the Normalized Data from the Liver of Female Rats

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

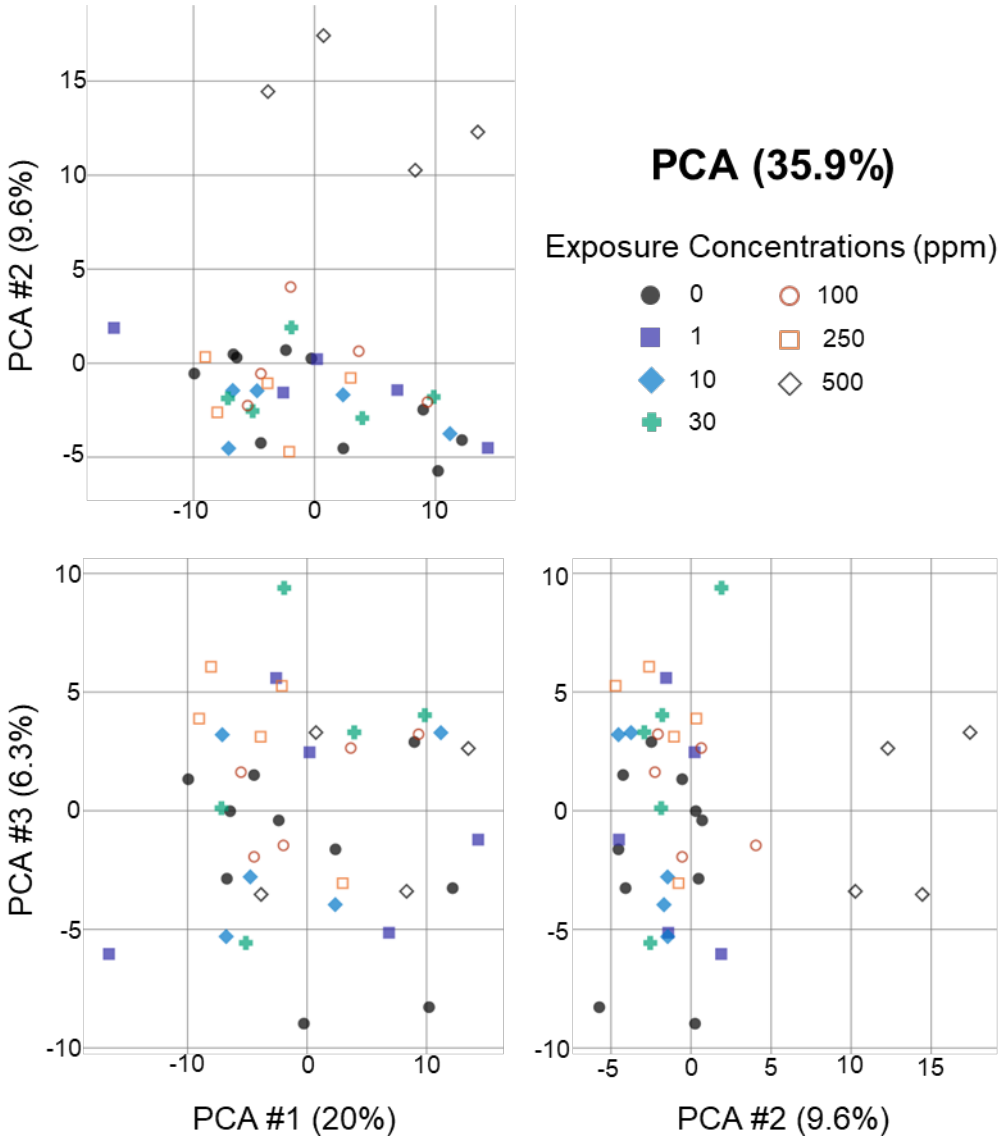

Figure D-4. A Principal Component Analysis of the Normalized Data from the Lung of Female Rats

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

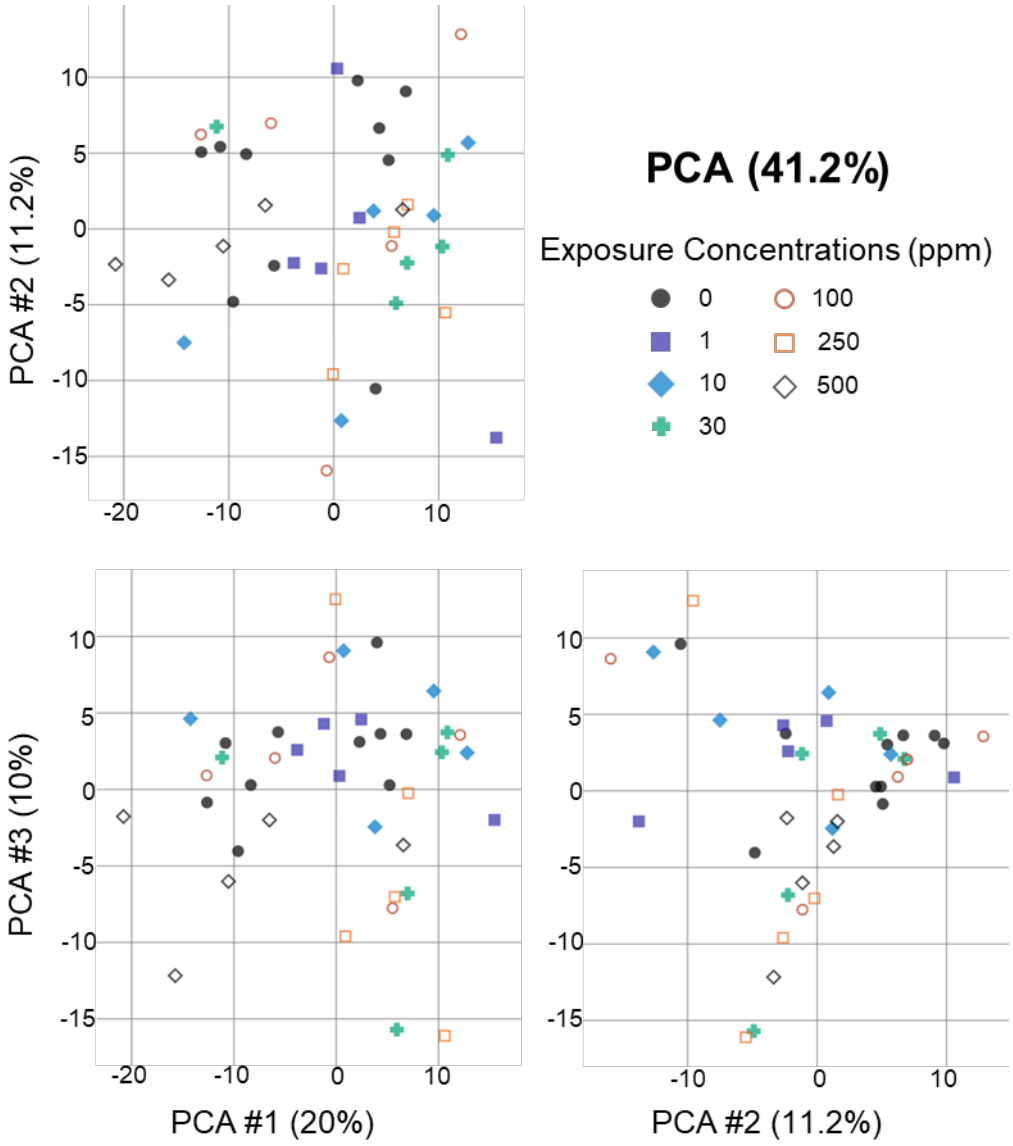

Figure D-5. A Principal Component Analysis of the Normalized Data from the Ovary of Female Rats

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

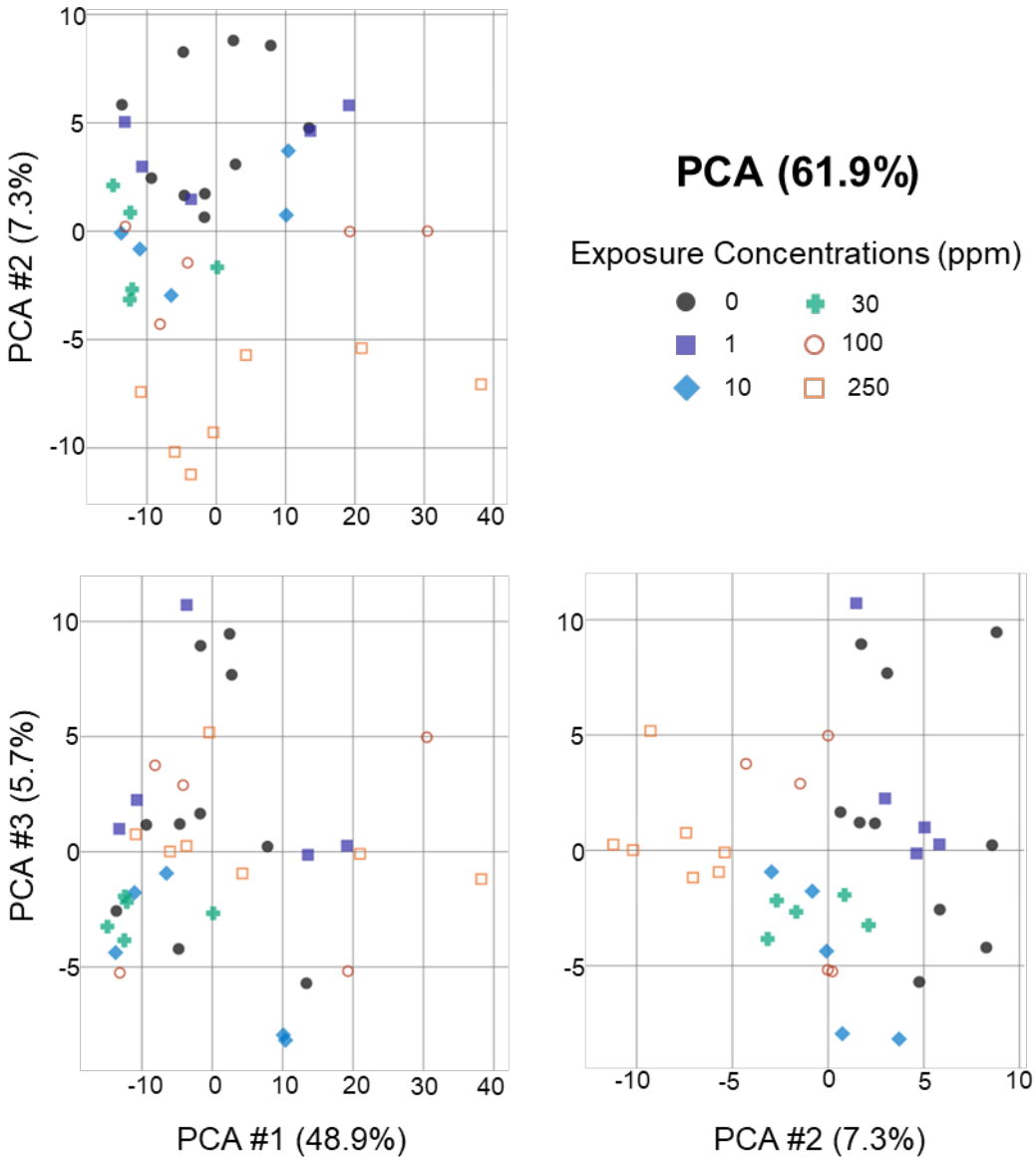

Figure D-6. A Principal Component Analysis of the Normalized Data from the Heart of Female Mice

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

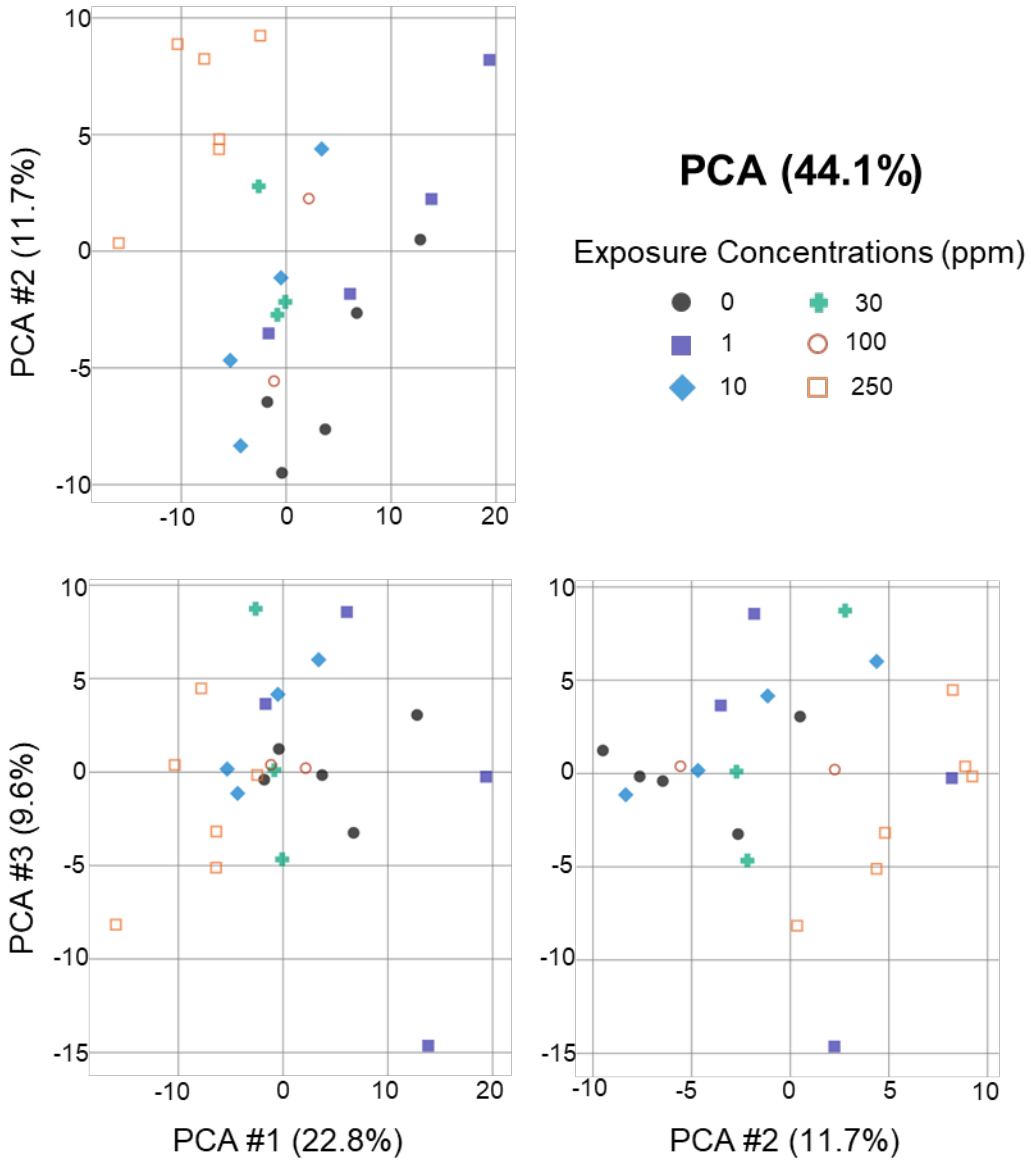

Figure D-7. A Principal Component Analysis of the Normalized Data from the Kidney of Female Mice

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

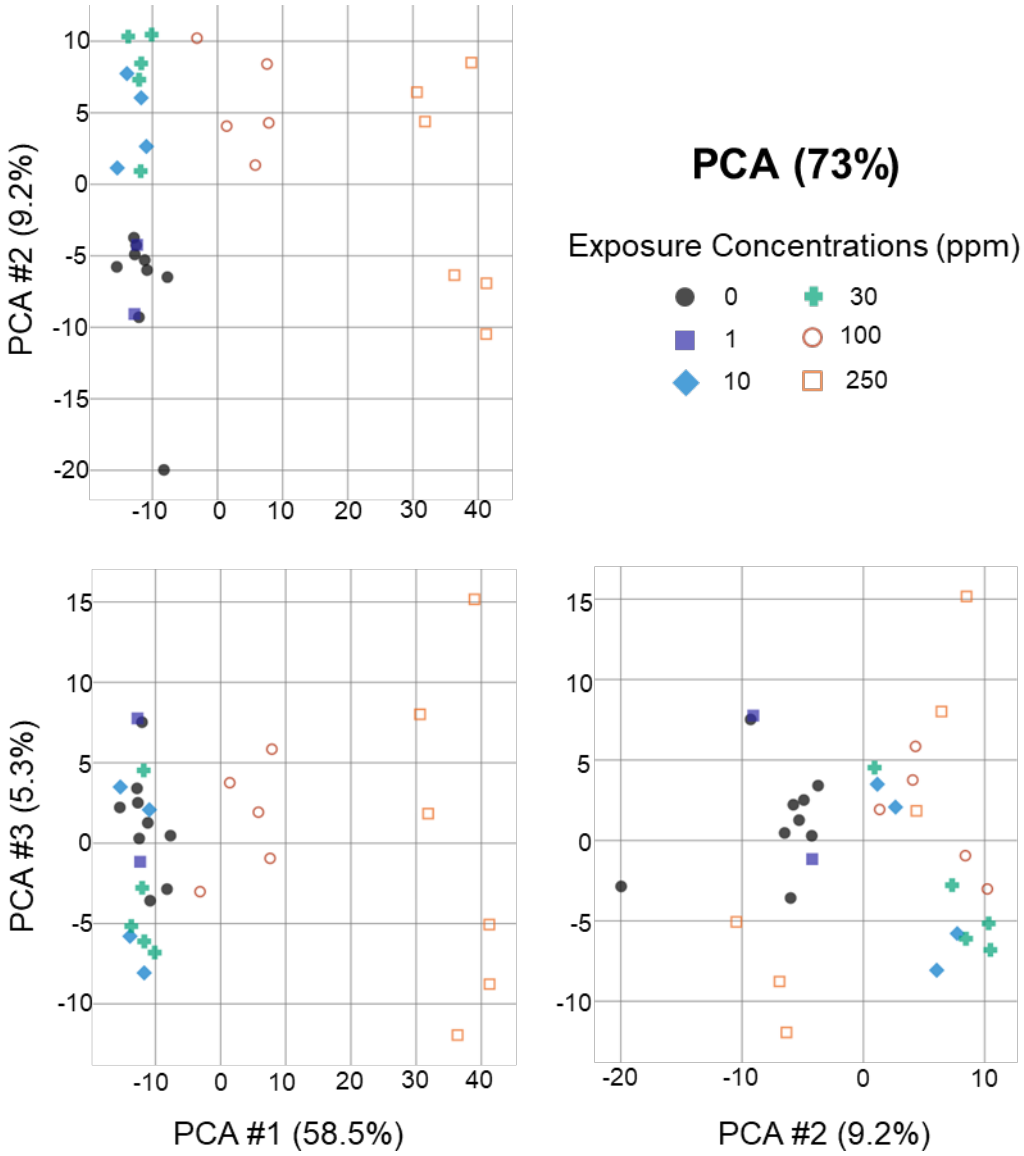

Figure D-8. A Principal Component Analysis of the Normalized Data from the Liver of Female Mice

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

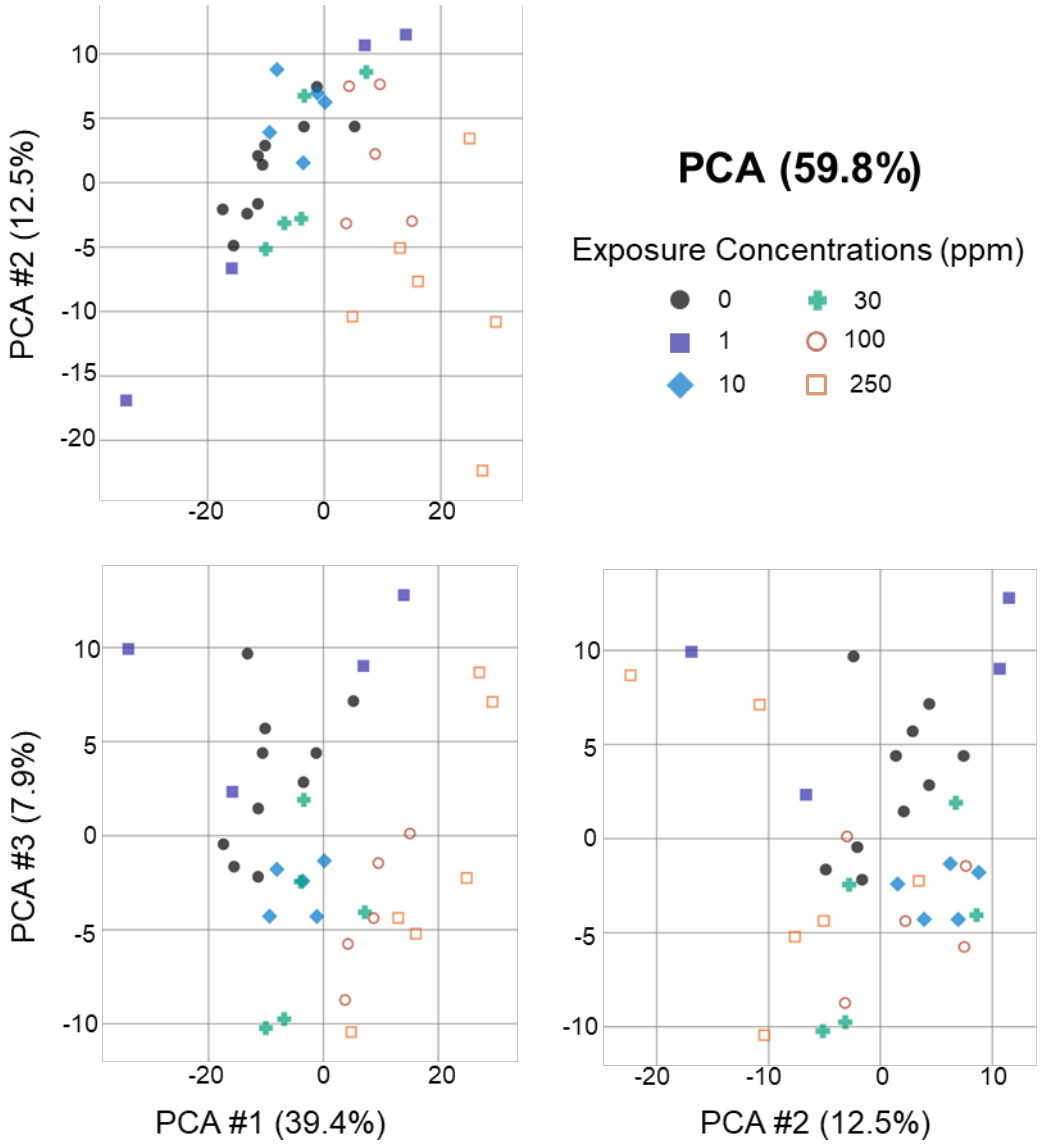

Figure D-9. A Principal Component Analysis of the Normalized Data from the Lung of Female Mice

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

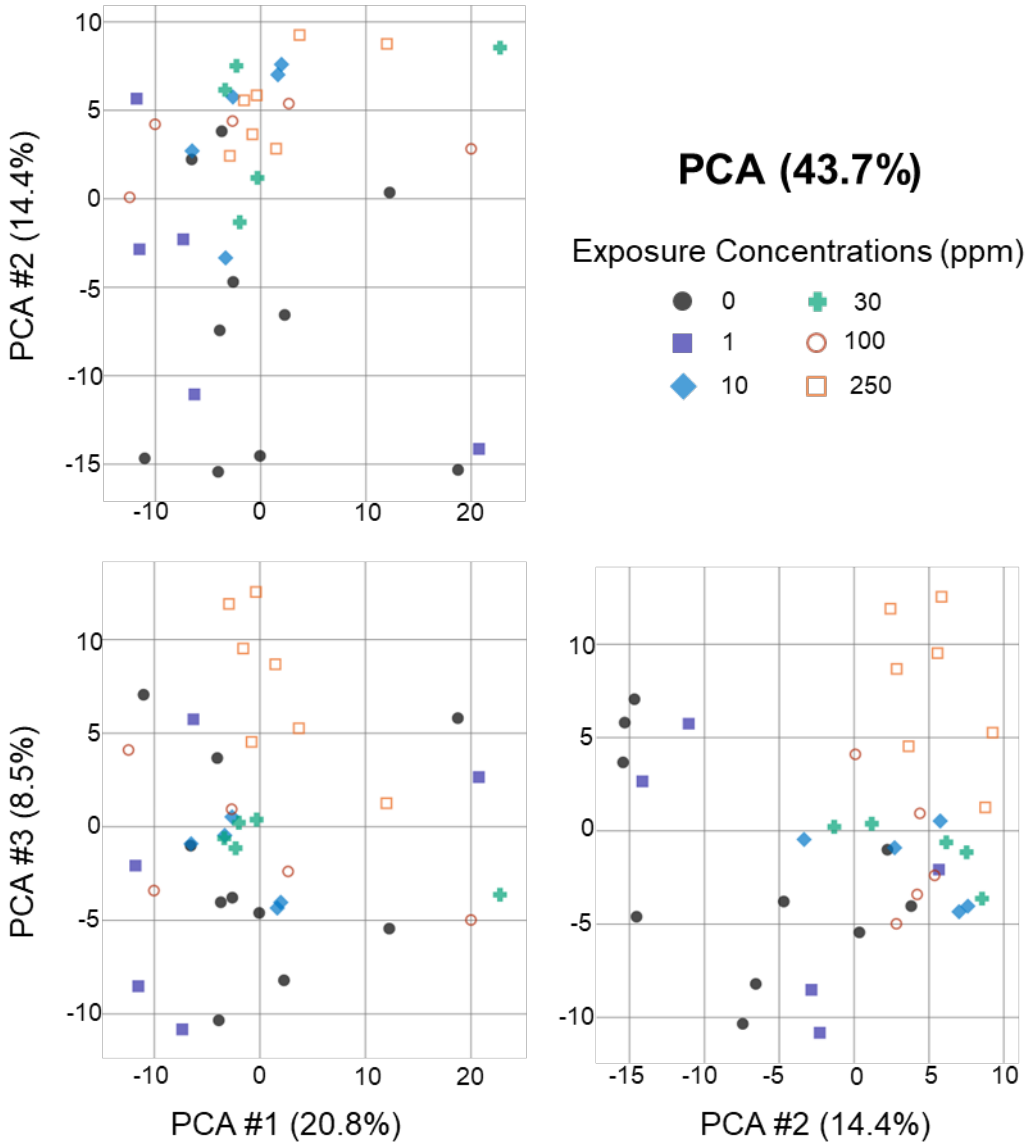

Figure D-10. A Principal Component Analysis of the Normalized Data from the Ovary of Female Mice

A principal component analysis (PCA) plot enables visualization of global transcriptional changes in two dimensions, with each plot showing a different angle on the basis of the principal components plotted. Global transcript data are shown for individual animals (dots) within each exposure group (designated by color). Dots that are spatially closer to each other indicate more similarity in global expression profiles; dots that are farther apart indicate dissimilarity in global expression profiles for those animals. The data represented in the plot are those employed in dose response modeling (i.e., if outliers were identified in the quality control process, they were removed from the data set and are not present in the plot). Visual inspection does not suggest subgrouping of the data other than exposure concentration-related changes, which indicates any technical batch-related effects are minimal.

D.2. Empirical False Discovery Rate

D.2.1. Methods

Synthetic null data were generated using the probe-filtered (i.e., "no 0") 0 ppm data from each tissue in the 1,2-dichlorobenzene rat and mouse studies. The synthetic null data were generated using the Synthetic and Null Data Generator (SaNDGen; https://rstudio.niehs.nih.gov/sandgen/), which employed the normal distribution method previously described for generating synthetic data.[29] In short, for each set of tissue/species, 0 ppm data were used to generate a distribution for each probe. This distribution was resampled to generate 1,000 values for each probe. These values were distributed into synthetic samples, which were then organized into 20 different experiments paralleling the distribution of samples in the experimental study, i.e., 10 samples in the 0 ppm group and 5 for each nonzero exposure concentration. Each of the 20 synthetic null experiments was processed through BMDExpress using the identical parameters used to analyze the experimental data. The resultant data were then used to determine the empirical false discovery rates (eFDR), which are reported as percentages of possible genes and Gene Ontology (GO) biological processes. The associated bm2 analysis files that are the basis of the empirical false discovery rate can be found in Appendix G.

D.2.2. Results

In all species-tissue combinations, the gene-level eFDR was <1%, with a range of 0% to 0.21% (Table D-1). Most gene-level eFDR values were below 0.5%. In all cases, the eFDR for GO biological processes was 0% across all species-tissue combinations.

Table D-1. Empirical False Discovery Results

Species	Tissue	Gene eFDR (%)		GO Biological Process eFDR (%)	
		Mean	Range	Mean	Range
Mouse	Heart	0.07	0–0.2	0	0
Mouse	Kidney	0.03	0–0.09	0	0
Mouse	Liver	0.05	0–0.21	0	0
Mouse	Lung	0.06	0–0.14	0	0
Mouse	Ovary	0.03	0–0.09	0	0
Rat	Heart	0.04	0–0.2	0	0
Rat	Kidney	0.02	0–0.005	0	0
Rat	Liver	0.03	0–0.01	0	0
Rat	Lung	0.03	0–0.007	0	0
Rat	Ovary	0.02	0–0.005	0	0

eFDR = empirical false discovery rate; GO = Gene Ontology.

Appendix E. Gene Set and Gene Definitions

Tables

Table E-1. Active Genes and Definitions within Top 10 Heart Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
Rats				
GO:0070301 cellular response to hydrogen peroxide	5/57 (8.8%)	*Tnfaip3; Nol3; Myh7; Lcn2; Bnip3*	148.7 (58.2–382.1)	Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a hydrogen peroxide (H_2O_2) stimulus.
GO:0044770 cell cycle phase transition	3/58 (5.2%)	*Ccnb2; Ccnb2-ps2; Ccna2*	161.5 (81.6–247.4)	The cell cycle process by which a cell commits to entering the next cell cycle phase.
GO:0044772 mitotic cell cycle phase transition	3/52 (5.8%)	*Ccnb2; Ccnb2-ps2; Ccna2*	161.5 (81.6–247.4)	The cell cycle process by which a cell commits to entering the next mitotic cell cycle phase.
GO:0048872 homeostasis of number of cells	5/58 (8.6%)	*Tnfaip3; Mef2c; Hmox1; Col6a1; Ccnb2*	171.1 (90.5–395.6)	Any biological process involved in the maintenance of the steady-state number of cells within a population of cells.
GO:0001568: blood vessel development	5/45 (11.1%)	*Mef2c; Loxl1; Col3a1; Col1a2; Apln*	174.1 (96.0–271.1)	The process whose specific outcome is the progression of a blood vessel over time, from its formation to the mature structure. The blood vessel is the vasculature carrying blood.
GO:0001936 regulation of endothelial cell proliferation	6/48 (12.5%)	*Xdh; Stat1; Sparc; Mef2c; Hmox1; Apln*	176.6 (107.9–257.6)	Any process that modulates the frequency, rate, or extent of endothelial cell proliferation.
GO:0010594 regulation of endothelial cell migration	6/58 (10.3%)	*Sparc; Mef2c; Lcn2; Hmox1; Glul; Fgf1*	176.6 (107.9–257.6)	Any process that modulates the rate, frequency, or extent of the orderly movement of an endothelial cell into the extracellular matrix to form an endothelium.
GO:0010634 positive regulation of epithelial cell migration	4/51 (7.8%)	*Sparc; Lcn2; Hmox1; Fgf1*	176.6 (107.9–257.6)	Any process that activates or increases the frequency, rate or extent of epithelial cell migration.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0071229 cellular response to acid chemical	5/54 (9.3%)	*Myh7; Col6a1; Col4a1; Col3a1; Col1a2*	179.7 (66.7–285.1)	Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus by the chemical structure of the anion portion of the dissociated acid (rather than the acid acting as a proton donor). The acid chemical may be in gaseous, liquid or solid form.
GO:0071230 cellular response to amino acid stimulus	5/50 (10.0%)	*Myh7; Col6a1; Col4a1; Col3a1; Col1a2*	179.7 (66.7–285.1)	Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of an amino acid stimulus. An amino acid is a carboxylic acid containing one or more amino groups.
Mice				
GO:0006575 cellular modified amino acid metabolic process	3/59 (5.1%)	*Gnmt; Mthfd1l; Gsta1*	4.4 (1.7–16.7)	The chemical reactions and pathways involving compounds derived from amino acids, organic acids containing one or more amino substituents.
GO:0030097 hemopoiesis	5/56 (8.9%)	*Alas1; Alas2; Slc11a2; Hba-a1; Hba-a2*	58.8 (17.9–116.1)	The process whose specific outcome is the progression of the myeloid and lymphoid derived organ/tissue systems of the blood and other parts of the body over time, from formation to the mature structure. The site of hemopoiesis is variable during development, but occurs primarily in bone marrow or kidney in many adult vertebrates.
GO:0002687 positive regulation of leukocyte migration	4/50 (8.0%)	*Serpine1; Ccl12; Ccl2; Ccl7*	69.1 (38.2–113.2)	Any process that activates or increases the frequency, rate, or extent of leukocyte migration.
GO:0030595 leukocyte chemotaxis	3/49 (6.1%)	*Ccl12; Ccl2; Ccl7*	69.1 (38.2–113.2)	The movement of a leukocyte in response to an external stimulus.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0043087 regulation of GTPase activity	3/59 (5.1%)	*Ccl12; Ccl2; Ccl7*	69.1 (38.2–113.2)	Any process that modulates the rate of GTP hydrolysis by a GTPase.
GO:0043547 positive regulation of GTPase activity	3/42 (7.1%)	*Ccl12; Ccl2; Ccl7*	69.1 (38.2–113.2)	Any process that activates or increases the activity of a GTPase.
GO:0097529 myeloid leukocyte migration	3/51 (5.9%)	*Ccl12; Ccl2; Ccl7*	69.1 (38.2–113.2)	The movement of a myeloid leukocyte within or between different tissues and organs of the body.
GO:0007178 transmembrane receptor protein serine/threonine kinase signaling pathway	4/52 (7.7%)	*Cldn5; Ccl2; Bambi; Stat3*	87.5 (41.0–425.7)	A series of molecular signals initiated by the binding of an extracellular ligand to a receptor on the surface of the target cell where the receptor possesses serine/ threonine kinase activity, and ending with regulation of a downstream cellular process, e.g., transcription.
GO:0009617 response to bacterium	6/114 (5.3%)	*Serpine1; Ccl2; Ifi44; Gsta1; Hba-a1; Hba-a2*	101.1 (52.8–140.7)	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus from a bacterium.
GO:0002685 regulation of leukocyte migration	5/69 (7.3%)	*Mif; Serpine1; Ccl12; Ccl2; Ccl7*	101.1 (54.7–142.2)	Any process that modulates the frequency, rate, or extent of leukocyte migration.

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology.

[a]Definitions of GO terms were adapted from the Gene Ontology Resource.[35] Official gene symbols from the Rat Genome Database[36] are shown in the "Active Genes" column.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

GO process descriptions and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Table E-2. Active Genes and Definitions within Top 10 Kidney Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
Rats				
GO:0048568 embryonic organ development	3/47 (6.4%)	*Slc6a8; Gatm; Atf4*	114.0 (73.9–NC)	Development, taking place during the embryonic phase, of a tissue or tissues that work together to perform a specific function or functions. Development pertains to the process whose specific outcome is the progression of a structure over time, from its formation to the mature structure. Organs are commonly observed as visibly distinct structures, but may also exist as loosely associated clusters of cells that work together to perform a specific function or functions.
GO:0072330 monocarboxylic acid biosynthetic process	6/56 (10.7%)	*Hsd17b10; Gstp1; Gstm2; Gatm; Cd74; Aldh1a1*	151.9 (109.0–183.8)	The chemical reactions and pathways resulting in the formation of monocarboxylic acids, any organic acid containing one carboxyl (-COOH) group.
GO:0120161 regulation of cold-induced thermogenesis	9/55 (16.4%)	*Prlr; Mfap2; Gnas; Gatm; Decr1; Cpt2; Atf4; Bmal1; Aldh1a1*	151.9 (109.0–183.8)	Any process that modulates the frequency, rate or extent of cold-induced thermogenesis.
GO:0098754 detoxification	12/58 (20.7%)	*Srxn1; Sod3; Slc22a2; Nqo1; Gstp1; Gstm2; Gsta3; Gsta2; Gpx3; Cat; Aldh1a1; Akr7a3*	163.7 (105.1–421.6)	Any process that reduces or removes the toxicity of a toxic substance. These may include transport of the toxic substance away from sensitive areas and to compartments or complexes whose purpose is sequestration of the toxic substance.
GO:1990748 cellular detoxification	10/51 (19.6%)	*Srxn1; Sod3; Slc22a2; Nqo1; Gstp1; Gstm2; Gsta2; Gpx3; Cat; Aldh1a1*	163.7 (105.1–421.6)	Any process carried out at the cellular level that reduces or removes the toxicity of a toxic substance. These may include transport of the toxic substance away from sensitive areas and to compartments or complexes whose purpose is sequestration of the toxic substance.
GO:0015718 monocarboxylic acid transport	8/64 (12.5%)	*Slc6a8; Slc5a6; Slc51a; Slc22a7; Slc22a2; Plin2; Cpt2; Abcc4*	165.8 (52.8–289.8)	The directed movement of monocarboxylic acids into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0006805 xenobiotic metabolic process	11/59 (18.6%)	*Gstp1; Gstm2; Gsta1; Gsta3; Gsta2; Gsta5; Fmo3; Cyp2f4; Cyp2e1; Acaa1b; Acaa1a*	169.1 (112.1–177.2)	The chemical reactions and pathways involving a xenobiotic compound, a compound foreign to living organisms. Used of chemical compounds, e.g., a xenobiotic chemical, such as a pesticide.
GO:0009062 fatty acid catabolic process	14/41 (34.2%)	*Pck1; Hsd17b10; Hao2; Gcdh; Ehhadh; Eci2; Eci1; Decr1; Cpt2; Acox1; Acadm; Acaa2; Acaa1b; Acaa1a*	169.1 (112.1–177.2)	The chemical reactions and pathways resulting in the breakdown of a fatty acid, any of the aliphatic monocarboxylic acids that can be liberated by hydrolysis from naturally occurring fats and oils. Fatty acids are predominantly straight-chain acids of 4 to 24 carbon atoms, which may be saturated or unsaturated; branched fatty acids and hydroxy fatty acids also occur, and very long chain acids of over 30 carbons are found in waxes.
GO:0072329 monocarboxylic acid catabolic process	15/49 (30.6%)	*Pck1; Hsd17b10; Hao2; Gcdh; Ehhadh; Eci2; Eci1; Decr1; Cryl1; Cpt2; Acox1; Acadm; Acaa2; Acaa1b; Acaa1a*	169.1 (112.1–177.2)	The chemical reactions and pathways resulting in the breakdown of monocarboxylic acids, any organic acid containing one carboxyl (-COOH) group.
GO:0098869 cellular oxidant detoxification	8/43 (18.6%)	*Srxn1; Sod3; Nqo1; Gstp1; Gstm2; Gsta2; Gpx3; Cat*	171.4 (84.7–903.1)	Any process carried out at the cellular level that reduces or removes the toxicity superoxide radicals or hydrogen peroxide.
Mice				
GO:0015850 organic hydroxy compound transport	6/58 (10.3%)	*Apoc3; Ldlr; Ceacam2; Abcc3; Tsku; Slc51a*	2.3 (0.6–219.7)	The directed movement of an organic hydroxy compound (organic alcohol) into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. An organic hydroxy compound is an organic compound having at least one hydroxy group attached to a carbon atom.
GO:0033559 unsaturated fatty acid metabolic process	7/40 (17.5%)	*Cyp2a5; Cd74; Cyp4a10; Gstp2; Ces2e; Ces2b; Cyp4a14*	2.6 (1.2–54.7)	The chemical reactions and pathways involving an unsaturated fatty acid, any fatty acid containing one or more double bonds between carbon atoms.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0055088 lipid homeostasis	5/52 (9.6%)	*Lpl; Insig1; Apoc3; Ldlr; Tsku*	2.7 (0.8–13.0)	Any process involved in the maintenance of an internal steady state of lipid within an organism or cell.
GO:0006869 lipid transport	11/77 (14.3%)	*Ppard; Apoc3; Ldlr; Ceacam2; Abcc3; Ace; Fabp7; Tsku; Slc22a7; Fabp1; Slc51a*	3.0 (0.8–9.9)	The directed movement of lipids into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. Lipids are compounds soluble in an organic solvent but not, or sparingly, in an aqueous solvent.
GO:0044242 cellular lipid catabolic process	7/44 (15.9%)	*Lpl; Ppard; Acadm; Apoc3; Acat2; Eci1; Hao2*	3.0 (0.8–401.3)	The chemical reactions and pathways resulting in the breakdown of lipids, as carried out by individual cells.
GO:1905952 regulation of lipid localization	4/52 (7.7%)	*Lpl; Ppard; Igfbp3; Apoc3*	3.0 (0.8–401.3)	Any process that modulates the frequency, rate or extent of lipid localization.
GO:0015718 monocarboxylic acid transport	8/52 (15.4%)	*Ppard; Ceacam2; Abcc3; Ace; Fabp7; Slc22a7; Fabp1; Slc51a*	3.0 (0.9–401.3)	The directed movement of monocarboxylic acids into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.
GO:0031589 cell-substrate adhesion	3/40 (7.5%)	*Ppard; Lamb1; Itga6*	3.1 (0.9–792.8)	The attachment of a cell to the underlying substrate via adhesion molecules.
GO:0031669 cellular response to nutrient levels	5/62 (8.1%)	*Lpl; Ppard; Cdkn1a; Bmf; Glul*	3.3 (1.0–408.4)	Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus reflecting the presence, absence, or concentration of nutrients.
GO:0090277 positive regulation of peptide hormone secretion	3/40 (7.5%)	*Apln; Ppard; Glul*	3.5 (1.2–24.1)	Any process that increases the rate, frequency, or extent of the regulated release of a peptide hormone from secretory granules.

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology; NC = nonconvergent.
[a]Definitions of GO terms were adapted from the Gene Ontology Resource.[35] Official gene symbols from the Rat Genome Database[36] are shown in the "Active Genes" column.
[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.
GO process descriptions and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Table E-3. Active Genes and Definitions within Top 10 Liver Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
Rats				
GO:0006805 xenobiotic metabolic process	31/59 (52.5%)	*Ugt2b1; Ugt1a3; Ugt1a2; Ugt1a6; Ugt1a1; Ugt1a9; Sult1c3; Sult1b1; Gstp1; Gstm7; Gstm2; Gsta4; Gsta1; Gsta3; Gsta2; Gsta5; Fmo3; Cyp3a2; Cyp2j4; Cyp2e1; Cyp2d5; Cyp2d3; Cyp2d1; Cyp2b3; Cyp2b1; Cyp1a2; Cbr1; Acaa1b; Acaa1a; Abcc2; Abcb11*	29.8 (25.3–89.7)	The chemical reactions and pathways involving a xenobiotic compound, a compound foreign to living organisms. Used of chemical compounds, e.g., a xenobiotic chemical, such as a pesticide.
GO:0098754 detoxification	30/58 (51.7%)	*Txnrd1; Srxn1; Sod3; Sod2; Ptgs1; Prdx3; Prdx2; Prdx1; Nqo1; Nfe2l2; Mt2a; Mt2-ps1; Mt1; Hp; Gstp1; Gstm7; Gstm2; Gsta3; Gsta2; Gsr; Gpx7; Gpx4; Gpx1; Fabp1; Cp; Apom; Ambp; Aldh1a1; Akr7a3; Abcc2*	33.8 (18.2–99.6)	Any process that reduces or removes the toxicity of a toxic substance. These may include transport of the toxic substance away from sensitive areas and to compartments or complexes whose purpose is sequestration of the toxic substance.
GO:1990748 cellular detoxification	23/51 (45.1%)	*Txnrd1; Srxn1; Sod3; Sod2; Ptgs1; Prdx3; Prdx2; Prdx1; Nqo1; Hp; Gstp1; Gstm7; Gstm2; Gsta2; Gsr; Gpx7; Gpx4; Gpx1; Fabp1; Cp; Apom; Ambp; Aldh1a1*	33.8 (18.2–99.6)	Any process carried out at the cellular level that reduces or removes the toxicity of a toxic substance. These may include transport of the toxic substance away from sensitive areas and to compartments or complexes whose purpose is sequestration of the toxic substance.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0048872 homeostasis of number of cells	20/58 (34.5%)	*Tuba1a; Slc40a1; Slc37a4; Rpa1; Ripk3; Rac1; Prdx2; Prdx1; Ppp2r3c; Polb; Map7; Ppp2ca; Ikbkb; Hmox1; Hif1a; Ccnb2; Casp3; Bax; Bak1; Abl1*	34.9 (10.5–93.0)	Any biological process involved in the maintenance of the steady-state number of cells within a population of cells.
GO:0006690 icosanoid metabolic process	20/50 (40.0%)	*Tnfrsf1a; Ptgs1; Mif; Mgll; Gstp1; Gsta1; Gsta2; Gpx4; Gpx1; Ephx1; Cyp4f4; Cyp2j4; Cyp2e1; Cyp2d5; Cyp2d3; Cyp2d1; Cyp2b3; Cyp2b1; Cyp1a2; Acox1*	35.9 (19.9–101.2)	The chemical reactions and pathways involving icosanoids, any of a group of C20 polyunsaturated fatty acids.
GO:0033559 unsaturated fatty acid metabolic process	24/54 (44.4%)	*Tnfrsf1a; Scd; Ptgs1; Mif; Mgll; Gstp1; Gstm7; Gstm2; Gsta1; Gsta2; Gpx4; Gpx1; Ephx1; Elovl5; Cyp4f4; Cyp2j4; Cyp2e1; Cyp2d5; Cyp2d3; Cyp2d1; Cyp2b3; Cyp2b1; Cyp1a2; Acox1*	35.9 (19.9–101.2)	The chemical reactions and pathways involving an unsaturated fatty acid, any fatty acid containing one or more double bonds between carbon atoms.
GO:1901136 carbohydrate derivative catabolic process	16/49 (32.7%)	*Vnn1; Pklr; Pgam1; Nudt9; Hprt1; Hmmr; Gapdh; Fuca1; Eno3; Dpys; Ces1f; Cd44; Amdhd2; Aldoa; Aldh1a1; Acot2*	36.3 (9.5–438.0)	The chemical reactions and pathways resulting in the breakdown of carbohydrate derivative.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0120254 olefinic compound metabolic process	28/64 (43.8%)	*Srd5a1; Rbp1; Ptgs1; Pnpla2; Mgll; Inhba; Gstp1; Gstm7; Gstm2; Gsta1; Gpx4; Gpx1; Ephx1; Dhrs7; Cyp4f4; Cyp3a2; Cyp2j4; Cyp2e1; Cyp2d5; Cyp2d3; Cyp2d1; Cyp2b3; Cyp2b1; Cyp1a2; Cyp17a1; Ces1f; Ces1d; Aldh1a1*	37.4 (9.5–455.2)	The chemical reactions and pathways involving an olefinic compound, any compound which contains a carbon-carbon double bond (aka C=C).
GO:0044770 cell cycle phase transition	26/58 (44.8%)	*Usp22; Rps6; Rb1; Pole; Mnat1; Itgb1; Hspa8; Gadd45a; Eif4ebp1; Eif4e; E2f1; Cks2; Chek2; Cdk6; Cdk4; Cdc25a; Cdc20; Ccng1; Ccnf; Ccnd1; Ccnb2; Ccnb2-ps2; Ccna2; Birc5; App; Akap8*	38.7 (14.3–79.1)	The cell cycle process by which a cell commits to entering the next cell cycle phase.
GO:0098869 cellular oxidant detoxification	22/43 (51.2%)	*Txnrd1; Srxn1; Sod3; Sod2; Ptgs1; Prdx3; Prdx2; Prdx1; Nqo1; Hp; Gstp1; Gstm7; Gstm2; Gsta2; Gsr; Gpx7; Gpx4; Gpx1; Fabp1; Cp; Apom; Ambp*	40.1 (11.7–95.6)	Any process carried out at the cellular level that reduces or removes the toxicity superoxide radicals or hydrogen peroxide.
Mice				
GO:0046889 positive regulation of lipid biosynthetic process	15/45 (33.3%)	*Apoe; Fgf1; Cd74; Elovl5; Igf1; Cyp7a1; Mapk9; Ppara; Il1b; Lpgat1; Por; Scp2; Abcd1; Apoa5; Abcg1*	1.5 (0.5–5.5)	Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways resulting in the formation of lipids.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0045834 positive regulation of lipid metabolic process	21/61 (34.4%)	*Gdf15; Apoe; Fgf1; Cd74; Elovl5; Igf1; Cyp7a1; Mapk9; Ppara; Ces1g; Il1b; Lpgat1; Por; Scp2; Nucb2; Mup1; Abcd1; Fabp1; Apoa5; Fgf21; Abcg1*	2.1 (0.7–5.0)	Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways involving lipids.
GO:0002708 positive regulation of lymphocyte mediated immunity	6/40 (15.0%)	*Rsad2; Stat5b; Il1b; C3; H2-t23; Lamp1*	2.2 (0.6–6.3)	Any process that activates or increases the frequency, rate, or extent of lymphocyte mediated immunity.
GO:0006805 xenobiotic metabolic process	25/56 (44.6%)	*Cyp2c38; Cyp2c39; Fmo5; Gsta2; Cyp2j5; Cyp2c29; Cyp2d9; Cbr1; Cyp3a41b; Cyp2b10; Gstp2; Gsta3; Cyp3a41a; Cyp2a4; Cyp2c54; Gstm5; Gsta1; Abcc2; Cyp2c55; Cyp3a44; Cyp2e1; Cyp1a2; Ugt1a6a; Ugt1a6b; Gstm2*	2.2 (0.3–88.7)	The chemical reactions and pathways involving a xenobiotic compound, a compound foreign to living organisms. Used of chemical compounds, e.g., a xenobiotic chemical, such as a pesticide.
GO:0048511 rhythmic process	25/89 (28.1%)	*Foxo3; Inhba; Ezh2; Afp; Src; Prkaa1; Dbp; Cdk1; Nfil3; Sgpl1; Igf1; Timeless; Gfpt1; Top2a; Suv39h1; Hlf; Mapk9; Ppara; Stat5b; Dnm1l; Mtnr1a; Egfr; Atf5; Mup1; Dtl*	2.9 (0.9–13.7)	Any process pertinent to the generation and maintenance of rhythms in the physiology of an organism.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0097191 extrinsic apoptotic signaling pathway	15/56 (26.8%)	*Foxo3; Hmgb2; Inhba; Mcl1; Igf1; Cttn; Krt8; Bex3; Lcn2; Parp2; Il1b; Tlr3; Bax; Prdx2; Krt18*	2.9 (0.9–13.7)	A series of molecular signals in which a signal is conveyed from the cell surface to trigger the apoptotic death of a cell. The pathway starts with either a ligand binding to a cell surface receptor, or a ligand being withdrawn from a cell surface receptor (e.g., in the case of signaling by dependence receptors), and ends when the execution phase of apoptosis is triggered.
GO:0019218 regulation of steroid metabolic process	17/46 (37.0%)	*Fmo5; Apoe; Fgf1; Insig1; Igf1; Pank2; Ephx2; Stub1; Dhcr7; Fgfr4; Cyp7a1; Stat5b; Ces1g; Por; Scp2; Asah1; Abcg1*	3.5 (0.9–4,078.8)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving steroids.
GO:0002705 positive regulation of leukocyte mediated immunity	7/47 (14.9%)	*Rsad2; Tyrobp; Stat5b; Il1b; C3; H2-t23; Lamp1*	3.7 (0.9–4,079.6)	Any process that activates or increases the frequency, rate, or extent of leukocyte mediated immunity.
GO:0002706 regulation of lymphocyte mediated immunity	8/59 (13.6%)	*Nckap1l; Rsad2; Stat5b; Il1b; C3; H2-t23; Ccr2; Lamp1*	3.7 (0.9–4,079.6)	Any process that modulates the frequency, rate, or extent of lymphocyte mediated immunity.
GO:0120254 olefinic compound metabolic process	26/51 (51%)	*Cyp2c38; Cyp2c39; Inhba; Afp; Gpx4; Cyp2j5; Adh1; Cyp2c29; Cyp2d9; Rdh7; Ephx2; Cyp2b10; Gstp2; Stat5b; Cyp2a4; Cyp2c54; Aldh1a1; Cyp2c55; Scp2; Ephx1; Cyp4a31; Hsd17b6; Cyp2e1; Cyp1a2; Srd5a1; Lrat*	3.7 (0.8–4,080.3)	The chemical reactions and pathways involving an olefinic compound, any compound which contains a carbon-carbon double bond (aka C=C).

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology.

[a]Definitions of GO terms were adapted from the Gene Ontology Resource.[35] Official gene symbols from the Rat Genome Database[36] are shown in the "Active Genes" column.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

GO process descriptions and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Table E-4. Active Genes and Definitions within Top 10 Lung Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
Rats				
GO:0098754 detoxification	11/58 (19.0%)	*Txnrd1; Srxn1; Selenow; Prdx1; Nqo1; Hbb-bs; Hbbl1; Hbb; Gstp1; Gsta3; Gpx4*	175.9 (109.3–224.0)	Any process that reduces or removes the toxicity of a toxic substance. These may include transport of the toxic substance away from sensitive areas and to compartments or complexes whose purpose is sequestration of the toxic substance.
GO:0098869 cellular oxidant detoxification	10/43 (23.3%)	*Txnrd1; Srxn1; Selenow; Prdx1; Nqo1; Hbb-bs; Hbbl1; Hbb; Gstp1; Gpx4*	175.9 (109.3–224.0)	Any process carried out at the cellular level that reduces or removes the toxicity superoxide radicals or hydrogen peroxide.
GO:1990748 cellular detoxification	10/51 (19.6%)	*Txnrd1; Srxn1; Selenow; Prdx1; Nqo1; Hbb-bs; Hbbl1; Hbb; Gstp1; Gpx4*	175.9 (109.3–224.0)	Any process carried out at the cellular level that reduces or removes the toxicity of a toxic substance. These may include transport of the toxic substance away from sensitive areas and to compartments or complexes whose purpose is sequestration of the toxic substance.
GO:0019362 pyridine nucleotide metabolic process	5/40 (12.5%)	*Txnrd1; Nqo1; Me1; Aldoc; Aldoa*	181.2 (46.6–348.4)	The chemical reactions and pathways involving a pyridine nucleotide, a nucleotide characterized by a pyridine derivative as a nitrogen base.
GO:0046496 nicotinamide nucleotide metabolic process	5/40 (12.5%)	*Txnrd1; Nqo1; Me1; Aldoc; Aldoa*	181.2 (46.6–348.4)	The chemical reactions and pathways involving nicotinamide nucleotides, any nucleotide that contains combined nicotinamide.
GO:0072524 pyridine-containing compound metabolic process	5/41 (12.2%)	*Txnrd1; Nqo1; Me1; Aldoc; Aldoa*	181.2 (37.4–352.1)	The chemical reactions and pathways involving a pyridine-containing compound, i.e., any compound that contains pyridine or a formal derivative thereof.
GO:0001676 long-chain fatty acid metabolic process	5/55 (9.1%)	*Gstp1; Gsta1; Gpx4; Cyp2e1; Cd36*	193.6 (60.7–298.8)	The chemical reactions and pathways involving long-chain fatty acids, A long-chain fatty acid is a fatty acid with a chain length between C13 and C22.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0006690 icosanoid metabolic process	5/50 (10.0%)	*Mif; Gstp1; Gsta1; Gpx4; Cyp2e1*	193.6 (60.7–298.8)	The chemical reactions and pathways involving icosanoids, any of a group of C20 polyunsaturated fatty acids.
GO:0006805 xenobiotic metabolic process	6/59 (10.2%)	*Gstp1; Gsta4; Gsta1; Gsta3; Cyp2e1; Cbr1*	193.6 (60.7–298.8)	The chemical reactions and pathways involving a xenobiotic compound, a compound foreign to living organisms. Used of chemical compounds, e.g., a xenobiotic chemical, such as a pesticide.
GO:0033559 unsaturated fatty acid metabolic process	5/54 (9.3%)	*Mif; Gstp1; Gsta1; Gpx4; Cyp2e1*	193.6 (60.7–298.8)	The chemical reactions and pathways involving an unsaturated fatty acid, any fatty acid containing one or more double bonds between carbon atoms.
Mice				
GO:0033559 unsaturated fatty acid metabolic process	8/40 (20.0%)	*Cyp2a5; Gpx4; Mif; Gstp2; Acox1; Ptgs2; Cyp2a4; Ephx1*	2.1 (0.7–4.8)	The chemical reactions and pathways involving an unsaturated fatty acid, any fatty acid containing one or more double bonds between carbon atoms.
GO:0008203 cholesterol metabolic process	8/48 (16.7%)	*Prkaa1; Sqle; G6pdx; Prkaa2; Ces1g; Il4; Tsku; Cat*	2.4 (0.7–5.8)	The chemical reactions and pathways involving cholesterol, cholest-5-en-3 beta-ol, the principal sterol of vertebrates and the precursor of many steroids, including bile acids and steroid hormones. It is a component of the plasma membrane lipid bilayer and of plasma lipoproteins and can be found in all animal tissues.
GO:0016125 sterol metabolic process	8/49 (16.3%)	*Prkaa1; Sqle; G6pdx; Prkaa2; Ces1g; Il4; Tsku; Cat*	2.4 (0.7–5.8)	The chemical reactions and pathways involving sterols, steroids with one or more hydroxyl groups and a hydrocarbon side-chain in the molecule.
GO:1902652 secondary alcohol metabolic process	10/53 (18.9%)	*Idh3g; Prkaa1; Sqle; G6pdx; Prkaa2; Ces1g; Il4; Idh3a; Tsku; Cat*	2.4 (0.7–5.8)	The chemical reactions and pathways involving secondary alcohol.
GO:0002708 positive regulation of lymphocyte mediated immunity	4/40 (10.0%)	*Rsad2; Pnp; Il4; Il6*	2.8 (0.9–843.5)	Any process that activates or increases the frequency, rate, or extent of lymphocyte mediated immunity.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0120254 olefinic compound metabolic process	11/51 (21.6%)	*Cyp2a5; Afp; Gpx4; Adh1; Gstp2; Ptgs2; Cyp2a4; Aldh1a1; Scp2; Ephx1; Lrat*	3.5 (0.7–53.9)	No description available.
GO:0006690 icosanoid metabolic process	8/43 (18.6%)	*Cyp2a5; Gpx4; Mif; Gstp2; Acox1; Ptgs2; Cyp2a4; Ephx1*	3.7 (0.9–11.6)	The chemical reactions and pathways involving icosanoids, any of a group of C20 polyunsaturated fatty acids.
GO:0006805 xenobiotic metabolic process	14/56 (25.0%)	*Cyp2a5; Gsta2; Fmo3; Cbr1; Gstp2; Gsta3; Ugt1a1; Cyp2a4; Gsta1; Cryz; Acaa1a; Ugt1a6a; Ugt1a6b; Gstm2*	3.8 (1.0–10.4)	The chemical reactions and pathways involving a xenobiotic compound, a compound foreign to living organisms. Used of chemical compounds, e.g., a xenobiotic chemical, such as a pesticide.
GO:0006066 alcohol metabolic process	22/90 (24.4%)	*Idh3g; Prkaa1; Adh1; Sqle; G6pdx; Prkaa2; Pck2; Tpi1; Abhd4; Pcbd1; Ces1g; Il4; Dpm2; Adh5; Aldh1a1; Idh3a; Aldh3a2; Scp2; Tsku; Ephx1; Cat; Lrat*	5.0 (0.9–60.7)	The chemical reactions and pathways involving alcohols, any of a class of compounds containing one or more hydroxyl groups attached to a saturated carbon atom.
GO:0015850 organic hydroxy compound transport	7/58 (12.1%)	*Slc16a3; Ces1g; Abcc4; Scp2; Tsku; Ceacam1; Slc16a7*	5.1 (1.3–26.0)	The directed movement of an organic hydroxy compound (organic alcohol) into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. An organic hydroxy compound is an organic compound having at least one hydroxy group attached to a carbon atom.

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology.

[a]Definitions of GO terms were adapted from the Gene Ontology Resource.[35] Official gene symbols from the Rat Genome Database[36] are shown in the "Active Genes" column.

[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.

GO process descriptions and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Table E-5. Active Genes and Definitions within Top 10 Ovary Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by 5th Percentile Benchmark Dose, for Female Rats and Mice Exposed to 1,2-Dichlorobenzene for Five Days

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
Rats				
GO:0001822 kidney development	4/57 (7.0%)	*Ace; Ret; Lef1; Gata3*	156.7 (50.8–280.4)	The process whose specific outcome is the progression of the kidney over time, from its formation to the mature structure. The kidney is an organ that filters the blood and/or excretes the end products of body metabolism in the form of urine.
GO:0008406 gonad development	3/51 (5.9%)	*Ace; Hmgcs1; Gata3*	156.7 (50.8–280.4)	The process whose specific outcome is the progression of the gonad over time, from its formation to the mature structure. The gonad is an animal organ that produces gametes; in some species it also produces hormones.
GO:0008584 male gonad development	3/46 (6.5%)	*Ace; Hmgcs1; Gata3*	156.7 (50.8–280.4)	The process whose specific outcome is the progression of the male gonad over time, from its formation to the mature structure.
GO:0045582 positive regulation of T cell differentiation	3/40 (7.5%)	*Tgfbr2; Lef1; Gata3*	160.7 (51.6–289.6)	Any process that activates or increases the frequency, rate or extent of T cell differentiation.
GO:0016055 Wnt signaling pathway	4/41 (9.8%)	*Prkaa2; Fzd7; Lef1; Gata3*	162.6 (54.1–417.6)	The series of molecular signals initiated by binding of a Wnt protein to a frizzled family receptor on the surface of the target cell and ending with a change in cell state.
GO:0030217 T cell differentiation	3/48 (6.3%)	*Fzd7; Lef1; Gata3*	162.6 (54.1–417.6)	The process in which a precursor cell type acquires characteristics of a more mature T-cell. A T cell is a type of lymphocyte whose defining characteristic is the expression of a T cell receptor complex.
GO:0030856 regulation of epithelial cell differentiation	3/53 (5.7%)	*Lef1; Gata3; Dmbt1*	163.7 (49.2–293.8)	Any process that modulates the frequency, rate or extent of epithelial cell differentiation.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0006694 steroid biosynthetic process	3/54 (5.6%)	*Prkaa2; Idi1; Hmgcs1*	175.7 (70.6–293.3)	The chemical reactions and pathways resulting in the formation of steroids, compounds with a 1,2,cyclopentanoperhydrophenanthrene nucleus; includes de novo formation and steroid interconversion by modification.
GO:0008203 cholesterol metabolic process	3/54 (5.6%)	*Prkaa2; Idi1; Hmgcs1*	175.7 (70.6–293.3)	The chemical reactions and pathways involving cholesterol, cholest-5-en-3 beta-ol, the principal sterol of vertebrates and the precursor of many steroids, including bile acids and steroid hormones. It is a component of the plasma membrane lipid bilayer and of plasma lipoproteins and can be found in all animal tissues.
GO:0016125 sterol metabolic process	3/56 (5.4%)	*Prkaa2; Idi1; Hmgcs1*	175.7 (70.6–293.3)	The chemical reactions and pathways involving sterols, steroids with one or more hydroxyl groups and a hydrocarbon side-chain in the molecule.
Mice				
GO:0030097 hemopoiesis	3/56 (5.4%)	*Tacc3; Hba-a2; Cdk6*	108.0 (35.9–NC)	The process whose specific outcome is the progression of the myeloid and lymphoid derived organ/tissue systems of the blood and other parts of the body over time, from formation to the mature structure. The site of hemopoiesis is variable during development, but occurs primarily in bone marrow or kidney in many adult vertebrates.
GO:0072594 establishment of protein localization to organelle	5/77 (6.5%)	*Mmp12; Sqstm1; Dnajc15; Cd68; Timm9*	110.3 (57.1–152.8)	The directed movement of a protein to a specific location on or in an organelle. Encompasses establishment of localization in the membrane or lumen of a membrane-bounded organelle.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0048167 regulation of synaptic plasticity	4/75 (5.3%)	*Apoe; Grin2c; Sqstm1; Syn1*	119.5 (80.8–248.7)	A process that modulates synaptic plasticity, the ability of synapses to change as circumstances require. They may alter function, such as increasing or decreasing their sensitivity, or they may increase or decrease in actual numbers.
GO:0050777 negative regulation of immune response	3/53 (5.7%)	*Nckap1l; Mmp12; Lilrb4b*	125.7 (60.1–425.4)	Any process that stops, prevents, or reduces the frequency, rate or extent of the immune response, the immunological reaction of an organism to an immunogenic stimulus.
GO:0006898 receptor-mediated endocytosis	3/48 (6.3%)	*Apoe; Hmmr; Lilrb4b*	136.8 (83.0–271.7)	An endocytosis process in which cell surface receptors ensure specificity of transport. A specific receptor on the cell surface binds tightly to the extracellular macromolecule (the ligand) that it recognizes; the plasma-membrane region containing the receptor-ligand complex then undergoes endocytosis, forming a transport vesicle containing the receptor-ligand complex and excluding most other plasma-membrane proteins. Receptor-mediated endocytosis generally occurs via clathrin-coated pits and vesicles.
GO:0043409 negative regulation of MAPK cascade	3/47 (6.4%)	*Apoe; Lilrb4b; Rgs2*	136.8 (83.0–271.7)	Any process that stops, prevents, or reduces the frequency, rate or extent of signal transduction mediated by the MAPKKK cascade.
GO:0045580 regulation of T cell differentiation	3/60 (5.0%)	*Nckap1l; Ctla2a; Lilrb4b*	149.2 (81.9–684.2)	Any process that modulates the frequency, rate or extent of T cell differentiation.

Category Name[a]	Input Genes/Platform Genes in Gene Set (% Coverage)	Active Genes	BMD_{rd25} 5th Percentile of Gene Set Transcripts (BMD_{Lrd25}–BMD_{Urd25}) (ppm)[b]	Definition
GO:0002761 regulation of myeloid leukocyte differentiation	3/50 (6.0%)	*Fbn1; Lilrb4b; Cdk6*	153.6 (37.5–NC)	Any process that modulates the frequency, rate, or extent of myeloid leukocyte differentiation.
GO:0016050 vesicle organization	3/54 (5.6%)	*Apoe; Sqstm1; Trappc6a*	155.0 (87.7–288.7)	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of a vesicle.
GO:0051235 maintenance of location	3/46 (6.5%)	*Apoe; Fbn1; Tacc3*	155.5 (74.0–NC)	Any process in which a cell, substance or cellular entity, such as a protein complex or organelle, is maintained in a location and prevented from moving elsewhere.

BMD_{rd25} = benchmark dose corresponding to a benchmark response set to a 25% change in the median response; BMD_{Lrd25} = benchmark dose lower confidence limit corresponding to a benchmark response set to a 25% change in the median response; BMD_{Urd25} = benchmark dose upper confidence limit corresponding to a benchmark response set to a 25% change in the median response; GO = Gene Ontology; NC = nonconvergent.
[a]Definitions of GO terms were adapted from the Gene Ontology Resource.[35] Official gene symbols from the Rat Genome Database[36] are shown in the "Active Genes" column.
[b]5th percentile = the value below which 5% of transcript benchmark dose values fall.
GO process descriptions and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Table E-6. Gene Identifiers and Definitions for Female Rats Exposed to 1,2-Dichlorobenzene for Five Days[a]

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Ace	24310	Ace_34123	Human Uniprot function (Human *ACE*): Converts angiotensin I to angiotensin II by release of the terminal His-Leu, this results in an increase of the vasoconstrictor activity of angiotensin. Also able to inactivate bradykinin, a potent vasodilator. Has also a glycosidase activity which releases GPI-anchored proteins from the membrane by cleaving the mannose linkage in the GPI moiety.
Acot12	170570	ACOT12_32718	Human Uniprot function (Human *ACOT12*): Acyl-CoA thioesterases are a group of enzymes that catalyze the hydrolysis of acyl-CoAs to the free fatty acid and coenzyme A (CoASH), providing the potential to regulate intracellular levels of acyl-CoAs, free fatty acids and CoASH (PubMed:16951743). Acyl-coenzyme A thioesterase 12/ACOT12 preferentially hydrolyzes acetyl-CoA (PubMed:16951743).\{ECO:0000269|PubMed:16951743, ECO:0000303|PubMed:16951743\}.
Ak2	24184	RGD1562178_32879	Human Uniprot function (Human *AK2*): Catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP. Plays an important role in cellular energy homeostasis and in adenine nucleotide metabolism. Adenylate kinase activity is critical for regulation of the phosphate utilization and the AMP de novo biosynthesis pathways. Plays a key role in hematopoiesis.\{ECO:0000255|HAMAP-Rule:MF_03168, ECO:0000269|PubMed:19043416\}.
Akr1b8/Akr1b15	286921	AKR1B8_8013	Human Uniprot function (Human *AKR1B10*): Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols (PubMed:9565553, PubMed:18087047, PubMed:12732097, PubMed:19013440, PubMed:19563777). Displays strong enzymatic activity toward all-trans-retinal, 9-cis-retinal, and 13-cis-retinal (PubMed:12732097, PubMed:18087047). Plays a critical role in detoxifying dietary and lipid-derived unsaturated carbonyls, such as crotonaldehyde, 4-hydroxynonenal, trans-2-hexenal, trans-2,4-hexadienal and their glutathione- conjugates carbonyls (GS-carbonyls) (PubMed:19013440, PubMed:19563777). Displays no reductase activity toward glucose (PubMed:12732097). \{ECO:0000269|PubMed:12732097, ECO:0000269|PubMed:18087047, ECO:0000269|PubMed:19013440, ECO:0000269|PubMed:19563777, ECO:0000269|PubMed:9565553\}.
Aldh1a1	24188	ALDH1A1_8022	Human Uniprot function (Human *ALDH1A1*): Can convert/oxidize retinaldehyde to retinoic acid. Binds free retinal and cellular retinol-binding protein-bound retinal (By similarity). May have a broader specificity and oxidize other aldehydes in vivo (PubMed:19296407, PubMed:26373694, PubMed:25450233). \{ECO:0000250|UniProtKB:P51647, ECO:0000269|PubMed:19296407, ECO:0000269|PubMed:25450233, ECO:0000269|PubMed:26373694\}.
Aldoc	24191	ALDOC_8034	Human Entrez Gene Summary (Human *ALDOC*): This gene encodes a member of the class I fructose-biphosphate aldolase gene family. Expressed specifically in the hippocampus and Purkinje cells of the brain, the encoded protein is a glycolytic enzyme that catalyzes the reversible aldol cleavage of fructose-1,6-biphosphate and fructose 1-phosphate to dihydroxyacetone phosphate and either glyceraldehyde-3-phosphate or glyceraldehyde, respectively. [provided by RefSeq, Jul 2008]

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Apln	58812	APLN_33320	Human Uniprot function (Human *APLN*): Endogenous ligand for the apelin receptor (APLNR) (PubMed:10525157). Drives internalization of the apelin receptor (By similarity). Apelin-36 dissociates more hardly than (pyroglu)apelin-13 from APLNR (By similarity). Hormone involved in the regulation of cardiac precursor cell movements during gastrulation and heart morphogenesis (By similarity). Has an inhibitory effect on cytokine production in response to T-cell receptor/CD3 cross-linking; the oral intake of apelin in the colostrum and the milk might therefore modulate immune responses in neonates (By similarity). Plays a role in early coronary blood vessels formation (By similarity). Mediates myocardial contractility in an ERK1/2-dependent manner (By similarity). May also have a role in the central control of body fluid homeostasis by influencing vasopressin release and drinking behavior (By similarity). \{ECO:0000250|UniProtKB:Q4TTN8, ECO:0000250|UniProtKB:Q9R0R3, ECO:0000250|UniProtKB:Q9R0R4, ECO:0000269|PubMed:10525157\}.; FUNCTION: (Microbial infection) Endogenous ligand for the apelin receptor (APLNR), an alternative coreceptor with CD4 for HIV-1 infection (PubMed:11090199). Inhibits HIV-1 entry in cells coexpressing CD4 and APLNR (PubMed:11090199). Apelin-36 has a greater inhibitory activity on HIV infection than other synthetic apelin derivatives (PubMed:11090199). \{ECO:0000269|PubMed:11090199\}.
Atf4	79255	ATF4_8096	Human Uniprot function (Human *ATF4*): Transcription factor that binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3') and acts both as a regulator of normal metabolic and redox processes, and as a master transcription factor during the integrated stress response (ISR) (PubMed:1847461, PubMed:16682973, PubMed:31444471, PubMed:32132707). Binds to asymmetric CRE's as a heterodimer and to palindromic CRE's as a homodimer (By similarity). Core effector of the ISR, which is required for adaptation to various stress, such as endoplasmic reticulum (ER) stress, amino acid starvation, mitochondrial stress or oxidative stress (PubMed:32132707). During the ISR, ATF4 protein is translated in response to eIF-2-alpha/EIF2S1 phosphorylation caused by stress, and acts as a master transcription factor of stress-responsive genes in order to promote cell recovery (PubMed:32132707). Protects cells against metabolic consequences of ER oxidation by promoting expression of genes linked to amino acid sufficiency and resistance to oxidative stress (By similarity). Regulates the induction of DDIT3/CHOP and asparagine synthetase (ASNS) in response to amino acid deprivation or endoplasmic reticulum (ER) stress (PubMed:11960987). Together with DDIT3/CHOP, mediates ER-mediated cell death by promoting expression of genes involved in cellular amino acid metabolic processes, mRNA translation and the unfolded protein response (UPR) in response to ER stress (By similarity). ATF4 and DDIT3/CHOP activate the transcription of TRIB3 and promote ER stress-induced neuronal cell-death by regulating the expression of BBC3/PUMA (PubMed:15775988, PubMed:18940792). During ER stress response, activates the transcription of NLRP1, possibly in concert with other factors (PubMed:26086088). Activates expression of genes required to promote cell recovery in response to mitochondrial stress (PubMed:32132706, PubMed:32132707). Independently of the ISR, also required for normal metabolic processes: plays a key role in embryonic lens formation, fetal liver hematopoiesis, bone development and synaptic plasticity (By similarity). Acts as a regulator of osteoblast differentiation in response to phosphorylation by RPS6KA3/RSK2: phosphorylation in osteoblasts enhances transactivation activity and promotes expression of osteoblast-specific genes and post-transcriptionally regulates the synthesis of Type I collagen, the main constituent of the bone matrix (PubMed:15109498). Cooperates with FOXO1 in osteoblasts to regulate glucose homeostasis through suppression of beta-cell production and decrease in insulin production (By

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
			similarity). Activates transcription of SIRT4 (By similarity). Regulates the circadian expression of the core clock component PER2 and the serotonin transporter SLC6A4 (By similarity). Binds in a circadian time-dependent manner to the cAMP response elements (CRE) in the SLC6A4 and PER2 promoters and periodically activates the transcription of these genes (By similarity). Mainly acts as a transcriptional activator in cellular stress adaptation, but it can also act as a transcriptional repressor: acts as a regulator of synaptic plasticity by repressing transcription, thereby inhibiting induction and maintenance of long-term memory (By similarity). Regulates synaptic functions via interaction with DISC1 in neurons, which inhibits ATF4 transcription factor activity by disrupting ATF4 dimerization and DNA-binding (PubMed:31444471). {ECO:0000250|UniProtKB:Q06507, ECO:0000269|PubMed:11960987, ECO:0000269|PubMed:15109498, ECO:0000269|PubMed:15775988, ECO:0000269|PubMed:16682973, ECO:0000269|PubMed:1847461, ECO:0000269|PubMed:18940792, ECO:0000269|PubMed:26086088, ECO:0000269|PubMed:31444471, ECO:0000269|PubMed:32132706, ECO:0000269|PubMed:32132707}.; FUNCTION: (Microbial infection) Binds to a Tax-responsive enhancer element in the long terminal repeat of HTLV-I. {ECO:0000269|PubMed:1847461}.
Ccnb2	363088	CCNB2_8223	Human Uniprot function (Human *CCNB2*): Essential for the control of the cell cycle at the G2/M (mitosis) transition.
Ccnb2-ps2	100364016	CCNB2_8223	No description available.
Ces1f	100125372	CESL1_32986	Human Uniprot function (Human *CES1*): Involved in the detoxification of xenobiotics and in the activation of ester and amide prodrugs (PubMed:7980644, PubMed:9169443, PubMed:9490062, PubMed:18762277). Hydrolyzes aromatic and aliphatic esters, but has no catalytic activity toward amides or a fatty acyl-CoA ester (PubMed:7980644, PubMed:9169443, PubMed:9490062, PubMed:18762277). Hydrolyzes the methyl ester group of cocaine to form benzoylecgonine (PubMed:7980644). Catalyzes the transesterification of cocaine to form cocaethylene (PubMed:7980644). Displays fatty acid ethyl ester synthase activity, catalyzing the ethyl esterification of oleic acid to ethyloleate (PubMed:7980644). Converts monoacylglycerides to free fatty acids and glycerol. Hydrolyzes of 2-arachidonoylglycerol and prostaglandins (PubMed:21049984). Hydrolyzes cellular cholesteryl esters to free cholesterols and promotes reverse cholesterol transport (RCT) by facilitating both the initial and final steps in the process (PubMed:18762277, PubMed:16024911, PubMed:11015575, PubMed:16971496). First of all, allows free cholesterol efflux from macrophages to extracellular cholesterol acceptors and secondly, releases free cholesterol from lipoprotein-delivered cholesteryl esters in the liver for bile acid synthesis or direct secretion into the bile (PubMed:18762277, PubMed:18599737, PubMed:16971496). \{ECO:0000269|PubMed:11015575, ECO:0000269|PubMed:16024911, ECO:0000269|PubMed:16971496, ECO:0000269|PubMed:18599737, ECO:0000269|PubMed:18762277, ECO:0000269|PubMed:21049984, ECO:0000269|PubMed:7980644, ECO:0000269|PubMed:9169443, ECO:0000269|PubMed:9490062\}.
Dbp	24309	DBP_8439	Human Uniprot function (Human *DBP*): This transcriptional activator recognizes and binds to the sequence 5'-RTTAYGTAAY-3' found in the promoter of genes such as albumin, *CYP2A4* and *CYP2A5*. It is not essential for circadian rhythm generation, but modulates important clock output genes. May be a direct target for regulation by the circadian pacemaker component clock. May affect circadian period and sleep regulation.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Ddit4	140942	DDIT4_8450	Human Uniprot function (Human *DDIT4*): Regulates cell growth, proliferation and survival via inhibition of the activity of the mammalian target of rapamycin complex 1 (mTORC1). Inhibition of mTORC1 is mediated by a pathway that involves DDIT4/REDD1, AKT1, the TSC1-TSC2 complex and the GTPase RHEB. Plays an important role in responses to cellular energy levels and cellular stress, including responses to hypoxia and DNA damage. Regulates p53/TP53-mediated apoptosis in response to DNA damage via its effect on mTORC1 activity. Its role in the response to hypoxia depends on the cell type; it mediates mTORC1 inhibition in fibroblasts and thymocytes, but not in hepatocytes (By similarity). Required for mTORC1-mediated defense against viral protein synthesis and virus replication (By similarity). Inhibits neuronal differentiation and neurite outgrowth mediated by NGF via its effect on mTORC1 activity. Required for normal neuron migration during embryonic brain development. Plays a role in neuronal cell death. \{ECO:0000250, ECO:0000269|PubMed:15545625, ECO:0000269|PubMed:15632201, ECO:0000269|PubMed:15988001, ECO:0000269|PubMed:17005863, ECO:0000269|PubMed:17379067, ECO:0000269|PubMed:19557001, ECO:0000269|PubMed:20166753, ECO:0000269|PubMed:21460850\}.
Elovl5	171400	ELOVL5_8556	Human Uniprot function (Human *ELOVL5*): Catalyzes the first and rate-limiting reaction of the four reactions that constitute the long-chain fatty acids elongation cycle. This endoplasmic reticulum-bound enzymatic process allows the addition of 2 carbons to the chain of long- and very long-chain fatty acids (VLCFAs) per cycle. Condensing enzyme that acts specifically toward polyunsaturated acyl-CoA with the higher activity toward C18:3(n-6) acyl-CoA. May participate in the production of monounsaturated and of polyunsaturated VLCFAs of different chain lengths that are involved in multiple biological processes as precursors of membrane lipids and lipid mediators (By similarity) (PubMed:10970790, PubMed:20937905). In conditions where the essential linoleic and alpha linoleic fatty acids are lacking it is also involved in the synthesis of Mead acid from oleic acid (By similarity). \{ECO:0000250|UniProtKB:Q8BHI7, ECO:0000255|HAMAP-Rule:MF_03205, ECO:0000269|PubMed:10970790, ECO:0000269|PubMed:20937905\}.
Ephx1	25315	EPHX1_8567	Human Uniprot function (Human *EPHX1*): Biotransformation enzyme that catalyzes the hydrolysis of arene and aliphatic epoxides to less reactive and more water soluble dihydrodiols by the trans addition of water (By similarity). Plays a role in the metabolism of endogenous lipids such as epoxide-containing fatty acids (PubMed:22798687). Metabolizes the abundant endocannabinoid 2-arachidonoylglycerol (2-AG) to free arachidonic acid (AA) and glycerol (PubMed:24958911). \{ECO:0000250|UniProtKB:P07687, ECO:0000269|PubMed:22798687, ECO:0000269|PubMed:24958911\}.
Fetub	83928	FETUB_33027	Human Uniprot function (Human *FETUB*): Protease inhibitor required for egg fertilization. Required to prevent premature zona pellucida hardening before fertilization, probably by inhibiting the protease activity of ASTL, a protease that mediates the cleavage of ZP2 and triggers zona pellucida hardening (By similarity). \{ECO:0000250\}.
Fgf13	84488	FGF13_32529	Human Uniprot function (Human *FGF13*): Microtubule-binding protein which directly binds tubulin and is involved in both polymerization and stabilization of microtubules (By similarity). Through its action on microtubules, may participate in the refinement of axons by negatively regulating axonal and leading processes branching (By similarity). Plays a crucial role in neuron polarization and migration in the cerebral cortex and the hippocampus (By similarity). May regulate voltage-gated sodium channels transport and function (PubMed:15282281). May also play a role in MAPK signaling (By similarity). Required for the

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
			development of axonal initial segment-targeting inhibitory GABAergic synapses made by chandelier neurons (By similarity). \{ECO:0000250|UniProtKB:P70377, ECO:0000269|PubMed:15282281\}.
Gria2	29627	GRIA2_33071	Human Uniprot function (Human *GRIA2*): Receptor for glutamate that functions as ligand-gated ion channel in the central nervous system and plays an important role in excitatory synaptic transmission. L-glutamate acts as an excitatory neurotransmitter at many synapses in the central nervous system. Binding of the excitatory neurotransmitter L-glutamate induces a conformation change, leading to the opening of the cation channel, and thereby converts the chemical signal to an electrical impulse. The receptor then desensitizes rapidly and enters a transient inactive state, characterized by the presence of bound agonist. In the presence of CACNG4 or CACNG7 or CACNG8, shows resensitization which is characterized by a delayed accumulation of current flux upon continued application of glutamate. Through complex formation with NSG1, GRIP1 and STX12 controls the intracellular fate of AMPAR and the endosomal sorting of the GRIA2 subunit toward recycling and membrane targeting (By similarity). \{ECO:0000250|UniProtKB:P19491, ECO:0000269|PubMed:20614889\}.
Gsta2	24422	GSTA2_8756	Human Uniprot function (Human *GSTA2*): Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.
Gsta5	494499	GSTA2_8756	Human Uniprot function (Human *GSTA5*): Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.
Gstp1	24426	GSTP1_8762	Human Uniprot function (Human *GSTP1*): Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles. Involved in the formation of glutathione conjugates of both prostaglandin A2 (PGA2) and prostaglandin J2 (PGJ2) (PubMed:9084911). Participates in the formation of novel hepoxilin regioisomers (PubMed:21046276). Regulates negatively CDK5 activity via p25/ p35 translocation to prevent neurodegeneration. \{ECO:0000269|PubMed:21046276, ECO:0000269|PubMed:21668448, ECO:0000269|PubMed:9084911\}.
Hmgcs1	29637	HMGCS1_8811	Human Uniprot function (Human *HMGCS1*): Catalyzes the condensation of acetyl-CoA with acetoacetyl-CoA to form HMG-CoA, which is converted by HMG-CoA reductase (HMGCR) into mevalonate, a precursor for cholesterol synthesis. \{ECO:0000269|PubMed:7913309\}.
Ipo13	116458	IPO13_8908	Human Uniprot function (Human *IPO13*): Functions in nuclear protein import as nuclear transport receptor. Serves as receptor for nuclear localization signals (NLS) in cargo substrates. Is thought to mediate docking of the importin/substrate complex to the nuclear pore complex (NPC) through binding to nucleoporin and the complex is subsequently translocated through the pore by an energy requiring, Ran-dependent mechanism. At the nucleoplasmic side of the NPC, Ran binds to the importin, the importin/substrate complex dissociates and importin is re-exported from the nucleus to the cytoplasm where GTP hydrolysis releases Ran. The directionality of nuclear import is thought to be conferred by an asymmetric distribution of the GTP- and GDP-bound forms of Ran between the cytoplasm and nucleus (By similarity). Mediates the nuclear import of UBC9, the RBM8A/MAGOH complex, PAX6 and probably other members of the paired homeobox family. Also mediates nuclear export of eIF-1A, and the cytoplasmic release of eIF-1A is triggered by the loading of import substrates onto IPO13. \{ECO:0000250, ECO:0000269|PubMed:11447110, ECO:0000269|PubMed:15143176\}.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Jchain	360922	IGJ_32511	Human Uniprot function (Human *JCHAIN*): Serves to link two monomer units of either IgM or IgA. In the case of IgM, the J chain-joined dimer is a nucleating unit for the IgM pentamer, and in the case of IgA it induces dimers and/or larger polymers. It also helps to bind these immunoglobulins to secretory component.
Lcn2	170496	LCN2_32481	Human Uniprot function (Human *LCN2*): Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development (PubMed:12453413, PubMed:27780864, PubMed:20581821). Binds iron through association with 2,5-dihydroxybenzoic acid (2,5-DHBA), a siderophore that shares structural similarities with bacterial enterobactin, and delivers or removes iron from the cell, depending on the context. Iron-bound form (holo-24p3) is internalized following binding to the SLC22A17 (24p3R) receptor, leading to release of iron and subsequent increase of intracellular iron concentration. In contrast, association of the iron-free form (apo-24p3) with the SLC22A17 (24p3R) receptor is followed by association with an intracellular siderophore, iron chelation and iron transfer to the extracellular medium, thereby reducing intracellular iron concentration. Involved in apoptosis due to interleukin-3 (IL3) deprivation: iron-loaded form increases intracellular iron concentration without promoting apoptosis, while iron-free form decreases intracellular iron levels, inducing expression of the proapoptotic protein BCL2L11/BIM, resulting in apoptosis (By similarity). Involved in innate immunity; limits bacterial proliferation by sequestering iron bound to microbial siderophores, such as enterobactin (PubMed:27780864). Can also bind siderophores from *M. tuberculosis* (PubMed:15642259, PubMed:21978368). \{ECO:0000250|UniProtKB:P11672, ECO:0000269|PubMed:12453413, ECO:0000269|PubMed:15642259, ECO:0000269|PubMed:20581821, ECO:0000269|PubMed:21978368, ECO:0000269|PubMed:27780864\}.
Lef1	161452	LEF1_32825	Human Uniprot function (Human *LEF1*): Transcription factor that binds DNA in a sequence-specific manner (PubMed:2010090). Participates in the Wnt signaling pathway (By similarity). Activates transcription of target genes in the presence of CTNNB1 and EP300 (By similarity). PIAG antagonizes both Wnt-dependent and Wnt-independent activation by LEF1 (By similarity). TLE1, TLE2, TLE3 and TLE4 repress transactivation mediated by LEF1 and CTNNB1 (PubMed:11266540). Regulates T-cell receptor alpha enhancer function (PubMed:19653274). Required for IL17A expressing gamma-delta T-cell maturation and development, via binding to regulator loci of BLK to modulate expression (By similarity). May play a role in hair cell differentiation and follicle morphogenesis (By similarity). {ECO:0000250|UniProtKB:P27782, ECO:0000269|PubMed:11266540, ECO:0000269|PubMed:19653274, ECO:0000269|PubMed:2010090}.; FUNCTION: [Isoform 1]: Transcriptionally activates MYC and CCND1 expression and enhances proliferation of pancreatic tumor cells. {ECO:0000269|PubMed:19653274}.; FUNCTION: [Isoform 3]: Lacks the CTNNB1 interaction domain and may therefore be an antagonist for Wnt signaling. {ECO:0000269| PubMed:11326276}.; FUNCTION: [Isoform 5]: Transcriptionally activates the fibronectin promoter, binds to and represses transcription from the E-cadherin promoter in a CTNNB1-independent manner, and is involved in reducing cellular aggregation and increasing cell migration of pancreatic cancer cells. {ECO:0000269|PubMed:19653274}.
Loc102549061/H2bc3	102549061	LOC102549061_32836	No description available.
Loc103689966	103689966	MARCKSL1_32320	No description available.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Lpar2	498609	LPAR2_9151	Human Uniprot function (Human *LPAR2*): Receptor for lysophosphatidic acid (LPA), a mediator of diverse cellular activities. Seems to be coupled to the G(i)/G(o), G(12)/G(13), and G(q) families of heteromeric G proteins. Plays a key role in phospholipase C-beta (PLC-beta) signaling pathway. Stimulates phospholipase C (PLC) activity in a manner that is independent of RALA activation. \{ECO:0000269|PubMed:15143197, ECO:0000269|PubMed:19306925\}.
Maob	25750	MAOB_9179	Human Uniprot function (Human *MAOB*): Catalyzes the oxidative deamination of biogenic and xenobiotic amines and has important functions in the metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues. MAOB preferentially degrades benzylamine and phenylethylamine.
Marcksl1	81520	MARCKSL1_32320	Human Uniprot function (Human *MARCKSL1*): Controls cell movement by regulating actin cytoskeleton homeostasis and filopodium and lamellipodium formation (PubMed:22751924). When unphosphorylated, induces cell migration (By similarity). When phosphorylated by MAPK8, induces actin bundles formation and stabilization, thereby reducing actin plasticity, hence restricting cell movement, including neuronal migration (By similarity). May be involved in coupling the protein kinase C and calmodulin signal transduction systems (By similarity). \{ECO:0000250|UniProtKB:P28667, ECO:0000269|PubMed:22751924\}.
Me1	24552	ME1_9215	Human Uniprot function (Human *ME1*): This gene encodes a cytosolic, NADP-dependent enzyme that generates NADPH for fatty acid biosynthesis. The activity of this enzyme, the reversible oxidative decarboxylation of malate, links the glycolytic and citric acid cycles. The regulation of expression for this gene is complex. Increased expression can result from elevated levels of thyroid hormones or by higher proportions of carbohydrates in the diet. [provided by RefSeq, Jul 2008]
Nqo1	24314	NQO1_33055	Human Uniprot function (Human *NQO1*): The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways as well as in biosynthetic processes such as the vitamin K-dependent gamma-carboxylation of glutamate residues in prothrombin synthesis.
Pcbd1	29700	PCBD1_9435	Human Uniprot function (Human *PCBD1*): Involved in tetrahydrobiopterin biosynthesis. Seems to both prevent the formation of 7-pterins and accelerate the formation of quinonoid-BH2. Coactivator for HNF1A-dependent transcription. Regulates the dimerization of homeodomain protein HNF1A and enhances its transcriptional activity.
Plk4	310344	PLK4_9505	Human Uniprot function (Human *PLK4*): Serine/threonine-protein kinase that plays a central role in centriole duplication. Able to trigger procentriole formation on the surface of the parental centriole cylinder, leading to the recruitment of centriole biogenesis proteins such as SASS6, CENPJ/CPAP, CCP110, CEP135 and gamma-tubulin. When overexpressed, it is able to induce centrosome amplification through the simultaneous generation of multiple procentrioles adjoining each parental centriole during S phase. Phosphorylates 'Ser-151' of FBXW5 during the G1/S transition, leading to inhibit FBXW5 ability to ubiquitinate SASS6. Its central role in centriole replication suggests a possible role in tumorigenesis, centrosome aberrations being frequently observed in tumors. Also involved in deuterosome-mediated centriole amplification in multiciliated that can generate more than 100 centrioles. Also involved in trophoblast differentiation by phosphorylating HAND1, leading to disrupt the interaction between HAND1 and MDFIC and activate HAND1. Phosphorylates CDC25C and CHEK2. Required for the recruitment of STIL to the centriole and for

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
			STIL-mediated centriole amplification (PubMed:22020124). \{ECO:0000269|PubMed:16244668, ECO:0000269|PubMed:16326102, ECO:0000269|PubMed:17681131, ECO:0000269|PubMed:18239451, ECO:0000269|PubMed:19164942, ECO:0000269|PubMed:21725316, ECO:0000269|PubMed:22020124, ECO:0000269|PubMed:27796307\}.
Prc1	308761	PRC1_9556	Human Uniprot function (Human *PRC1*): Key regulator of cytokinesis that cross-links antiparallel microtubules at an average distance of 35 nM. Essential for controlling the spatiotemporal formation of the midzone and successful cytokinesis. Required for KIF14 localization to the central spindle and midbody. Required to recruit PLK1 to the spindle. Stimulates PLK1 phosphorylation of RACGAP1 to allow recruitment of ECT2 to the central spindle. Acts as an oncogene for promoting bladder cancer cells proliferation, apoptosis inhibition and carcinogenic progression (PubMed:17409436). \{ECO:0000269|PubMed:12082078, ECO:0000269|PubMed:15297875, ECO:0000269|PubMed:15625105, ECO:0000269|PubMed:16431929, ECO:0000269|PubMed:17409436, ECO:0000269|PubMed:19468300, ECO:0000269|PubMed:20691902, ECO:0000269|PubMed:9885575\}.
Pxmp2	29533	PXMP2_9629	Human Uniprot function (Human *PXMP2*): Seems to be involved in pore-forming activity and may contribute to the unspecific permeability of the peroxisomal membrane.
Slc37a4	29573	SLC37A4_9871	Human Uniprot function (Human *SLC37A4*): Inorganic phosphate and glucose-6-phosphate antiporter of the endoplasmic reticulum. Transports cytoplasmic glucose-6-phosphate into the lumen of the endoplasmic reticulum and translocates inorganic phosphate into the opposite direction . Forms with glucose-6-phosphatase the complex responsible for glucose production through glycogenolysis and gluconeogenesis. Hence, it plays a central role in homeostatic regulation of blood glucose levels. \{ECO:0000269|PubMed:10026167, ECO:0000269|PubMed:21949678\}.
Slc51a	303879	SLC51A_33157	Human Uniprot function (Human *SLC51A*): Essential component of the Ost-alpha/Ost-beta complex, a heterodimer that acts as the intestinal basolateral transporter responsible for bile acid export from enterocytes into portal blood. Efficiently transports the major species of bile acids. \{ECO:0000269|PubMed:16317684\}.
Slc5a6	170551	SLC5A6_9881	Human Uniprot function (Human *SLC5A6*): Transports pantothenate, biotin and lipoate in the presence of sodium.
Slc6a8	50690	SLC6A8_32561	Human Uniprot function (Human *SLC6A8*): Required for the uptake of creatine in muscles and brain.
Srxn1	296271	SRXN1_9943	Human Uniprot function (Human *SRXN1*): Contributes to oxidative stress resistance by reducing cysteine-sulfinic acid formed under exposure to oxidants in the peroxiredoxins PRDX1, PRDX2, PRDX3 and PRDX4. Does not act on PRDX5 or PRDX6. May catalyze the reduction in a multi-step process by acting both as a specific phosphotransferase and a thioltransferase. \{ECO:0000269|PubMed:15448164, ECO:0000269|PubMed:15590625\}.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Sult1c3	65185	SULT1C3_9971	Human Uniprot function (Human *SULT1C3*): [Isoform 1]: Sulfotransferase that utilizes 3'-phospho-5'-adenylyl sulfate (PAPS) as sulfonate donor. Has sulfotransferase activity toward various substrates, such as bile acids, thyroid hormones and toward xenobiotic compounds such as chlorophenols and hydroxypyrenes. Lithocholic acid appears to be the best substrate among the endogenous compounds tested and 3,3',5,5'-tetrachloro-4,4'-biphenyldiol shows the highest specific activity among the xenobiotic compounds. \{ECO:0000269|PubMed:17425406, ECO:0000269|PubMed:17936463, ECO:0000269|PubMed:28992322\}.; FUNCTION: [Isoform 2]: Exhibits weak sulphating activity and only toward chlorophenols (pentachlorophenol and 3,3',5,5'-tetrachloro-4,4'-biphenyldiol). \{ECO:0000269|PubMed:28992322\}.
Svs5	171027	SVS5_33083	Rat Entrez Gene Summary (Rat *Svs5*): Androgen-regulated serine-rich structural protein [RGD, Feb 2006]
Ugt2b37	29623	UGT2B15_33121	Human Uniprot function (Human *UGT2B28*): [Isoform 1]: UDP-glucuronosyltransferase (UGT) that catalyzes phase II biotransformation reactions in which lipophilic substrates are conjugated with glucuronic acid to increase the metabolite's water solubility, thereby facilitating excretion into either the urine or bile (PubMed:11300766). Essential for the elimination and detoxification of drugs, xenobiotics and endogenous compounds (PubMed:11300766). Catalyzes the glucuronidation of endogenous steroid hormones such as androgens (androsterone, 3alpha-androstanediol) and estrogens (estradiol, estrone) (PubMed:11300766). Catalyzes the glucuronidation of bile acid substrates, which are natural detergents for dietary lipids absorption (PubMed:11300766). Displays glucuronidation activity toward the phenolic compounds eugenol (PubMed:11300766). \{ECO:0000269|PubMed:11300766\}.; FUNCTION: [Isoform 2]: Lack UDP-glucuronosyltransferase (UGT) activity. \{ECO:0000269|PubMed:11300766\}.
Unc93b1	361689	UNC93B1_10134	Human Uniprot function (Human *UNC93B1*): Plays an important role in innate and adaptive immunity by regulating nucleotide-sensing Toll-like receptor (TLR) signaling. Required for the transport of a subset of TLRs (including TLR3, TLR7 and TLR9) from the endoplasmic reticulum to endolysosomes where they can engage pathogen nucleotides and activate signaling cascades. May play a role in autoreactive B-cells removal. \{ECO:0000269|PubMed:19006693\}.

[a]Descriptions of orthologous human genes are shown due to the increased detail available in public resources such as UniprotKB[37] and Entrez Gene.[38] Gene definitions adapted from Human UniprotKB were used as the primary resource due to the greater breadth of annotation and depth of functional detail provided. Gene definitions adapted from Rat UniprotKB were used as the secondary resource if the primary source did not provide a detailed description of function. Human Entrez Gene was used as the third resource. Rat Entrez Gene was used as the fourth resource.
[b]In some cases, a probe may map to more than one gene, resulting in duplicate reporting of that probe mapped to different genes.
Gene definition version and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Table E-7. Gene Identifiers and Definitions for Female Mice Exposed to 1,2-Dichlorobenzene for Five Days[a]

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Adh1	11522	Adh1_84160	Human Entrez Gene Summary (Human *ADH1C*): This gene encodes class I alcohol dehydrogenase, gamma subunit, which is a member of the alcohol dehydrogenase family. Members of this enzyme family metabolize a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. Class I alcohol dehydrogenase, consisting of several homo- and heterodimers of alpha, beta, and gamma subunits, exhibits high activity for ethanol oxidation to acetaldehyde, thus playing a major role in ethanol catabolism. Three genes encoding alpha, beta and gamma subunits are tandemly organized in a genomic segment as a gene cluster. An association between ADH1C polymorphism and alcohol dependence has not been established. [provided by RefSeq, Sep 2019]
Adm	11535	Adm_30365	Human Uniprot function (Human *ADM*): AM and PAMP are potent hypotensive and vasodilatator agents. Numerous actions have been reported most related to the physiologic control of fluid and electrolyte homeostasis. In the kidney, am is diuretic and natriuretic, and both am and pamp inhibit aldosterone secretion by direct adrenal actions. In pituitary gland, both peptides at physiologically relevant doses inhibit basal ACTH secretion. Both peptides appear to act in brain and pituitary gland to facilitate the loss of plasma volume, actions which complement their hypotensive effects in blood vessels.
Bhlhe40	20893	Bhlhe40_32105	Human Uniprot function (Human *BHLHE40*): Transcriptional repressor involved in the regulation of the circadian rhythm by negatively regulating the activity of the clock genes and clock-controlled genes (PubMed:12397359, PubMed:18411297). Acts as the negative limb of a novel autoregulatory feedback loop (DEC loop) which differs from the one formed by the PER and CRY transcriptional repressors (PER/CRY loop) (PubMed:14672706). Both these loops are interlocked as it represses the expression of PER1/2 and in turn is repressed by PER1/2 and CRY1/2 (PubMed:15193144). Represses the activity of the circadian transcriptional activator: CLOCK-ARNTL/BMAL1|ARNTL2/BMAL2 heterodimer by competing for the binding to E-box elements (5'-CACGTG-3') found within the promoters of its target genes (PubMed:15560782). Negatively regulates its own expression and the expression of DBP and BHLHE41/DEC2 (PubMed:14672706). Acts as a corepressor of RXR and the RXR-LXR heterodimers and represses the ligand-induced RXRA and NR1H3/LXRA transactivation activity (PubMed:19786558). May be involved in the regulation of chondrocyte differentiation via the cAMP pathway (PubMed:19786558). Represses the transcription of NR0B2 and attenuates the transactivation of NR0B2 by the CLOCK-ARNTL/BMAL1 complex (PubMed:28797635). Drives the circadian rhythm of blood pressure through transcriptional repression of ATP1B1 in the cardiovascular system (PubMed:30012868). \{ECO:0000269|PubMed:12397359, ECO:0000269|PubMed:14672706, ECO:0000269|PubMed:15193144, ECO:0000269|PubMed:15560782, ECO:0000269|PubMed:18411297, ECO:0000269|PubMed:19786558, ECO:0000269|PubMed:28797635, ECO:0000269|PubMed:30012868\}.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Cdk2	12566	Cdk2_31446	Human Uniprot function (Human *CDK2*): Serine/threonine-protein kinase involved in the control of the cell cycle; essential for meiosis, but dispensable for mitosis. Phosphorylates CTNNB1, USP37, p53/TP53, NPM1, CDK7, RB1, BRCA2, MYC, NPAT, EZH2. Triggers duplication of centrosomes and DNA. Acts at the G1-S transition to promote the E2F transcriptional program and the initiation of DNA synthesis, and modulates G2 progression; controls the timing of entry into mitosis/meiosis by controlling the subsequent activation of cyclin B/CDK1 by phosphorylation, and coordinates the activation of cyclin B/CDK1 at the centrosome and in the nucleus. Crucial role in orchestrating a fine balance between cellular proliferation, cell death, and DNA repair in human embryonic stem cells (hESCs). Activity of CDK2 is maximal during S phase and G2; activated by interaction with cyclin E during the early stages of DNA synthesis to permit G1-S transition, and subsequently activated by cyclin A2 (cyclin A1 in germ cells) during the late stages of DNA replication to drive the transition from S phase to mitosis, the G2 phase. EZH2 phosphorylation promotes H3K27me3 maintenance and epigenetic gene silencing. Phosphorylates CABLES1 (By similarity). Cyclin E/CDK2 prevents oxidative stress-mediated Ras-induced senescence by phosphorylating MYC. Involved in G1-S phase DNA damage checkpoint that prevents cells with damaged DNA from initiating mitosis; regulates homologous recombination-dependent repair by phosphorylating BRCA2, this phosphorylation is low in S phase when recombination is active, but increases as cells progress toward mitosis. In response to DNA damage, double-strand break repair by homologous recombination a reduction of CDK2-mediated BRCA2 phosphorylation. Phosphorylation of RB1 disturbs its interaction with E2F1. NPM1 phosphorylation by cyclin E/CDK2 promotes its dissociates from unduplicated centrosomes, thus initiating centrosome duplication. Cyclin E/CDK2-mediated phosphorylation of NPAT at G1-S transition and until prophase stimulates the NPAT-mediated activation of histone gene transcription during S phase. Required for vitamin D-mediated growth inhibition by being itself inactivated. Involved in the nitric oxide- (NO) mediated signaling in a nitrosylation/activation-dependent manner. USP37 is activated by phosphorylation and thus triggers G1-S transition. CTNNB1 phosphorylation regulates insulin internalization. Phosphorylates FOXP3 and negatively regulates its transcriptional activity and protein stability (By similarity). Phosphorylates CDK2AP2 (PubMed:12944431). Phosphorylates ERCC6 which is essential for its chromatin remodeling activity at DNA double-strand breaks (PubMed:29203878).\{ECO:0000250|UniProtKB:P97377, ECO:0000269|PubMed:10499802, ECO:0000269|PubMed:10884347, ECO:0000269|PubMed:10995386, ECO:0000269|PubMed:10995387, ECO:0000269|PubMed:11051553, ECO:0000269|PubMed:11113184, ECO:0000269|PubMed:12944431, ECO:0000269|PubMed:15800615, ECO:0000269|PubMed:17495531, ECO:0000269|PubMed:18372919, ECO:0000269|PubMed:19966300, ECO:0000269|PubMed:20079829, ECO:0000269|PubMed:20147522, ECO:0000269|PubMed:20195506, ECO:0000269|PubMed:20935635, ECO:0000269| PubMed:21262353, ECO:0000269|PubMed:21319273, ECO:0000269|PubMed:21596315, ECO:0000269|PubMed:28666995, ECO:0000269|PubMed:29203878\}.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Cdkn1a	12575	Cdkn1a_30669	Human Uniprot function (Human *CDKN1A*): May be involved in p53/TP53 mediated inhibition of cellular proliferation in response to DNA damage. Binds to and inhibits cyclin-dependent kinase activity, preventing phosphorylation of critical cyclin-dependent kinase substrates and blocking cell cycle progression. Functions in the nuclear localization and assembly of cyclin D-CDK4 complex and promotes its kinase activity toward RB1. At higher stoichiometric ratios, inhibits the kinase activity of the cyclin D-CDK4 complex. Inhibits DNA synthesis by DNA polymerase delta by competing with POLD3 for PCNA binding (PubMed:11595739). Plays an important role in controlling cell cycle progression and DNA damage-induced G2 arrest (PubMed:9106657). \{ECO:0000269|PubMed:11595739, ECO:0000269|PubMed:8242751, ECO:0000269|PubMed:9106657\}.
Ces1g	12623	Ces1g_30475	No description available.
Chil3	12655	Chil3_30648	Mouse Uniprot function (Mouse *Chil3*): Lectin that binds saccharides with a free amino group, such as glucosamine or galactosamine. Binding to oligomeric saccharides is much stronger than binding to mono- or disaccharides. Also binds chitin and heparin. Has weak hexosaminidase activity but no chitinase activity. Has chemotactic activity for T-lymphocytes, bone marrow cells and eosinophils. May play a role in inflammation and allergy.
Chka	12660	Chka_29307	Human Uniprot function (Human *CHKA*): Has a key role in phospholipid biosynthesis and may contribute to tumor cell growth. Catalyzes the first step in phosphatidylcholine biosynthesis. Contributes to phosphatidylethanolamine biosynthesis. Phosphorylates choline and ethanolamine. Has higher activity with choline. \{ECO:0000269|PubMed:19915674\}.
Cldn5	12741	Cldn5_31612	Human Uniprot function (Human *CLDN5*): Plays a major role in tight junction-specific obliteration of the intercellular space. \{ECO:0000250\}.
Creld2	76737	Creld2_31000	Human Uniprot function (Human *CRELD2*): Protein disulfide isomerase (By similarity). Might play a role in the unfolded protein response (By similarity). May regulate transport of alpha4-beta2 neuronal acetylcholine receptor (PubMed:16238698). \{ECO:0000250|UniProtKB:Q9CYA0, ECO:0000269|PubMed:16238698\}.
Cyp2a4	13086	Cyp2a4_30021	Mouse Uniprot function (Mouse *Cyp2a4*): Highly active in the 15-alpha-hydroxylation of testosterone. Also active in the 15-alpha-hydroxylation of progesterone and androstenedione. Little or no activity on corticosterone, pregnenolone, dehydroepiandrosterone, estradiol or estriol.
Cyp2a5	13087	Cyp2a5_31713	Mouse Uniprot function (Mouse *Cyp2a5*): Exhibits a high coumarin 7-hydroxylase activity.
Cyp2d9	13105	Cyp2d9_31050	Mouse Uniprot function (Mouse *Cyp2d9*): Cytochromes P450 are a group of heme-thiolate monooxygenases. In liver microsomes, this enzyme is involved in an NADPH dependent electron transport pathway. It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics.
Cyp3a44	337924	Cyp3a44_29466	Mouse Uniprot function (Mouse *Cyp3a44*): Cytochromes P450 are a group of heme-thiolate monooxygenases. In liver microsomes, this enzyme is involved in an NADPH-dependent electron transport pathway. It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Cyp7a1	13122	Cyp7a1_30680	Human Uniprot function (Human *CYP7A1*): A cytochrome P450 monooxygenase involved in the metabolism of endogenous cholesterol and its oxygenated derivatives (oxysterols) (PubMed:11013305, PubMed:12077124, PubMed:19965590, PubMed:2384150, PubMed:21813643). Mechanistically, uses molecular oxygen inserting one oxygen atom into a substrate, and reducing the second into a water molecule, with two electrons provided by NADPH via cytochrome P450 reductase (CPR; NADPH-ferrihemoprotein reductase) (PubMed:2384150, PubMed:11013305, PubMed:12077124, PubMed:19965590, PubMed:21813643). Functions as a critical regulatory enzyme of bile acid biosynthesis and cholesterol homeostasis. Catalyzes the hydroxylation of carbon hydrogen bond at 7-alpha position of cholesterol, a rate-limiting step in cholesterol catabolism and bile acid biosynthesis (PubMed:12077124, PubMed:19965590, PubMed:2384150). 7-alpha hydroxylates several oxysterols, including 4beta-hydroxycholesterol and 24-hydroxycholesterol (PubMed:11013305, PubMed:12077124). Catalyzes the oxidation of the 7,8 double bond of 7-dehydrocholesterol and lathosterol with direct and predominant formation of the 7-keto derivatives (PubMed:21813643). \{ECO:0000269|PubMed:11013305, ECO:0000269|PubMed:12077124, ECO:0000269|PubMed:19965590, ECO:0000269|PubMed:21813643, ECO:0000269|PubMed:2384150\}.
Dbp	13170	Dbp_31391	Human Uniprot function (Human *DBP*): This transcriptional activator recognizes and binds to the sequence 5'-RTTAYGTAAY-3' found in the promoter of genes such as albumin, *CYP2A4* and *CYP2A5*. It is not essential for circadian rhythm generation, but modulates important clock output genes. May be a direct target for regulation by the circadian pacemaker component clock. May affect circadian period and sleep regulation.
Ecm1	13601	Ecm1_29959	Human Uniprot function (Human *ECM1*): Involved in endochondral bone formation as negative regulator of bone mineralization. Stimulates the proliferation of endothelial cells and promotes angiogenesis. Inhibits MMP9 proteolytic activity. \{ECO:0000269|PubMed:11165938, ECO:0000269|PubMed:11292659, ECO:0000269|PubMed:16512877\}.
Gnmt	14711	Gnmt_32228	Human Uniprot function (Human *GNMT*): Catalyzes the methylation of glycine by using S-adenosylmethionine (AdoMet) to form N-methylglycine (sarcosine) with the concomitant production of S-adenosylhomocysteine (AdoHcy). Possible crucial role in the regulation of tissue concentration of AdoMet and of metabolism of methionine. \{ECO:0000269|PubMed:15340920, ECO:0000269|PubMed:17660255\}.
Hlf	217082	Hlf_30768	Human Entrez Gene Summary (Human *HLF*): This gene encodes a member of the proline and acidic-rich (PAR) protein family, a subset of the bZIP transcription factors. The encoded protein forms homodimers or heterodimers with other PAR family members and binds sequence-specific promoter elements to activate transcription. Chromosomal translocations fusing portions of this gene with the E2A gene cause a subset of childhood B-lineage acute lymphoid leukemias. Alternatively spliced transcript variants have been described, but their biological validity has not been determined. [provided by RefSeq, Jul 2008]

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Hsd3b1	15492	Hsd3b1_30309	Human Uniprot function (Human *HSD3B1*): A bifunctional enzyme responsible for the oxidation and isomerization of 3beta-hydroxy-Delta(5)-steroid precursors to 3-oxo-Delta(4)-steroids, an essential step in steroid hormone biosynthesis. Specifically catalyzes the conversion of pregnenolone to progesterone, 17alpha-hydroxypregnenolone to 17alpha-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to 4-androstenedione, and androstenediol to testosterone. Additionally, catalyzes the interconversion between 3beta-hydroxy and 3-oxo-5alpha-androstane steroids controlling the bioavailability of the active forms. Specifically converts dihydrotestosterone to its inactive form 5alpha-androstanediol, that does not bind androgen receptor/AR. Also converts androstanedione, a precursor of testosterone and estrone, to epiandrosterone (PubMed:1401999, PubMed:2139411). Expected to use NAD(+) as preferred electron donor for the 3beta-hydroxy-steroid dehydrogenase activity and NADPH for the 3-ketosteroid reductase activity (Probable). \{ECO:0000269|PubMed:1401999, ECO:0000269|PubMed:2139411, ECO:0000305|PubMed:1401999\}.
Ifi44	99899	Ifi44_29813	Human Uniprot function (Human *IFI44*): This protein aggregates to form microtubular structures. \{ECO:0000250\}.
Ifit3	15959	Ifit3_29860	Human Uniprot function (Human *IFIT3*): IFN-induced antiviral protein which acts as an inhibitor of cellular as well as viral processes, cell migration, proliferation, signaling, and viral replication. Enhances MAVS-mediated host antiviral responses by serving as an adapter bridging TBK1 to MAVS which leads to the activation of TBK1 and phosphorylation of IRF3 and phosphorylated IRF3 translocates into nucleus to promote antiviral gene transcription. Exhibits an antiproliferative activity via the up-regulation of cell cycle negative regulators CDKN1A/p21 and CDKN1B/p27. Normally, CDKN1B/p27 turnover is regulated by COPS5, which binds CDKN1B/p27 in the nucleus and exports it to the cytoplasm for ubiquitin-dependent degradation. IFIT3 sequesters COPS5 in the cytoplasm, thereby increasing nuclear CDKN1B/p27 protein levels. Upregulates CDKN1A/p21 by downregulating MYC, a repressor of CDKN1A/p21. Can negatively regulate the apoptotic effects of IFIT2. \{ECO:0000269|PubMed:17050680, ECO:0000269|PubMed:20686046, ECO:0000269|PubMed:21190939, ECO:0000269|PubMed:21642987, ECO:0000269|PubMed:21813773\}.
Il1b	16176	Il1b_30437	Human Uniprot function (Human *IL1B*): Potent proinflammatory cytokine. Initially discovered as the major endogenous pyrogen, induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production, and fibroblast proliferation and collagen production. Promotes Th17 differentiation of T-cells. Synergizes with IL12/ interleukin-12 to induce IFNG synthesis from T-helper 1 (Th1) cells (PubMed:10653850). Plays a role in angiogenesis by inducing VEGF production synergistically with TNF and IL6 (PubMed:12794819). \{ECO:0000269|PubMed:10653850, ECO:0000269|PubMed:12794819, ECO:0000269|PubMed:3920526\}.
Il4	16189	Il4_31858	Human Uniprot function (Human *IL4*): Participates in at least several B-cell activation processes as well as of other cell types (PubMed:3016727). It is a costimulator of DNA-synthesis. It induces the expression of class II MHC molecules on resting B-cells. It enhances both secretion and cell surface expression of IgE and IgG1. It also regulates the expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes. Positively regulates IL31RA expression in macrophages (By similarity). Stimulates autophagy in dendritic cells by interfering with mTORC1 signaling and through the induction of RUFY4 (By similarity). \{ECO:0000250|UniProtKB:P07750, ECO:0000269|PubMed:3016727\}.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Mthfd1l	270685	Mthfd1l_32017	Human Uniprot function (Human *MTHFD1L*): May provide the missing metabolic reaction required to link the mitochondria and the cytoplasm in the mammalian model of one-carbon folate metabolism in embryonic an transformed cells complementing thus the enzymatic activities of MTHFD2. \{ECO:0000250, ECO:0000269|PubMed:16171773\}.
Ndrg1	17988	Ndrg1_31127	Human Uniprot function (Human *NDRG1*): Stress-responsive protein involved in hormone responses, cell growth, and differentiation. Acts as a tumor suppressor in many cell types. Necessary but not sufficient for p53/TP53-mediated caspase activation and apoptosis. Has a role in cell trafficking, notably of the Schwann cell, and is necessary for the maintenance and development of the peripheral nerve myelin sheath. Required for vesicular recycling of CDH1 and TF. May also function in lipid trafficking. Protects cells from spindle disruption damage. Functions in p53/TP53-dependent mitotic spindle checkpoint. Regulates microtubule dynamics and maintains euploidy. \{ECO:0000269|PubMed:15247272, ECO:0000269|PubMed:15377670, ECO:0000269|PubMed:17786215, ECO:0000269|PubMed:9766676\}.
Nfil3	18030	Nfil3_31222	Human Uniprot function (Human *NFIL3*): Acts as a transcriptional regulator that recognizes and binds to the sequence 5'-[GA]TTA[CT]GTAA[CT]-3', a sequence present in many cellular and viral promoters. Represses transcription from promoters with activating transcription factor (ATF) sites. Represses promoter activity in osteoblasts (By similarity). Represses transcriptional activity of PER1 (By similarity). Represses transcriptional activity of PER2 via the B-site on the promoter (By similarity). Activates transcription from the interleukin-3 promoter in T-cells. Competes for the same consensus-binding site with PAR DNA-binding factors (DBP, HLF and TEF) (By similarity). Component of the circadian clock that acts as a negative regulator for the circadian expression of PER2 oscillation in the cell-autonomous core clock (By similarity). Protects pro-B cells from programmed cell death (By similarity). Represses the transcription of CYP2A5 (By similarity). Positively regulates the expression and activity of CES2 by antagonizing the repressive action of NR1D1 on CES2 (By similarity). \{ECO:0000250|UniProtKB:O08750, ECO:0000269|PubMed:1620116, ECO:0000269|PubMed:7565758, ECO:0000269|PubMed:8836190\}.
Nqo1	18104	Nqo1_29503	Human Uniprot function (Human *NQO1*): The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways as well as in biosynthetic processes such as the vitamin K-dependent gamma-carboxylation of glutamate residues in prothrombin synthesis.
Pcolce	18542	Pcolce_31229	Human Uniprot function (Human *PCOLCE*): Binds to the C-terminal propeptide of type I procollagen and enhances procollagen C-proteinase activity.; FUNCTION: C-terminal processed part of PCPE (CT-PCPE) may have an metalloproteinase inhibitory activity.
Pecam1	18613	Pecam1_30950	Human Uniprot function (Human *PECAM1*): Cell adhesion molecule which is required for leukocyte transendothelial migration (TEM) under most inflammatory conditions (PubMed:19342684, PubMed:17580308). Tyr-690 plays a critical role in TEM and is required for efficient trafficking of PECAM1 to and from the lateral border recycling compartment (LBRC) and is also essential for the LBRC membrane to be targeted around migrating leukocytes (PubMed:19342684). Trans-homophilic interaction may play a role in endothelial cell-cell adhesion via cell junctions (PubMed:27958302). Heterophilic interaction with CD177 plays a role in transendothelial migration of neutrophils (PubMed:17580308). Homophilic ligation of PECAM1 prevents macrophage-mediated phagocytosis of neighboring viable leukocytes by transmitting a detachment signal (PubMed:12110892). Promotes macrophage-mediated phagocytosis of apoptotic

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
			leukocytes by tethering them to the phagocytic cells; PECAM1-mediated detachment signal appears to be disabled in apoptotic leukocytes (PubMed:12110892). Modulates bradykinin receptor BDKRB2 activation (PubMed:18672896). Regulates bradykinin- and hyperosmotic shock-induced ERK1/2 activation in endothelial cells (PubMed:18672896). Induces susceptibility to atherosclerosis (By similarity). \{ECO:0000250|UniProtKB:Q08481, ECO:0000269|PubMed:12110892, ECO:0000269|PubMed:17580308, ECO:0000269|PubMed:18672896, ECO:0000269|PubMed:19342684, ECO:0000269|PubMed:27958302\}.; FUNCTION: [Isoform Delta15]: Does not protect against apoptosis. \{ECO:0000269|PubMed:18388311\}.
Por	18984	Por_29647	Human Uniprot function (Human *POR*): This enzyme is required for electron transfer from NADP to cytochrome P450 in microsomes. It can also provide electron transfer to heme oxygenase and cytochrome B5. \{ECO:0000255|HAMAP-Rule:MF_03212\}.
Prc1	233406	Prc1_29718	Human Uniprot function (Human *PRC1*): Key regulator of cytokinesis that cross-links antiparallel microtubules at an average distance of 35 nM. Essential for controlling the spatiotemporal formation of the midzone and successful cytokinesis. Required for KIF14 localization to the central spindle and midbody. Required to recruit PLK1 to the spindle. Stimulates PLK1 phosphorylation of RACGAP1 to allow recruitment of ECT2 to the central spindle. Acts as an oncogene for promoting bladder cancer cells proliferation, apoptosis inhibition and carcinogenic progression (PubMed:17409436). \{ECO:0000269|PubMed:12082078, ECO:0000269|PubMed:15297875, ECO:0000269|PubMed:15625105, ECO:0000269|PubMed:16431929, ECO:0000269|PubMed:17409436, ECO:0000269|PubMed:19468300, ECO:0000269|PubMed:20691902, ECO:0000269|PubMed:9885575\}.
Rgs2	19735	Rgs2_29422	Human Uniprot function (Human *RGS2*): Regulates G protein-coupled receptor signaling cascades. Inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits, thereby driving them into their inactive GDP-bound form (PubMed:11063746, PubMed:19478087). It is involved in the negative regulation of the angiotensin-activated signaling pathway (PubMed:28784619). Plays a role in the regulation of blood pressure in response to signaling via G protein-coupled receptors and GNAQ. Plays a role in regulating the constriction and relaxation of vascular smooth muscle (By similarity). Binds EIF2B5 and blocks its activity, thereby inhibiting the translation of mRNA into protein (PubMed:19736320). \{ECO:0000250|UniProtKB:O08849, ECO:0000269|PubMed:11063746, ECO:0000269|PubMed:11278586, ECO:0000269|PubMed:17901199, ECO:0000269|PubMed:19736320, ECO:0000269|PubMed:28784619, ECO:0000305|PubMed:7643615\}.
Rsad2	58185	Rsad2_31088	Human Uniprot function (Human *RSAD2*): Interferon-inducible iron-sulfur (4FE-4S) cluster-binding antiviral protein which plays a major role in the cell antiviral state induced by type I and type II interferon. Can inhibit a wide range of DNA and RNA viruses, including human cytomegalovirus (HCMV), hepatitis C virus (HCV), west Nile virus (WNV), dengue virus, sindbis virus, influenza A virus, sendai virus, vesicular stomatitis virus (VSV), and human immunodeficiency virus (HIV-1). Displays antiviral activity against influenza A virus by inhibiting the budding of the virus from the plasma membrane by disturbing the lipid rafts. This is accomplished, at least in part, through binding and inhibition of the enzyme farnesyl diphosphate synthase (FPPS), which is essential for the biosynthesis of isoprenoid-derived lipids. Promotes TLR7 and TLR9-dependent production of IFN-beta production in plasmacytoid dendritic cells (pDCs) by facilitating Lys-63'-linked ubiquitination of IRAK1. Plays a role in CD4+ T-cells activation and differentiation. Facilitates T-cell receptor (TCR)-mediated GATA3 activation and optimal T-helper 2 (Th2)

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
			cytokine production by modulating NFKB1 and JUNB activities. Can inhibit secretion of soluble proteins. \{ECO:0000269|PubMed:11752458, ECO:0000269|PubMed:16108059, ECO:0000269|PubMed:16982913, ECO:0000269|PubMed:17686841, ECO:0000269|PubMed:18005719, ECO:0000269|PubMed:19074433\}.
Slc51a	106407	Slc51a_29138	Human Uniprot function (Human *SLC51A*): Essential component of the Ost-alpha/Ost-beta complex, a heterodimer that acts as the intestinal basolateral transporter responsible for bile acid export from enterocytes into portal blood. Efficiently transports the major species of bile acids. \{ECO:0000269|PubMed:16317684\}.
Slfn4	20558	Slfn4_31065	Rat Entrez Gene Summary (Rat *Slfn4*): Regulates cell growth; involved in T cell development [RGD, Feb 2006]
Syn1	20964	Syn1_30750	Human Uniprot function (Human *SYN1*): Neuronal phosphoprotein that coats synaptic vesicles, binds to the cytoskeleton, and is believed to function in the regulation of neurotransmitter release. The complex formed with NOS1 and CAPON proteins is necessary for specific nitric-oxide functions at a presynaptic level.
Tcap	21393	Tcap_31038	Human Uniprot function (Human *TCAP*): Muscle assembly regulating factor. Mediates the antiparallel assembly of titin (TTN) molecules at the sarcomeric Z-disk.
Tk1	21877	Tk1_29710	Human Entrez Gene Summary (Human *TK1*): The protein encoded by this gene is a cytosolic enzyme that catalyzes the addition of a gamma-phosphate group to thymidine. This creates dTMP and is the first step in the biosynthesis of dTTP, which is one component required for DNA replication. The encoded protein, whose levels fluctuate depending on the cell cycle stage, can act as a low activity dimer or a high activity tetramer. High levels of this protein have been used as a biomarker for diagnosing and categorizing many types of cancers. [provided by RefSeq, Oct 2016]
Tmem182	381339	Tmem182_30628	No description available.

Gene Symbol	Entrez Gene IDs	Probe IDs[b]	Definition
Tsku	244152	Tsku_31948,Tsku_29473	Human Uniprot function (Human *TSKU*): Contributes to various developmental events and other processes such as wound healing and cholesterol homeostasis through its interactions with multiple signaling pathways. Wnt signaling inhibitor which competes with WNT2B for binding to Wnt receptor FZD4 and represses WNT2B-dependent development of the peripheral eye. Plays a role in regulating the hair cycle by controlling TGFB1 signaling. Required for the development of the anterior commissure in the brain by inhibiting neurite outgrowth. Essential for terminal differentiation of hippocampal neural stem cells. Plays a role in regulating bone elongation and bone mass by modulating growth plate chondrocyte function and overall body size. Required for development of the inner ear through its involvement in stereocilia formation in inner hair cells. Facilitates wound healing by inhibiting secretion of TGFB1 from macrophages which prevents myofibroblast differentiation, maintaining inflammatory cell quiescence. Plays a role in cholesterol homeostasis by reducing circulating high-density lipoprotein cholesterol, lowering cholesterol efflux capacity and decreasing cholesterol-to-bile acid conversion in the liver. In one study, shown to negatively regulate sympathetic innervation in brown fat, leading to reduced energy expenditure. In another study, shown not to affect brown fat thermogenic capacity, body weight gain or glucose homeostasis.
Tspan4	64540	Tspan4_30708,Tspan4_32011	Human Entrez Gene Summary (Human *TSPAN4*): The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. Most of these members are cell-surface proteins that are characterized by the presence of four hydrophobic domains. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. This encoded protein is a cell surface glycoprotein and is similar in sequence to its family member CD53 antigen. It is known to complex with integrins and other transmembrane 4 superfamily proteins. Alternatively spliced transcript variants encoding different isoforms have been identified. [provided by RefSeq, Jul 2008]

[a]Descriptions of orthologous human genes are shown due to the increased detail available in public resources such as UniprotKB[37] and Entrez Gene.[38] Gene definitions adapted from Human UniprotKB were used as the primary resource due to the greater breadth of annotation and depth of functional detail provided. Gene definitions adapted from Mouse UniprotKB were used as the secondary resource if the primary source did not provide a detailed description of function. Human Entrez Gene was used as the third resource. Mouse Entrez Gene was used as the fourth resource.
[b]In some cases, a probe may map to more than one gene, resulting in duplicate reporting of that probe mapped to different genes.
Gene definition version and retrieval dates: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.

Appendix F. Organ Weight Descriptions

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F.1. Organ Weight Descriptions

Heart: The heart drives the circulatory system, supplying oxygen and essential macro- and micronutrients to the tissues. Increased heart weight in subacute studies would indicate severe cardiotoxicity, compensatory myocardial hypertrophy, and/or pulmonary injury. Decreased heart weight in subacute studies is often of unknown toxicological significance; however, it may be caused by decreased load on the heart from dehydration or modulation of contractility.

Kidney: The kidneys remove waste products and xenobiotics from the body, balance blood electrolytes, regulate blood pressure through the release of hormones, synthesize the active form of vitamin D, and control the production of erythropoiesis. In subacute studies, changes in kidney weight may reflect renal toxicity (particularly if accompanied by increases in other markers of kidney toxicity, e.g., increased Kim-1) and/or tubular hypertrophy. Decreased kidney weights in subacute studies are typically of unknown toxicological significance.

Liver: The liver carries out biotransformation and excretion of endogenous and xenobiotic substances, regulation of blood sugar, enzymatic transformation of essential nutrients, generation of blood proteins involved in fluid balance and clotting, and bile production for digestion and absorption of fats. Liver weight changes can be an indication of chemical-induced stress. Specifically, in subacute studies, increases in liver weight in response to low doses of toxicants typically stem from increases in xenobiotic metabolizing enzymes and associated hepatocyte hypertrophy or peroxisome proliferation. Increased liver weight, particularly when accompanied by evidence of leakage of liver-specific enzymes into blood, likely reflects hemodynamic changes related to severe hepatotoxicity. Higher liver weight relative to body weight may also occur at any dose level that causes a slowed rate of body growth and does not necessarily indicate liver toxicity. Decreased liver weight in subacute studies is typically of unknown toxicological significance but in rare cases may be related to glycogen depletion.

Lung: The lung is the main respiratory organ and is primarily responsible for gas exchange but also aids in regulating blood pH and protecting the body from pathogens. An increase in lung weight following short-term exposure is a sensitive indicator of direct pulmonary toxicity. This change is typically driven by an acute inflammatory response, characterized by edema (fluid accumulation) and the infiltration of immune cells like neutrophils and macrophages. It is considered an adverse finding that signals local irritation and cellular injury. While the magnitude of the weight increase often correlates with the dose, a definitive interpretation of the nature and severity of the damage requires correlation with histopathological examination of the lung tissue. Conversely, a decrease in absolute lung weight is less common and usually points to significant systemic toxicity rather than a direct lung effect. The primary cause is often severe body weight loss (inanition), which leads to a proportional, catabolic reduction in the mass of most organs. In this scenario, the toxicological interpretation hinges on the relative lung-to-body-weight ratio, as an increase in this ratio can unmask a direct pulmonary effect that is otherwise obscured by the animal's poor overall condition.

Ovary: The ovary is the female gamete-producing organ and produces and releases essential sex hormones. Changes in ovarian weight are a key indicator of reproductive toxicity, often resulting from hormonal disruption. A decrease in weight is the more frequent and concerning finding, typically signaling an adverse impact on fertility. This can be caused by a direct toxic effect on ovarian cells, leading to follicular atresia, or by disruption of the Hypothalamic-Pituitary-

Gonadal (HPG) axis. It may also occur as a secondary consequence of severe systemic toxicity and body weight loss, which suppresses reproductive function. An increase in ovarian weight is less common but also indicates significant reproductive perturbation, possibly from hormonal overstimulation, inflammation, or cysts. For any change in weight, the interpretation is critically dependent on context. The animal's estrous cycle stage must be considered, as weight fluctuates naturally. Ultimately, a definitive conclusion about the cause and severity of the effect (either increased or decreased weight) is not possible without histopathological correlation to examine the underlying microscopic structure of the ovary.

Appendix G. Supplemental Data

The following supplemental files are available at https://doi.org/10.22427/NIEHS-DATA-NIEHS-12.

G.1. Materials and Methods

Materials and Methods
Materials_and_Methods.pdf

G.2. Theoretical Inhaled Dose

Theoretical Inhaled Dose
Theoretical_Inhaled_Dose.pdf

G.3. Rats

G.3.1. Apical Benchmark Dose Analysis

BMD, NOEL and LOEL Summary for Apical Endpoints
108020006_BMD_NOEL_and_LOEL_Summary_for_Apical_Endpoints.pdf

BMDExpress Project File_Rat_Apical (bm2 format)
108020006_2D_Rat_NonGenomic.bm2

Clinical Chemistry Summary
108020006_Clinical_Chemistry_Summary.pdf

Mean Body Weights Summary
108020006_Mean_Body_Weights_Summary.pdf

Organ Weights Summary
108020006_OrganWeights_Summary.pdf

G.3.2. Genomic Benchmark Dose Analysis

GEO/SRA: https://www.ncbi.nlm.nih.gov/bioproject/1304578

All Organ Principal Components Analysis Files
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Animal and Fastaq Metadata
108020006_Animal_and_Fastaq_Metadata.pdf

Attenuation-adjusted Raw Read Counts Rat Study
108020006_Attenuation-adjustedRawReadCountsRatStudy.zip

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108020006_Benchmark_Dose_Analysis_Rat_Heart.txt

Benchmark Dose Analysis Rat Kidney
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Benchmark Dose Analysis Rat Liver
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Benchmark Dose Analysis Rat Lung
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Benchmark Dose Analysis Rat Ovary
108020006_Benchmark_Dose_Analysis_Rat_Ovary.txt

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BMDExpress Expression Data Kidney
108020006_BMDExpress_Expression_Data_Kidney.txt

BMDExpress Expression Data Liver
108020006_BMDExpress_Expression_Data_Liver.txt

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BMDExpress GO Biological Process BMD Results Rat Heart
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BMDExpress Individual Gene BMD Results Rat Ovary
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BMDExpress Project File Rat Genomic (bm2 format)
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BMDExpress Project File Rat Genomic (JSON format)
108020006_2D_Rat_Genomic.json

Curve Fit Rat Heart
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Curve Fit Rat Kidney
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Curve Fit Rat Ovary
108020006_Curve_Fit_Rat_Ovary.txt

Most Potent GO Biological Process In Each Tissue
108020006_Most_Potent_GO_Biological_Process_In_Each_Tissue.pdf

One-way ANOVA_Rat_Heart
108020006_One-way_ANOVA_Rat_Heart.txt

One-way ANOVA_Rat_Kidney
108020006_One-way_ANOVA_Rat_Kidney.txt

One-way ANOVA_Rat_Liver
108020006_One-way_ANOVA_Rat_Liver.txt

One-way ANOVA_Rat_Lung
108020006_One-way_ANOVA_Rat_Lung.txt

One-way ANOVA_Rat_Ovary
108020006_One-way_ANOVA_Rat_Ovary.txt

Rat DCB eFDR Synthetic Null Analysis
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TempO-Seq S1500v1.2 Full Manifest Rattus Norvegicus
108020006_TempO-SeqS1500v1.2FullManifestRattusNorvegicus.csv

Top 10 Genes Ranked by Potency of Perturbation Heart
108020006_Top_10_Genes_Ranked_by_Potency_of_Perturbation_Heart.pdf

Top 10 Genes Ranked by Potency of Perturbation Kidney
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Top 10 GO Biological Process Gene Sets Kidney
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Top 10 GO Biological Process Gene Sets Liver
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Top 10 GO Biological Process Gene Sets Lung
108020006_Top_10_GO_Biological_Process_Gene_Sets_Lung.pdf

Top 10 GO Biological Process Gene Sets Ovary
108020006_Top_10_GO_Biological_Process_Gene_Sets_Ovary.pdf

Transcriptomics Data QC Report
108020006_TranscriptomicsDataQCReport.zip

G.3.3. Study Tables

I01 - Animal Removal Summary
108020006_I01_Animal_Removal_Summary.pdf

I02 - Animal Removals
108020006_I02_Animal_Removals.pdf

I04 - Mean Body Weights and Survival
108020006_I04_Mean_Body_Weights_and_Survival.pdf

I04G - Mean Body Weight Gain
108020006_I04G_Mean_Body_Weight_Gain.pdf

I05 - Clinical Observations Summary
108020006_I05_Clinical_Observations_Summary.pdf

PA06 - Organ Weights Summary
108020006_PA06_Organ_Weights_Summary.pdf

PA41 - Clinical Chemistry Summary
108020006_PA41_Clinical_Chemistry_Summary.pdf

PA43 - Hematology Summary
108020006_PA43_Hematology_Summary.pdf

PA48 - Blood and Tissue Concentration
108020006_Blood_and_Tissue_Concentration.pdf

G.3.4. Individual Animal Data

Individual Animal Blood and Tissue Concentration Data
108020006_Individual_Animal_Blood_and_Tissue_Concentration_Data.xlsx

Individual Animal Body Weight Data
108020006_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Chemistry Data
108020006_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Clinical Observations Data
108020006_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Hematology Data
108020006_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
108020006_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Removal Reasons Data
108020006_Individual_Animal_Removal_Reasons_Data.xlsx

G.4. Mice

G.4.1. Apical Benchmark Dose Analysis

BMD, NOEL and LOEL Summary for Apical Endpoints
108020007_BMD_NOEL_and_LOEL_Summary_for_Apical_Endpoints_Mouse.pdf

BMDExpress Project File Mouse Apical (bm2 format)
108020007_2D_NonGenomic_Mouse.bm2

Clinical Chemistry Summary
108020007_Clinical_Chemistry_Summary.pdf

Mean Body Weights Summary
108020007_Mean_Body_Weights_Summary.pdf

Organ Weights Summary
108020007_Organ_Weights_Summary.pdf

G.4.2. Genomic Benchmark Dose Analysis

GEO/SRA: https://www.ncbi.nlm.nih.gov/bioproject/1304935

All Organ Principal Components Analysis Files
108020007_All_Organ_Principal_Components_Analysis_Files.zip

Animal and Fastaq Metadata
108020007_Animal_and_Fastaq_Metadata.pdf

Attenuation-adjusted Raw Read Counts Mouse Study
108020007_Attenuation-adjustedRawReadCountsMouseStudy.zip

Benchmark Dose Analysis Mouse Heart
108020007_Benchmark_Dose_Analysis_Mouse_Heart.txt

Benchmark Dose Analysis Mouse Kidney
108020007_Benchmark_Dose_Analysis_Mouse_Kidney.txt

Benchmark Dose Analysis Mouse Liver
108020007_Benchmark_Dose_Analysis_Mouse_Liver.txt

Benchmark Dose Analysis Mouse Lung
108020007_Benchmark_Dose_Analysis_Mouse_Lung.txt

Benchmark Dose Analysis Mouse Ovary
108020007_Benchmark_Dose_Analysis_Mouse_Ovary.txt

BMDExpress Expression Data Heart
108020007_BMDExpress_Expression_Data_Heart.txt

BMDExpress Expression Data Kidney
108020007_BMDExpress_Expression_Data_Kidney.txt

BMDExpress Expression Data Liver
108020007_BMDExpress_Expression_Data_Liver.txt

BMDExpress Expression Data Lung
108020007_BMDExpress_Expression_Data_Lung.txt

BMDExpress Expression Data Ovary
108020007_BMDExpress_Expression_Data_Ovary.txt

BMDExpress GO Biological Process BMD Results Mouse Heart
108020007_BMDExpress_GO_Biological_Process_BMD_Results_Mouse_Heart.txt

BMDExpress GO Biological Process BMD Results Mouse Kidney
108020007_BMDExpress_GO_Biological_Process_BMD_Results_Mouse_Kidney.txt

BMDExpress GO Biological Process BMD Results Mouse Liver
108020007_BMDExpress_GO_Biological_Process_BMD_Results_Mouse_Liver.txt

BMDExpress GO Biological Process BMD Results Mouse Lung

108020007_BMDExpress_GO_Biological_Process_BMD_Results_Mouse_Lung.txt

BMDExpress GO Biological Process BMD Results Mouse Ovary
108020007_BMDExpress_GO_Biological_Process_BMD_Results_Mouse_Ovary.txt

BMDExpress Individual Gene BMD Results Mouse Heart
108020007_BMDExpress_Individual_Gene_BMD_Results_Mouse_Heart.txt

BMDExpress Individual Gene BMD Results Mouse Kidney
108020007_BMDExpress_Individual_Gene_BMD_Results_Mouse_Kidney.txt

BMDExpress Individual Gene BMD Results Mouse Liver
108020007_BMDExpress_Individual_Gene_BMD_Results_Mouse_Liver.txt

BMDExpress Individual Gene BMD Results Mouse Lung
108020007_BMDExpress_Individual_Gene_BMD_Results_Mouse_Lung.txt

BMDExpress Individual Gene BMD Results Mouse Ovary
108020007_BMDExpress_Individual_Gene_BMD_Results_Mouse_Ovary.txt

BMDExpress Project File Mouse Genomic (bm2 format)
108020007_2D_Mouse_Genomic.bm2

BMDExpress Project File Mouse Genomic (JSON format)
108020007_2D_Mouse_Genomic.json

Curve Fit Mouse Heart
108020007_Curve_Fit_Mouse_Heart.txt

Curve Fit Mouse Kidney
108020007_Curve_Fit_Mouse_Kidney.txt

Curve Fit Mouse Liver
108020007_Curve_Fit_Mouse_Liver.txt

Curve Fit Mouse Lung
108020007_Curve_Fit_Mouse_Lung.txt

Curve Fit Mouse Ovary
108020007_Curve_Fit_Mouse_Ovary.txt

Mouse DCB eFDR Synthetic Null Analysis
108020007_Mouse_DCB_eFDR_Synthetic_Null_Analysis.zip

Most Potent GO Biological Process In Each Tissue
108020007_Most_Potent_GO_Biological_Process_In_Each_Tissue.pdf

One-way ANOVA_Mouse_Heart
108020007_One-way_ANOVA_Mouse_Heart.txt

One-way ANOVA_Mouse_Kidney
108020007_One-way_ANOVA_Mouse_Kidney.txt

One-way ANOVA_Mouse_Liver
108020007_One-way_ANOVA_Mouse_Liver.txt

One-way ANOVA_Mouse_Lung
108020007_One-way_ANOVA_Mouse_Lung.txt

One-way ANOVA_Mouse_Ovary
108020007_One-way_ANOVA_Mouse_Ovary.txt

TempO-Seq S1500v1.2 Full Manifest Mus Musculus
108020007_TempO-SeqS1500v1.2FullManifestMus_Musculus.csv

Top 10 Genes Ranked by Potency of Perturbation Heart
108020007_Top_10_Genes_Ranked_by_Potency_of_Perturbation_Heart.pdf

Top 10 Genes Ranked by Potency of Perturbation Kidney
108020007_Top_10_Genes_Ranked_by_Potency_of_Perturbation_Kidney.pdf

Top 10 Genes Ranked by Potency of Perturbation Liver
108020007_Top_10_Genes_Ranked_by_Potency_of_Perturbation_Liver.pdf

Top 10 Genes Ranked by Potency of Perturbation Lung
108020007_Top_10_Genes_Ranked_by_Potency_of_Perturbation_Lung.pdf

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Top 10 GO Biological Process Gene Sets Kidney
108020007_Top_10_GO_Biological_Process_Gene_Sets_Kidney.pdf

Top 10 GO Biological Process Gene Sets Liver
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Top 10 GO Biological Process Gene Sets Lung
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Top 10 GO Biological Process Gene Sets Ovary
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G.4.4. Individual Animal Data

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108020007_Individual_Animal_Body_Weight_Data.xlsx

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108020007_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Removal Reasons Data
108020007_Individual_Animal_Removal_Reasons_Data.xlsx

G.5. Software

G.5.1. Analysis Pipeline Evaluation

Analysis Pipeline Evaluation
AnalysisPipelineEvaluation.zip

DTT tBMD Pipeline bm2
DTT_tBMD_Pipelinebm2.zip

EPA tBMD Pipeline bm2
EPA_tBMD_Pipelinebm2.zip

G.5.2. BMDExpress Preview Software

BMDExpress Preview Software
https://github.com/auerbachs/BMDExpress-3/releases/download/3.20.141/BMDExpress3_windows-x64_3_20_0141.exe

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Environmental Health Sciences
Division of Translational Toxicology
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