National Institute of
Environmental Health Sciences

NIEHS Report on the In Vivo Repeat Dose Biological Potency Study of Tricresyl Phosphate (CASRN 1330-78-5) in Male Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies)

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Foreword

The National Institute of Environmental Health Sciences (NIEHS) is one of 27 institutes and centers of the National Institutes of Health, which is part of the U.S. Department of Health and Human Services. The NIEHS mission is to discover how the environment affects people in order to promote healthier lives. NIEHS works to accomplish its mission by conducting and funding research on human health effects of environmental exposures; developing the next generation of environmental health scientists; and providing critical research, knowledge, and information to citizens and policymakers who are working to prevent hazardous exposures and reduce the risk of disease and disorders connected to the environment. NIEHS is a foundational leader in environmental health sciences and committed to ensuring that its research is directed toward a healthier environment and healthier lives for all people.

The NIEHS Report series began in 2022. 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.

NIEHS reports are available free of charge on the NIEHS/DTT 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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About This Report

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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 (Hsd:Sprague Dawley® SD®) Rats (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 Study of Tricresyl Phosphate (CASRN 1330-78-5)in Male Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies)* was not subjected to further external peer review.

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Abstract

Background: Tricresyl phosphate (TCP) is an organophosphate flame retardant currently on the market that is used as a replacement for phased-out polybrominated diphenyl ethers. TCP is a mixture of three isomeric compounds, including TCP, dicresyl phenyl phosphate, and cresyl diphenyl phosphate. Toxicological information on this class of chemicals is sparse. A short-term, in vivo transcriptomic study was used to assess the biological potency of TCP.

Methods: Scientists at the Division of Translational Toxicology, National Institute of Environmental Health Sciences conducted this short-term in vivo biological potency study on TCP in young adult male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats. TCP was formulated in corn oil and administered once daily for 4 consecutive days by gavage. TCP was tested at six doses (0, 62, 125, 249, 497, and 995 mg/kg body weight [mg/kg] corresponding to 0, 0.169, 0.338, 0.675, 1.35, and 2.7 mmol/kg). On study day 4, animals were euthanized, standard toxicological measures were assessed, and the liver was assayed in gene expression studies using Affymetrix microarrays. Modeling was conducted to identify the benchmark doses (BMDs) associated with apical toxicological endpoints and transcriptional changes in the liver. A benchmark response of one standard deviation was used to model all endpoints.

Results: Several clinical pathology and organ weight measurements showed dose-related changes from which BMD values could be obtained. The effects include increased serum alanine aminotransferase activity, decreased serum albumin concentration, increased relative liver weight, decreased serum albumin/globulin ratio, increased absolute liver weight, increased serum high-density lipoprotein cholesterol concentration, increased serum total cholesterol concentration, increased serum low-density lipoprotein cholesterol concentration, and increased serum globulin concentration. The BMDs and benchmark dose lower confidence limits (BMD_{LS}) were 31.1 (10.1), 62.5 (28.4), 129.5 (78.4), 172.8 (134.5), 223.8 (97.1), 241.8 (175.1), 243.8 (193.1), 335.6 (245.7), and 344.8 (255.8) mg/kg, respectively. Although serum cholinesterase activity was significantly decreased in all dosed groups (43%–78% decrease), beginning with 62 mg/kg (the lowest-observed-effect level), its BMD value was below the lower limit of extrapolation (<20.7 mg/kg).

No Gene Ontology biological processes had BMD median values <20.7 mg/kg. The most sensitive gene sets for which a reliable estimate of the BMD could be made were cellular response to dexamethasone stimulus and cellular response to glucocorticoid stimulus, both with median BMDs of 58.5 mg/kg and median BMD_{LS} of 34.0 mg/kg. Two individual genes, *Fer1l5* and *Gsta3*, had median BMD values <20.7 mg/kg. *Gsta3* was upregulated, while *Fer1l5* was downregulated. The most sensitive upregulated genes with reliable BMD estimates included *Ces2c*, *Abcc3*, *Orm1*, *Mnd1*, *App*, *Cyp7a1*, *Ddit4*, and *Gstt3*, with BMDs (BMD_{LS}) of 27.7 (18.7), 32.1 (22.0), 32.6 (10.4), 33.8 (14.8), 37.7 (20.8), 39.4 (17.3), 41.6 (9.5), and 66.0 (28.7) mg/kg, respectively. Other than *Fer1l5*, no other responding genes had decreased expression among the top 10 most sensitive genes.

Summary: Taken together, the most sensitive gene set BMD (BMD_L) median, individual gene BMD (BMD_L), and apical endpoint BMD (BMD_L) values that could be reliably determined occurred at 58.5 (34.0), 27.7 (18.7), and 31.1 (10.1) mg/kg, respectively. The BMDs (BMD_{LS}) could not be determined for 2 of the top 10 most sensitive individual genes and were estimated to be <20.7 mg/kg. Serum cholinesterase inhibition was also estimated to be <20.7 mg/kg. Future

studies investigating lower doses would be helpful to obtain more accurate estimates of BMD values for the most sensitive individual genes and for serum cholinesterase inhibition.

Background

Tricresyl phosphate (TCP) is an organophosphate flame retardant (OPFR). TCP is a mixture of three isomeric compounds, including TCP, dicresyl phenyl phosphate, and cresyl diphenyl phosphate. OPFRs are organic phosphate esters used in a diverse collection of products to interrupt or hinder combustion.[1] OPFRs can leach from treated materials and persist in the environment.[2] They have been detected in indoor air, household dust, wastewater treatment plant effluent, drinking water, and wildlife samples.[3-6] The literature contains little information on the incidence and potency of health effects associated with exposure to this chemical class. For this reason, OPFRs were nominated to the National Institute of Environmental Health Sciences for toxicological characterization.

Reported here are the results of a repeat dose study of TCP performed in male rats. The goal of this study is to provide a rapid assessment of in vivo biological potency by evaluating a combination of traditional toxicological endpoints and transcriptomics analysis to broadly query biological space for any dose-related change. The justification for using this type of assessment relates to the observation that gene set benchmark dose values from short-term transcriptomic studies have been shown to approximate dose responsiveness of the most sensitive apical endpoints from resource intensive guideline toxicological assessments (e.g., carcinogenicity).[7; 8] Importantly, the study reported here is not intended to assess or identify hazards. In particular, any observations related to traditional toxicological hazards gleaned from qualitative interpretation of the transcriptomics data should be considered hypotheses requiring further evaluation.

Materials and Methods

Study Design

Young adult male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Harlan Laboratories, Inc. (now Envigo, Indianapolis, IN). Males were selected because of the historical precedent of using males in transcriptomic studies to avoid challenges with hormonal cyclicity in female rats that can affect interpretation of gene expression data. On receipt, the rats were 7–8 weeks of age. Animals were quarantined for 7 days, and then randomly assigned to one of six dose groups, each containing five or six rats. The rats in each dose group then were administered tricresyl phosphate (TCP) by gavage in corn oil at a dose level of 0, 62, 125, 249, 497, or 995 mg/kg body weight. These doses correspond to molar equivalencies of 0, 0.169, 0.338, 0.675, 1.35, and 2.7 mmol/kg. Corn oil was selected as the vehicle on the basis of physical and chemical properties that indicated the test article would exhibit maximal solubility in corn oil relative to other commonly used vehicles. Dosing of the animals with the test article occurred on 4 consecutive days. Dosage volume was 5 mL/kg body weight and was based on the most recently measured body weight. Euthanasia, blood/serum collection, and tissue sample collection were completed on the day following the final administration of the test article (study day 4). Animal identification numbers and microarray data file names for each animal are presented in Appendix A.

Dose Selection Rationale

Dose selection was informed by National Toxicology Program (NTP) subchronic studies of TCP. At dose levels of approximately 1,000 mg per kg body weight per day (mg/kg/day) for 90 days, TCP produced significant histopathological manifestations in the liver of rats, which indicated the animals were adequately challenged. An equimolar dose of TCP was estimated to be approximately 995 mg/kg/day; thus, this dose was selected as the highest dose in the present study.

Chemistry

TCP ($C_{21}H_{21}O_4P$; molar mass 368.36 g/mol) was obtained from Acros Organics via Fisher Scientific (Waltham, MA; lot A0271226). CASRN 1330-78-5 specifies the TCP isomer mixture. The identity was confirmed using gas chromatography coupled with mass spectrometry. Ten components were observed with relative chromatographic peak areas >0.05%. Of these, four were identified as isomers of TCP, three as isomers of dicresyl phenyl phosphate, two as isomers of cresyl diphenyl phosphate, and one as triphenyl phosphate. The combined relative areas of the components indicate that the lot consisted of approximately 68% of the isomers of TCP, 31% of isomers of cresyl diphenyl phosphate and dicresyl phenyl phosphates, and 1% of triphenyl phosphate.

Dose formulations were prepared in corn oil at target concentrations of 0 (vehicle), 0.0338, 0.0676, 0.135, 0.270, and 0.540 mmol/mL, analyzed by gas chromatography with flame ionization detection, and shipped to Alion (Research Triangle Park, NC). All formulations were within ±10% of target concentrations, and no TCP was detected in any control formulation. The stability of the corn oil formulations was assessed using the 0.0338 mmol/mL concentration for

up to 21 days when stored at ambient temperature in sealed glass bottles under inert gas; the measured concentration was within 10% of the nominal concentration on day 0, demonstrating the stability during the period of use. All chemistry activities were conducted by MRIGlobal (Kansas City, MO).

Clinical Examinations and Sample Collection

Clinical Observations

Standard clinical observations were performed within 4 hours post dosing on all study days. Animals were observed for signs of cholinesterase inhibition with specific signs recorded, such as weakness, lethargy, tremors, eye-bulging, salivation, lacrimation, and diarrhea.

Body and Organ Weights

Animals were weighed on the first day of dosing and on the day of necropsy. During necropsy, the entire liver and brain were removed, and organ weights were recorded for each animal.

Clinical Pathology

Animals were terminated in random order by CO_2/O_2 (70%/30%) anesthesia one day after the final day of dosing. Blood samples were taken via cardiocentesis. Five mL of blood was collected into a tube void of anticoagulant and the serum harvested for clinical chemistry, total thyroxine (T_4), and cholinesterase measurements. The following clinical chemistry parameters were measured on an Olympus AU400e chemistry analyzer (Olympus America, Inc., Irvin, TX) using reagents obtained from Beckman Coulter (Brea, CA) or Diazyme (Poway, CA): urea nitrogen, creatinine, total protein, albumin, sorbitol dehydrogenase, alanine aminotransferase, aspartate aminotransferase, bile acids, cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and cholinesterase. Total T_4 was measured using an MP Biomedical T4 radioimmunoassay kit with an Apex automatic gamma counter (ICN Micromedic Systems, Inc., Huntsville, AL). Toxicological study data tables are presented in Appendix B.

Transcriptomics

Sample Collection for Transcriptomics

Liver transcriptomics were performed on samples taken from three animals per dose group (randomly selected). Half the left liver lobe was processed for RNA isolation. Specifically, three pieces (3-mm cubes) were dissected and transferred to a weigh boat containing liquid nitrogen. Once flash frozen, the liver tissue for each animal was placed into a single, prechilled 2-mL cryotube and stored at or below −70°C. Frozen liver samples were shipped to the Battelle Biomedical Research Center (West Jefferson, OH) on dry ice.

RNA Isolation and cDNA Synthesis

The frozen liver tissues were submerged in 10 volumes of prechilled RNA*later*®-ICE (Life Technologies, Carlsbad, CA) and stored at −20°C ± 10°C for a minimum of 16 hours. The tissues were removed from the RNA*later*®-ICE and weighed. Each liver tissue sample, weighing

between 21 and 30 mg, was added to lysis buffer and homogenized using plastic disposable pestles (Fisher Scientific, Pittsburgh, PA). Following homogenization, samples were stored at −70°C ± 10°C until RNA was isolated. Samples were thawed and centrifuged. RNA was extracted from the supernatant, subjected to DNase digestion, and isolated using the Qiagen RNeasy Mini Kit (Cat #: 74104; Qiagen, Valencia, CA). Each RNA sample was analyzed for quantity and purity by UV analysis using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples were evaluated for RNA integrity using an RNA 6000 Nano Kit with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA) and then stored at −70°C ± 10°C until further processing.

Total RNA (100 ng), isolated from each liver sample, was used to synthesize single-stranded DNA, which was subsequently converted into a double-stranded complementary DNA (cDNA) template for transcription. An in vitro transcription (IVT) reaction, which incorporates biotinylated ribonucleotide analogs, then was used to create labeled amplified RNA (aRNA). This RNA target preparation was performed using the Affymetrix GeneChip® 3' IVT Express Kit (Cat #: 901228; Affymetrix Inc., Santa Clara, CA) and an Eppendorf Mastercycler® thermal cycler (Eppendorf, Hamburg, Germany).

Labeled aRNA was fragmented and subsequently hybridized to the Affymetrix Rat Genome 230 2.0 Array (Cat #: 900505; 31,099 probe sets) using an Affymetrix GeneChip® Hybridization Oven 645. The arrays were washed and stained using the Affymetrix GeneChip® Hybridization Wash and Stain kit (Cat #: 900720) and a Fluidics Station 450 according to the Affymetrix-recommended protocol (FS450_0001). After washing and staining, arrays were scanned using an Affymetrix GeneChip® Scanner 3000 7G, and the raw microarray data (.CEL files) were acquired using Affymetrix GeneChip® Command Console® Software. The Rat Genome 230 2.0 Array provides coverage of more than 30,000 known transcripts; although the array provides comprehensive coverage of global transcript expression, of note is that it does not cover the entirety of the rat transcriptome.

Analysis of GeneChip Data Quality

Quality control measurements were evaluated to determine if the data generated from each Affymetrix GeneChip® array were of sufficient quality. Affymetrix-recommended guidelines for evaluating quality were used to evaluate the output files for each GeneChip® array using the R/Bioconductor package, Simpleaffy.[9] The following quality control parameters were evaluated for each array: average background, scale factor, percentage of genes scored as present, 3' to 5' ratios for the internal control genes beta-actin and glyceraldehyde-3-phosphate dehydrogenase, values for hybridization control transcripts, and values for poly (A) controls.

For samples that failed to pass quality control evaluation due to insufficient data quality, an additional round of RNA isolation and cDNA synthesis was performed and additional GeneChip® arrays were run, which were designated with –R after each sample number.

Data Analysis

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

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control 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[10; 11] and Dunnett.[12] Hormone data and clinical chemistry, which typically have skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[13] and Dunn.[14] The Jonckheere test[15] 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[16] were examined by National Institute of Environmental Health Sciences staff. Values from animals suspected of illness due to causes other than experimental exposure and values that the laboratory indicated as inadequate due to measurement problems were eliminated from the analysis.

A no-observed-effect level (NOEL) was identified as the highest dose not showing a significant ($p \leq 0.05$) pairwise difference relative to the vehicle control group. A lowest-observed-effect level (LOEL) was identified as the lowest dose demonstrating a significant ($p \leq 0.05$) pairwise difference relative to the vehicle control group.

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

Clinical pathology, body weight, and organ weight endpoints that exhibited a significant trend and pairwise test were submitted in batch for automated benchmark dose (BMD) modeling analysis. For body weight, the BMD and benchmark dose lower confidence limit (BMD_L) were reported as not determined when there were no significant results. BMD modeling and analysis was conducted using a modification of Benchmark Dose Modeling Software (BMDS) version 2.7.0. Data sets were executed using the Python BMDS interface (https://pypi.python.org/pypi/bmds; version 0.11), which allows for batch processing of multiple data sets. Data for all endpoints submitted were continuous. A default benchmark response (BMR) of 1 standard deviation (relative to control) was used for all data sets. The following BMDS 2.7.0 models were used to model the means of the data sets:

- Linear
- Polynomial 2°, 3°, 4°, 5°
- Power
- Hill
- Exponential M2, M3, M4, M5

Multiple versions of the polynomial model were executed, from a polynomial of degree 2 to a polynomial of degree equal to the number of dose groups minus 1 (e.g., if a data set had five dose groups, a 2°, 3°, and 4° polynomial model would be executed). Models were initialized using BMDS 2.7.0 model defaults, including restricting the power parameter of the power model and n-parameter of the Hill model to >1 and the beta parameters of the polynomial model to positive or negative, depending on the mean response direction of the data set. For all models, either a constant or nonconstant variance model was selected as outlined in U.S. Environmental Protection Agency (EPA) BMD technical guidance[17] and was implemented in the BMDS 2.7.0 software.

After model execution, BMDs were selected using the model recommendation procedures generally described,[17] and the automated decision logic described in Wignall et al.[18] and summarized in Appendix D, Table D-1. Models were placed into one of four possible bins, depending on the results and the bin recommendation logic:

1. **Failure:** model did not successfully complete
2. **Nonviable model (NVM):** model successfully completed but with serious issues
3. **Not reportable (NR)**: model is identified and meets all acceptability criteria with the exception of the estimated BMD being below the lower limit of extrapolation (<1/3 the lowest nonzero dose tested); BMD reported as <1/3 the lowest nonzero dose tested and BMDL is not reportable
4. **Viable model:** candidate for recommended model without warning

If only one model was in the viable model bin, it was selected as the best-fitting model. If the viable bin had more than one model, consistent with EPA guidance,[17] either the model with the lowest Akaike information criterion (AIC) or lowest BMD_L was selected. If the range of BMD_L values was sufficiently close (less than threefold different), the AIC value was used; otherwise, the BMD_L value was used. If no model was recommended, no BMD was presented in the results. Details on the analysis criteria and decision tree are provided in Appendix D, Table D-1 and Figure D-1, respectively. To avoid effects of model extrapolation, BMD values derived from viable models that were threefold lower than the lowest nonzero dose tested were reported as <1/3 the lowest nonzero dose tested and corresponding BMD_L values were not reported.

Benchmark Dose Analysis of Transcriptomics Data

The BMD analysis of the transcriptomic data was performed in accordance with NTP 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*.[19]

Probe set intensities from raw microarray data (.CEL files from Affymetrix Rat Genome 230 2.0 Arrays) were normalized by applying the Robust Multi-array Average (RMA) algorithm from the genomics analysis tool, GeneSpring GX 12.6 (Agilent Technology, Foster City, CA). The microarray studies of multiple organophosphate phosphates (data to be reported elsewhere) were performed at the same time such that .CEL files from those related studies were normalized together with the data sets collected in this study. Principal component analysis (PCA) of the primary RMA-normalized data indicated a batch effect; due to randomization of the samples in the processing and detailed metadata capture, the source of the batch effect could be identified as

the hybridization date. To correct the batch effect, the primary normalized data were loaded into Partek Genomic Suite version 6.16.0812 (St. Louis, MO) and annotated with chemical exposure/dose group and hybridization date annotations. The ANOVA-based remove batch effect function in Partek Genomic Suite then was used to remove quantitative impacts from the hybridization date batch effect. Quality control of the batch-corrected, normalized data was performed by visual inspection, using a PCA plot and normalized intensity histograms (Appendix C).

Dose-response analyses of RMA-normalized, batch-corrected probe set intensities from the TCP study samples were performed using BMDExpress 2.20.0148 beta[20] (https://github.com/auerbachs/BMDExpress-2/releases), an updated version of BMDExpress 1.41 that uses an updated modeling approach. First, control genes (AFFX-) were removed from each data set. A trend test (the Williams trend test,[10; 11] $p \leq 0.05$) and fold change filter (1.5-fold change up or down relative to the vehicle control group for probe sets) was applied to the data set to remove probe sets demonstrating no response to chemical exposure from subsequent analysis. These filter criteria were empirically determined, with the goal of balancing false discovery with reproducibility. The criteria are consistent with the MicroArray Quality Control recommendations to combine the nominal p value threshold with a fold change filter to maximize replicability of transcriptomic findings across labs. The following dose-response models were fit to the probe sets that passed the trend test and fold change filter:

- Hill
- Power
- Linear
- Polynomial 2°
- Exponential M2, M3, M4, M5

All gene expression data analyzed in BMDExpress were log2 transformed, and thus nearly all probe sets exhibit constant variance across the doses. For this reason and for efficiency purposes, each model was run assuming constant variance. Lacking any broadly applicable guidance regarding the level of change in gene expression considered to be biologically significant, a BMR of 1 standard deviation (relative to the fit at control) was used in this study. This approach enables standardization of the BMR between apical endpoints and transcriptomic endpoints and provides a standard for use across multiple chemicals tested in this rapid screening paradigm. The expression direction (upregulated or downregulated) for each probe set was determined by a trend test intrinsic to the model executables (provided by EPA) contained in BMDExpress.

To identify the best-fit model for each fitted probe set, the AIC values for each fitted model were compared and the model with the lowest AIC selected. The best model for each probe set was used to calculate the BMD, BMD_L, and BMD upper confidence limit (BMD_U). The specific parameter settings, selected from the BMDExpress software when performing probe set-level BMD analysis, were as follows: maximum iterations – 250, confidence level – 0.95, BMR factor – 1 (the multiplier of the SD that defined the BMD), restrict power – no restriction, and constant variance – selected. The specific model selection setting in the BMDExpress software when performing probe set-level BMD analysis was as follows: best poly model test – lowest AIC, flag Hill model with ‘k’ parameters – <1/3 the lowest nonzero dose tested, and best model selection with flagged Hill model – include flagged Hill model. The inclusion of the flagged

models is a deviation from EPA guidance. The justification for this deviation relates to subsequent use of the data in which the probe set BMD values are grouped into gene sets from which a median BMD is derived. If the probe sets were removed from the analysis or forced to another model, the probe set might not be counted in the gene set analysis and could lead to loss of "active" gene sets. Importantly, most of the probe sets that produce flagged Hill models show highly potent responses and should therefore be counted in the analysis.

To perform Gene Ontology (GO; annotation accession date: 03/09/18) gene set analysis, only GO terms with ≥10 and ≤250 annotated genes measured on the gene expression platform were considered. Before sorting genes into the GO terms, the best-fit model for each probe set was subjected to a filtering process to remove those probe sets (1) with a BMD >highest dose tested, (2) that mapped to more than one gene, (3) that had a global goodness-of-fit p value ≤0.1, and (4) with a BMD_U/BMD_L ratio >40. GO terms that were at least 5% populated and contained three genes that passed the above criteria were considered "active" (i.e., responsive to chemical exposure). For this report, GO terms populated with identical sets of differentially expressed genes were filtered to limit redundancy in reporting based on the following selection criteria: (1) highest percentage populated and (2) most specific/highest GO level. Redundant GO terms failing to differentiate on the basis of these criteria were retained and reported. A complete list of "active" GO terms can be found in Appendix F. To avoid effects of model extrapolation, GO terms exhibiting BMD values below the lower limit of extrapolation (<1/3 the lowest nonzero dose tested) were reported as <1/3 the lowest nonzero dose tested and corresponding BMD_L and BMD_U values were not reported.

To perform Individual Gene Analysis, a Defined Category Analysis in BMDExpress was performed that mapped probe sets to genes using a probe-to-gene annotation file. In short, the best-fit model for each probe set was subjected to a filtering process to remove those probe sets (1) with a BMD >highest dose tested, (2) that mapped to more than one gene, (3) that had a global goodness-of-fit p value ≤0.1, and (4) with a BMD_U/BMD_L ratio >40. For genes that had more than one probe set represented on the microarray and passed the above filtering, a median BMD was used to estimate the BMD, BMD_L, and BMD_U values. To ensure only genes with a robust response were assessed for potency, genes with probe sets that had a median fold change <|2| were removed prior to reporting. A complete list of genes and their corresponding metrics can be found in Appendix F. To avoid effects of model extrapolation, genes exhibiting BMD values below the lower limit of extrapolation (<1/3 the lowest nonzero dose tested) were reported as <1/3 the lowest nonzero dose tested and corresponding BMD_L and BMD_U values were not reported.

A summary of the BMDExpress gene expression analysis pipeline used in this study is shown in Figure D-2.

Data Accessibility

Primary and analyzed data used in this study are available to the public at https://doi.org/10.22427/NIEHS-DATA-NIEHS-06.[21]

Results

Animal Condition, Body Weights, and Organ Weights

All rats administered tricresyl phosphate (TCP) survived to the end of the study. One rat in the 995 mg/kg group was noted with diarrhea on study days 2 and 4; no other clinical observations were noted. A negative trend was found in terminal body weight, although there were no significant pairwise comparisons (Table 1).

Table 1. Body Weight Summary

Study Day	0 mg/kg[a,b] n = 5	62 mg/kg n = 6	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 5	BMD_{1Std} (mg/kg)	$BMDL_{1Std}$ (mg/kg)
0	225.8 ± 3.7	226.0 ± 1.2	222.1 ± 3.5	224.3 ± 4.6	225.1 ± 4.7	224.9 ± 4.8	ND	ND
4	247.2 ± 3.8*	245.0 ± 1.6	239.5 ± 3.9	245.7 ± 4.8	240.4 ± 4.1	232.2 ± 6.4	ND	ND

Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$.
Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; study day 0 = the first day of dosing; study day 4 = the day of necropsy; 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.

At necropsy, a significant increase in absolute and relative liver weights occurred in dose groups ≥497 mg/kg and ≥249 mg/kg, respectively; both endpoints had positive trends (Table 2). The benchmark dose (benchmark dose lower confidence limit)—BMD (BMD_L)—for increased absolute liver weight was 223.8 (97.1) mg/kg and for relative liver weight was 129.5 (78.4) mg/kg. Significant trend and pairwise comparisons were not observed in absolute or relative brain weights (Appendix B).

Clinical Chemistry

Serum albumin concentration was significantly decreased in dose groups ≥125 mg/kg with a negative trend and a BMD (BMD_L) of 62.5 (28.4) (Table 3), while serum globulin concentration was significantly increased in the 995 mg/kg group with a positive trend and a BMD (BMD_L) of 344.8 (255.8). The combination of these changes resulted in a negative trend in the albumin/globulin ratio and significant pairwise comparisons in the ≥125 mg/kg groups with a BMD (BMD_L) of 172.8 (134.5) mg/kg. Low-density lipoprotein (LDL) cholesterol concentration was significantly increased in the 995 mg/kg group and total cholesterol and high-density lipoprotein (HDL) cholesterol concentrations were significantly increased in dose groups ≥249 mg/kg; all three endpoints had positive trends with BMDs (BMD_Ls) ranging from 241.8 (175.1) mg/kg to 335.6 (245.7) mg/kg. Alanine aminotransferase activity was significantly increased in dose groups ≥125 mg/kg, with a positive trend and a BMD (BMD_L) of 31.1 (10.1) mg/kg. There were no other clinical chemistry findings that exhibited significant trend and pairwise comparisons (Appendix B).

Hormones and Enzymes

Serum cholinesterase activity was significantly decreased in all dosed groups by 43%–78%, beginning with the 62 mg/kg group (Table 4); a BMD_L was not reportable because the BMD was

below the lower limit of extrapolation (<20.7 mg/kg). Testing lower doses in future studies will therefore be necessary to calculate a BMD associated with decreased cholinesterase in the context of TCP exposure. No significant trend and pairwise comparisons were observed in total thyroxine concentration (Appendix B).

Table 2. Organ Weights Summary[a]

Endpoint	0 mg/kg[b,c] n = 5	62 mg/kg n = 4–6[d]	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 4–5[d]	BMD_{1Std} (mg/kg)	$BMDL_{1Std}$ (mg/kg)
Terminal Body Weight (g)	247.2 ± 3.8*	245.0 ± 1.6	239.5 ± 3.9	245.7 ± 4.8	240.4 ± 4.1	232.2 ± 6.4	ND	ND
Liver Weight Absolute (g)	10.35 ± 0.23**	10.51 ± 0.35	10.02 ± 0.30	11.10 ± 0.22	11.43 ± 0.29*	11.57 ± 0.44**	223.8	97.1
Liver Weight Relative[e] (mg/g)	41.90 ± 0.77**	42.96 ± 1.23	41.83 ± 0.96	45.19 ± 0.35*	47.53 ± 1.05**	50.11 ± 0.75**	129.5	78.4

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; ND = not determined.
[a]Descriptions of organ weight endpoints and changes are provided in Appendix E.
[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]Changes in n reflect exclusions based on outlier analysis or organ weights not recorded at necropsy. Liver weight from one animal was removed as an outlier in the 62 mg/kg group. Liver weight was not measured for one animal in the 62 mg/kg group and one animal in the 995 mg/kg group.
[e]Relative organ weights (organ weight-to-body weight ratios) are given as mg organ weight/g body weight.

Table 3. Clinical Chemistry Summary

Endpoint	0 mg/kg[a,b] n = 5	62 mg/kg n = 5	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 5	BMD_{1Std} (mg/kg)	BMD_{L1Std} (mg/kg)
Globulin (g/dL)	2.54 ± 0.07*	2.58 ± 0.04	2.52 ± 0.04	2.58 ± 0.02	2.62 ± 0.04	2.82 ± 0.05*	344.8	255.8
A/G Ratio	1.35 ± 0.01**	1.30 ± 0.02	1.29 ± 0.01*	1.24 ± 0.02**	1.24 ± 0.02**	1.11 ± 0.01**	172.8	134.5
Albumin (g/dL)	3.42 ± 0.06**	3.36 ± 0.05	3.26 ± 0.02*	3.20 ± 0.04*	3.24 ± 0.02*	3.14 ± 0.04**	62.5	28.4
Cholesterol (mg/dL)	113.2 ± 5.1**	125.6 ± 5.7	114.0 ± 5.6	135.6 ± 1.6**	140.6 ± 4.9**	152.8 ± 3.3**	243.8	193.1
LDL Cholesterol (mg/dL)	23.2 ± 1.3**	22.0 ± 1.2	22.2 ± 1.0	23.8 ± 0.9	26.8 ± 1.0	28.6 ± 0.9*	335.6	245.7
HDL Cholesterol (mg/dL)	52.0 ± 2.2**	58.4 ± 2.6	54.2 ± 3.0	64.8 ± 0.9**	63.6 ± 1.7**	65.6 ± 2.6**	241.8	175.1
Alanine Aminotransferase (IU/L)	59.6 ± 4.0**	89.8 ± 28.6	78.0 ± 7.6*	83.8 ± 9.6*	98.0 ± 11.9**	104.6 ± 14.2**	31.1	10.1

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; A/G Ratio = ratio of albumin to globulin; LDL = low-density lipoprotein; HDL = high-density lipoprotein.
[a]Data are displayed as mean ± standard error of the mean.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Table 4. Hormones and Enzymes Summary

Endpoint	0 mg/kg[a,b] n = 5	62 mg/kg n = 5	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 5	BMD_{1Std} (mg/kg)	BMD_{L1Std} (mg/kg)
Cholinesterase (IU/L)	285.4 ± 16.1**	164.0 ± 10.8**	138.6 ± 6.8**	111.0 ± 5.7**	80.4 ± 8.2**	63.4 ± 7.9**	<20.7[c]	NR

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; NR = BMD_L is not reportable because the BMD is below the lower limit of extrapolation (<1/3 the lowest nonzero dose tested).
[a]Data are displayed as mean ± standard error of the mean.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]<20.7 = a best-fit model was identified and a BMD was estimated that was <1/3 the lowest nonzero dose tested.

Apical Endpoint Benchmark Dose Summary

A summary of the calculated BMDs for each toxicological endpoint is provided in Table 5. The 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 <20.7 mg/kg.

Table 5. BMD, BMD_L, LOEL, and NOEL Summary for Apical Endpoints, Sorted by BMD or LOEL from Low to High

Endpoint	BMD_{1Std} (mg/kg)	BMD_{L1Std} (mg/kg)	LOEL (mg/kg)	NOEL (mg/kg)	Direction of Change
Cholinesterase	<20.7[a]	NR	62	ND	DOWN
Alanine Aminotransferase	31.1	10.1	125	62	UP
Albumin	62.5	28.4	125	62	DOWN
Liver Weight Relative	129.5	78.4	249	125	UP
A/G Ratio	172.8	134.5	125	62	DOWN
Liver Weight Absolute	223.8	97.1	497	249	UP
HDL Cholesterol	241.8	175.1	249	125	UP
Cholesterol	243.8	193.1	249	125	UP
LDL Cholesterol	335.6	245.7	995	497	UP
Globulin	344.8	255.8	995	497	UP

Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; LOEL = lowest-observed-effect level; NOEL = no-observed-effect level; NR = BMD_L is not reportable because the BMD is below the lower limit of extrapolation (<1/3 the lowest nonzero dose tested); ND = not determined; A/G Ratio = ratio of albumin to globulin; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
[a]<20.7 = a best-fit model was identified and a BMD was estimated that was <1/3 the lowest nonzero dose tested.

Gene Set Benchmark Dose Analysis

Chemical-induced alterations in liver gene transcript expression were examined to determine those gene sets most sensitive to TCP 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 liver. This analysis used transcript-level BMD data to assess an aggregate score of gene set potency (median transcript BMD) and enrichment.

The "active" gene sets with the lowest BMD median values are shown in Table 6. The gene sets in Table 6 should be interpreted with caution from the standpoint of the underlying biology and any relationship to toxicity or toxic agents referenced in the GO term definitions. The data primarily should be considered 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.

No gene sets had estimated BMD median values <20.7 mg/kg. The most sensitive GO biological processes for which a BMD value could be reliably calculated were cellular response to

dexamethasone stimulus (GO:0071549) and cellular response to glucocorticoid stimulus (GO:0071385), both with BMDs (BMD_{LS}) of 58.5 (34.0) mg/kg. The full list of affected gene sets can be found in Appendix F.

Table 6. Top 10 Gene Ontology Biological Process Gene Sets Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median[a]

Category Name	Input Genes/Platform Genes in Gene Set	% Gene Set Coverage	Active Genes	BMD_{1std} Median of Gene Set Transcripts (mg/kg)	Median BMD_{L1Std}–BMD_{U1Std} (mg/kg)	Genes with Changed Direction Up	Genes with Changed Direction Down
GO:0071549 cellular response to dexamethasone stimulus	4/67	6%	*Igfals*; *Errfi1*; *Hnrnpu*; *Ddit4*	58.5	34.0–168.5	1	3
GO:0071385 cellular response to glucocorticoid stimulus	6/107	6%	*Igfals*; *Errfi1*; *Hnrnpu*; *Lmnb1*; *Orm1*; *Ddit4*	58.5	34.0–168.5	3	3
GO:0035176 social behavior	3/50	6%	*Mss51*; *Avpr1a*; *Anxa7*	63.3	45.9–305.5	1	2
GO:0051705 multi-organism behavior	5/72	7%	*Mss51*; *LOC100910823*; *App*; *Avpr1a*; *Anxa7*	63.3	45.9–305.5	3	2
GO:0071548 response to dexamethasone	5/97	5%	*Igfals*; *Cldn1*; *Errfi1*; *Hnrnpu*; *Ddit4*	70.6	50.3–185.5	1	4
GO:2000272 negative regulation of receptor activity	3/25	12%	*Pcsk9*; *Errfi1*; *App*	70.6	50.3–116.6	1	2
GO:0042058 regulation of epidermal growth factor receptor signaling pathway	5/61	8%	*Rhbdf2*; *Errfi1*; *App*; *Dab2ip*; *Ceacam1*	71.6	51.1–116.6	2	3
GO:0032651 regulation of interleukin-1 beta production	4/46	9%	*Casp4*; *Tnfaip3*; *Errfi1*; *Orm1*	82.2	57.2–145.1	3	1

Category Name	Input Genes/Platform Genes in Gene Set	% Gene Set Coverage	Active Genes	BMD_{1std} Median of Gene Set Transcripts (mg/kg)	Median BMD_{L1Std}–BMD_{U1Std} (mg/kg)	Genes with Changed Direction Up	Genes with Changed Direction Down
GO:0042632 cholesterol homeostasis	4/57	7%	*Pcsk9*; *Abcg5*; *Abcg8*; *Cyp7a1*	85.3	59.2–147.8	1	3
GO:0032375 negative regulation of cholesterol transport	3/12	25%	*Pcsk9*; *Abcg5*; *Abcg8*	92.5	63.3–165.3	0	3

Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; BMD_U = benchmark dose upper confidence limit; GO = Gene Ontology.
[a]Definitions of GO terms were adapted from the Gene Ontology Resource.[22] Official gene symbols from the Rat Genome Database[23] are shown in the "Active Genes" column.
GO process description version: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.[24]
GO:0071549 cellular response to dexamethasone stimulus: 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 dexamethasone stimulus.
GO:0071385 cellular response to glucocorticoid stimulus: 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 glucocorticoid stimulus. Glucocorticoids are hormonal C21 corticosteroids synthesized from cholesterol with the ability to bind with the cortisol receptor and trigger similar effects. Glucocorticoids act primarily on carbohydrate and protein metabolism and have anti-inflammatory effects.
GO:0035176 social behavior: Behavior directed toward society or taking place between members of the same species. Occurs predominantly, or only, in individuals that are part of a group.
GO:0051705 multi-organism behavior: Any process in which an organism has a behavioral effect on another organism of the same or different species.
GO:0071548 response to dexamethasone: 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 dexamethasone stimulus.
GO:2000272 negative regulation of signaling receptor activity: Any process that stops, prevents or reduces the frequency, rate or extent of a signaling receptor activity.
GO:0042058 regulation of epidermal growth factor receptor signaling pathway: Any process that modulates the frequency, rate or extent of epidermal growth factor receptor signaling pathway activity.
GO:0032651 regulation of interleukin-1 beta production: Any process that modulates the frequency, rate, or extent of interleukin-1 beta production.
GO:0042632 cholesterol homeostasis: Any process involved in the maintenance of an internal steady state of cholesterol within an organism or cell.
GO:0032375 negative regulation of cholesterol transport: Any process that stops, prevents, or reduces the frequency, rate or extent of the directed movement of cholesterol into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore.

Gene Benchmark Dose Analysis

The top 10 genes (fold change >|2|, significant Williams trend test, global goodness of fit p value >0.1, and $BMD_U/BMD_L < 40$), ranked by estimated BMD are shown in Table 7. As with the GO analysis, the biological or toxicological significance of the changes in gene expression shown in Table 7 should be interpreted with caution. The data primarily should be considered 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.

The most sensitive gene, exhibiting increased expression, was *Gsta3* (glutathione S-transferase alpha 3) with an estimated BMD median value <20.7 mg/kg. The most sensitive upregulated

genes with a calculated BMD <40 mg/kg were *Ces2c* (carboxylesterase 2c), *Abcc3* (canalicular multispecific organic anion transporter 2), *Orm1* (orosomucoid 1), *Mnd1* (meiotic nuclear division protein 1 homolog), *App* (amyloid beta precursor protein), and *Cyp7a1* (cytochrome P450 family 7 subfamily A member 1). The most sensitive gene exhibiting a decrease in expression was *Fer1l5* (Fer-1-like protein 5) with a BMD <20.7 mg/kg. No other responding genes with decreased expression occurred in the top 10 genes.

Table 7. Top 10 Genes Ranked by Potency of Perturbation, Sorted by Benchmark Dose Median[a]

Gene Symbol	Entrez Gene IDs	Probe IDs	BMD_{1Std} (BMD_{L1std}–BMD_{U1std}) in mg/kg	Maximum Fold Change	Direction of Expression Change
Fer1l5	679806	1396729_at	<20.7[b] (NR)	2.2	DOWN
Gsta3	494500	1371089_at	<20.7 (NR)	3.3	UP
Ces2c	171118	1368905_at	27.7 (18.7–48.5)	7.6	UP
Abcc3	140668	1369698_at	32.1 (22.0–52.8)	7.3	UP
Orm1	24614	1368731_at	32.6 (10.4–123.2)	2.2	UP
Mnd1	295160	1391626_at	33.8 (14.8–91.6)	2.3	UP
App	54226	1371571_at,1371572_at,1380533_at	37.7 (20.8–91.8)	3.3	UP
Cyp7a1	25428	1368458_at	39.4 (17.3–109.9)	4.7	UP
Ddit4	140942	1368025_at	41.6 (9.5–185.5)	3.7	UP
Gstt3	499422	1371942_at	66.0 (28.7–178.7)	2.2	UP

Benchmark response set at 1 standard deviation from the mean.
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; BMD_U = benchmark dose upper confidence limit; NR = the BMD_L-BMD_U range is not reportable because the BMD median is below the lower limit of extrapolation (<1/3 the lowest nonzero dose tested).
[a]Descriptions of orthologous human genes are shown due to the increased detail available in public resources such as UniprotKB[25] and Entrez Gene.[26] Human UniprotKB was used as the primary resource due to the greater breadth of annotation and depth of functional detail provided. Rat UniprotKB was used as the secondary resource if the primary source did not provide a detailed description of function. Human Entrez Gene Summary was used as the third resource. Rat Entrez Gene Summary was used as the fourth resource.
[b]<20.7 = a best-fit model was identified and a BMD was estimated that was <1/3 the lowest nonzero dose tested.
Gene definition version: https://doi.org/10.22427/NTP-DATA-002-00600-0002-000-0.[24]
Fer1l5: Human Uniprot function (Human *FER1L5*): Plays a role in myoblast fusion; probable mediator of endocytic recycling for membrane trafficking events during myotube formation. {ECO0000250}.
Gsta3: Human Uniprot function (Human *GSTA1*): Glutathione S-transferase that catalyzes the nucleophilic attack of the sulfur atom of glutathione on the electrophilic groups of a wide range of exogenous and endogenous compounds (probable). Involved in the formation of glutathione conjugates of both prostaglandin A2 (PGA2) and prostaglandin J2 (PGJ2) (PubMed9084911). It also catalyzes the isomerization of D5-androstene-3,17-dione (AD) into D4-androstene-3,17-dione and may therefore play an important role in hormone biosynthesis (PubMed11152686). Through its glutathione-dependent peroxidase activity toward the fatty acid hydroperoxide (13S)-hydroperoxy-(9Z,11E)-octadecadienoate/13-HPODE, it is also involved in the metabolism of oxidized linoleic acid (PubMed16624487). {ECO0000269|PubMed11152686, ECO0000269|PubMed16624487, ECO0000269|PubMed9084911, ECO0000305|PubMed20606271}.

***Ces2c*:** Human Uniprot function (Human *CES2*): Involved in the detoxification of xenobiotics and in the activation of ester and amide prodrugs (PubMed9169443). Shows high catalytic efficiency for hydrolysis of cocaine, 4-methylumbelliferyl acetate, heroin, and 6-monoacetylmorphine (PubMed9169443). Hydrolyzes aspirin, substrates with large alcohol group and small acyl group, and endogenous lipids such as triacylglycerol (PubMed28677105). Converts monoacylglycerides to free fatty acids and glycerol. Hydrolyzes of 2-arachidonoylglycerol and prostaglandins (PubMed21049984). {ECO0000269|PubMed21049984, ECO0000269|PubMed9169443, ECO0000303|PubMed28677105}.
***Abcc3*:** Human Uniprot function (Human *ABCC3*): May act as an inducible transporter in the biliary and intestinal excretion of organic anions. Acts as an alternative route for the export of bile acids and glucuronides from cholestatic hepatocytes (by similarity). {ECO0000250}.
***Orm1*:** Human Uniprot function (Human *ORM1*): Functions as transport protein in the blood stream. Binds various ligands in the interior of its beta-barrel domain. Also binds synthetic drugs and influences their distribution and availability in the body. Appears to function in modulating the activity of the immune system during the acute-phase reaction. {ECO0000269|PubMed17008009, ECO0000269|PubMed17321687}.
***Mnd1*:** Human Uniprot function (Human *MND1*): Required for proper homologous chromosome pairing and efficient cross-over and intragenic recombination during meiosis (by similarity). Stimulates both DMC1- and RAD51-mediated homologous strand assimilation, which is required for the resolution of meiotic double-strand breaks. {ECO0000250|UniProtKBQ8K396, ECO0000269|PubMed16407260}.
***App*:** Human Uniprot function (Human *APP*): Functions as a cell surface receptor and performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion, and axonogenesis. Interaction between APP molecules on neighboring cells promotes synaptogenesis (PubMed25122912). Involved in cell mobility and transcription regulation through protein-protein interactions. Can promote transcription activation through binding to APBB1-KAT5 and inhibits Notch signaling through interaction with Numb. Couples to apoptosis-inducing pathways such as those mediated by G(O) and JIP. Inhibits G(o) alpha ATPase activity (by similarity). Acts as a kinesin I membrane receptor, mediating the axonal transport of beta-secretase and presenilin 1 (by similarity). By acting as a kinesin I membrane receptor, plays a role in axonal anterograde transport of cargo toward synapses in axons (PubMed17062754, PubMed23011729). Involved in copper homeostasis/oxidative stress through copper ion reduction. In vitro, copper-metallated APP induces neuronal death directly or is potentiated through Cu^{2+}-mediated low-density lipoprotein oxidation. Can regulate neurite outgrowth through binding to components of the extracellular matrix such as heparin and collagen I and IV. The splice isoforms that contain the BPTI domain possess protease inhibitor activity. Induces an AGER-dependent pathway that involves activation of p38 MAPK, resulting in internalization of amyloid-beta peptide and leading to mitochondrial dysfunction in cultured cortical neurons. Provides Cu^{2+} ions for GPC1 that are required for release of nitric oxide (NO) and subsequent degradation of the heparan sulfate chains on GPC1. {ECO0000250, ECO0000250|UniProtKBP12023, ECO0000269|PubMed17062754, ECO0000269|PubMed23011729, ECO0000269|PubMed25122912}; FUNCTION Amyloid-beta peptides are lipophilic metal chelators with metal-reducing activity. Bind transient metals such as copper, zinc and iron. In vitro, can reduce Cu^{2+} and Fe^{3+} to Cu^{+} and Fe^{2+}, respectively. Amyloid-beta protein 42 is a more effective reductant than amyloid-beta protein 40. Amyloid-beta peptides bind to lipoproteins and apolipoproteins E and J in the CSF and to HDL particles in plasma, inhibiting metal-catalyzed oxidation of lipoproteins. APP42-beta may activate mononuclear phagocytes in the brain and elicit inflammatory responses. Promotes both tau aggregation and TPK II-mediated phosphorylation. Interaction with overexpressed HADH2 leads to oxidative stress and neurotoxicity. Also binds GPC1 in lipid rafts; FUNCTION Appicans elicit adhesion of neural cells to the extracellular matrix and may regulate neurite outgrowth in the brain. {ECO0000250}; FUNCTION The gamma-CTF peptides as well as the caspase-cleaved peptides, including C31, are potent enhancers of neuronal apoptosis; FUNCTION N-APP binds TNFRSF21, triggering caspase activation and degeneration of both neuronal cell bodies (via caspase-3) and axons (via caspase-6).
***Cyp7a1*:** Human Uniprot function (Human *CYP7A1*): A cytochrome P450 monooxygenase involved in the metabolism of endogenous cholesterol and its oxygenated derivatives (oxysterols) (PubMed11013305, PubMed12077124, PubMed19965590, PubMed2384150, PubMed21813643). 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) (PubMed2384150, PubMed11013305, PubMed12077124, PubMed19965590, PubMed21813643). 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 (PubMed12077124, PubMed19965590, PubMed2384150). 7-alpha hydroxylates several oxysterols, including 4beta-hydroxycholesterol and 24-hydroxycholesterol (PubMed11013305, PubMed12077124). Catalyzes the oxidation of the 7,8 double bond of 7-dehydrocholesterol and lathosterol with direct and predominant formation of the 7-keto derivatives (PubMed21813643). {ECO0000269|PubMed11013305, ECO0000269|PubMed12077124, ECO0000269|PubMed19965590, ECO0000269|PubMed21813643, ECO0000269|PubMed2384150}.
***Ddit4*:** 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. {ECO0000250, ECO0000269|PubMed15545625, ECO0000269|PubMed15632201, ECO0000269|PubMed15988001, ECO0000269|PubMed17005863, ECO0000269|PubMed17379067, ECO0000269|PubMed19557001, ECO0000269|PubMed20166753, ECO0000269|PubMed21460850}.

***Gstt3*:** Human Uniprot function (Human *GSTT1*): Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles. Acts on 1,2-epoxy-3-(4-nitrophenoxy)propane, phenethylisothiocyanate 4-nitrobenzyl chloride and 4-nitrophenethyl bromide. Displays glutathione peroxidase activity with cumene hydroperoxide. GSTT1_HUMAN, P30711.

Summary

Tricresyl phosphate (TCP) is an organophosphorus flame retardant with widespread human exposure. The literature contains few toxicological data for estimating the potential adverse health effects of TCP. This study used a transcriptomic approach and standard toxicological endpoints to estimate the in vivo biological potency of TCP.

Serum cholinesterase activity was significantly and markedly decreased for all dosed groups. These findings are consistent with several reports that show the classic cholinesterase inhibition in organophosphates.[27] As the lowest-observed-effect level for the study, cholinesterase inhibition appeared to be the most sensitive apical measure; the estimated benchmark dose (BMD) was below the lower limit of extrapolation (<20.7 mg/kg). Further studies are warranted to assess cholinesterase effects at doses <20.7 mg/kg to obtain an accurate point of departure. The most sensitive apical endpoint for which a BMD could be determined was increased serum alanine aminotransferase activity with a BMD and benchmark dose lower confidence limit (BMD_L) of 31.1 (10.1) mg/kg. The next most sensitive apical endpoints observed were a decrease in serum albumin concentration and an increase in relative liver weight with BMDs (BMD_Ls) of 62.5 (28.4) and 129.5 (78.4) mg/kg, respectively.

Gene set-level transcriptional changes in the liver following TCP exposure were estimated to occur at a BMD (BMD_L) as low as 58.5 (34.0) mg/kg, which corresponded to cellular response to dexamethasone stimulus (GO:0071549) and cellular response to glucocorticoid stimulus (GO:0071385). Two genes (*Fer1l5* and *Gsta3*) exhibited changes in expression at dose levels below which a reliable estimate of potency could be achieved (<20.7 mg/kg). The most sensitive genes for which a reliable BMD could be determined were *Ces2c*, with a BMD (BMD_L) of 27.7 (18.7) mg/kg, and *Abcc3*, with a BMD (BMD_L) of 32.1 (22.0) mg/kg.

Under the conditions of this short-duration transcriptomic study in Sprague Dawley (Hsd:Sprague Dawley® SD®) rats, the most sensitive point of departure with a reliable estimate was a transcriptional change in a gene, *Ces2c*, with a BMD (BMD_L) of 27.7 (18.7) mg/kg. Gene set and apical endpoints provided potency estimates slightly higher than *Ces2c*. Follow-up studies that investigate transcriptional and apical endpoint changes at lower doses will be a useful future direction to determine the biological potency of TCP more accurately.

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Appendix A. Animal Identifiers

Tables

Table A-1. Animal Numbers and Microarray Data File Names A-2

Table A-1. Animal Numbers and Microarray Data File Names

Animal Number	Group	Dose (mmol/kg/day)	Dose (mg/kg/day)	Survived to Study Termination	Array ID
77	Corn Oil	0	0	Yes	057-052114-MW_(Rat230_2).CEL
100	Corn Oil	0	0	Yes	084-052714-JAP_(Rat230_2).CEL
104	Corn Oil	0	0	Yes	013-051914-MW_(Rat230_2).CEL
122	Corn Oil	0	0	Yes	017-051914-MW_(Rat230_2).CEL
133	Corn Oil	0	0	Yes	049-052114-MW_(Rat230_2).CEL
69	TCP	0.169	62	Yes	085-052714-JAP_(Rat230_2).CEL
78	TCP	0.169	62	Yes	014-051914-MW_(Rat230_2).CEL
79	TCP	0.169	62	Yes	NA
112	TCP	0.169	62	Yes	NA
119	TCP	0.169	62	Yes	050-052114-MW_(Rat230_2).CEL
138	TCP	0.169	62	Yes	NA
87	TCP	0.338	125	Yes	015-051914-MW_(Rat230_2).CEL
89	TCP	0.338	125	Yes	051-052114-MW_(Rat230_2).CEL
94	TCP	0.338	125	Yes	NA
102	TCP	0.338	125	Yes	NA
142	TCP	0.338	125	Yes	086-052714-JAP_(Rat230_2).CEL
75	TCP	0.675	249	Yes	16R-082514-MW_(Rat230_2).CEL
92	TCP	0.675	249	Yes	052-052114-MW_(Rat230_2).CEL
99	TCP	0.675	249	Yes	NA
136	TCP	0.675	249	Yes	NA
139	TCP	0.675	249	Yes	087-052714-JAP_(Rat230_2).CEL
70	TCP	1.35	497	Yes	018-051914-MW_(Rat230_2).CEL
95	TCP	1.35	497	Yes	053-052114-MW_(Rat230_2).CEL
113	TCP	1.35	497	Yes	NA
123	TCP	1.35	497	Yes	NA
134	TCP	1.35	497	Yes	088-052714-JAP_(Rat230_2).CEL
76	TCP	2.7	995	Yes	019-051914-MW_(Rat230_2).CEL
88	TCP	2.7	995	Yes	054-052114-MW_(Rat230_2).CEL
91	TCP	2.7	995	Yes	NA
98	TCP	2.7	995	Yes	089-052814-MW_(Rat230_2).CEL
109	TCP	2.7	995	Yes	NA

NA = no transcriptomics data collected for selected animal.

Appendix B. Toxicology Data Tables

Tables

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Table B-2. PA06: Organ Weights Summary B-3
Table B-3. PA41: Clinical Chemistry Summary B-4
Table B-4. R07: Hormones and Enzymes Summary B-5

Table B-1. I04: Body Weight Summary[a,b]

Study Day	0 mg/kg n = 5	62 mg/kg n = 6	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 5
0	225.8 ± 3.7	226.0 ± 1.2	222.1 ± 3.5	224.3 ± 4.6	225.1 ± 4.7	224.9 ± 4.8
4	247.2 ± 3.8*	245.0 ± 1.6	239.5 ± 3.9	245.7 ± 4.8	240.4 ± 4.1	232.2 ± 6.4

Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$.
Study day 0 = the first day of dosing; study day 4 = the day of necropsy.
[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.

Table B-2. PA06: Organ Weights Summary[a,b,c]

Endpoint	0 mg/kg n = 5	62 mg/kg n = 4–6[d]	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 4–5[d]
Terminal Body Weight (g)	247.2 ± 3.8*	245.0 ± 1.6	239.5 ± 3.9	245.7 ± 4.8	240.4 ± 4.1	232.2 ± 6.4
Brain Weight Absolute (g)	1.68 ± 0.04	1.79 ± 0.02	1.76 ± 0.01	1.72 ± 0.02	1.73 ± 0.02	1.68 ± 0.07
Brain Weight Relative[e] (mg/g)	6.82 ± 0.22	7.28 ± 0.08	7.34 ± 0.10	7.01 ± 0.09	7.22 ± 0.17	7.22 ± 0.14
Liver Weight Absolute (g)	10.35 ± 0.23**	10.51 ± 0.35	10.02 ± 0.30	11.10 ± 0.22	11.43 ± 0.29*	11.57 ± 0.44**
Liver Weight Relative (mg/g)	41.90 ± 0.77**	42.96 ± 1.23	41.83 ± 0.96	45.19 ± 0.35*	47.53 ± 1.05**	50.11 ± 0.75**

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

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

[a]Descriptions of organ weight endpoints and changes are provided in Appendix E.

[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]Changes in n reflect exclusions based on outlier analysis or organ weights not recorded at necropsy. Liver weight from one animal was removed as an outlier in the 62 mg/kg group. Liver weight was not measured for one animal in the 62 mg/kg group and one animal in the 995 mg/kg group.

[e]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Table B-3. PA41: Clinical Chemistry Summary[a,b]

Endpoint	0 mg/kg n = 5	62 mg/kg n = 5	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 5
Urea Nitrogen (mg/dL)	9.6 ± 0.2	10.8 ± 0.7	10.4 ± 0.9	13.2 ± 1.6	9.4 ± 0.4	11.0 ± 0.5
Creatinine (mg/dL)	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.3 ± 0.01
Total Protein (g/dL)	5.96 ± 0.12	5.94 ± 0.08	5.78 ± 0.06	5.78 ± 0.04	5.86 ± 0.04	5.96 ± 0.09
Globulin (g/dL)	2.54 ± 0.07*	2.58 ± 0.04	2.52 ± 0.04	2.58 ± 0.02	2.62 ± 0.04	2.82 ± 0.05*
A/G Ratio	1.35 ± 0.01**	1.3 ± 0.02	1.29 ± 0.01*	1.24 ± 0.02**	1.24 ± 0.02**	1.11 ± 0.01**
Albumin (g/dL)	3.42 ± 0.06**	3.36 ± 0.05	3.26 ± 0.02*	3.2 ± 0.04*	3.24 ± 0.02*	3.14 ± 0.04**
Cholesterol (mg/dL)	113.2 ± 5.1**	125.6 ± 5.7	114.0 ± 5.6	135.6 ± 1.6**	140.6 ± 4.9**	152.8 ± 3.3**
Triglyceride (mg/dL)	52.2 ± 1.1	55.8 ± 6.2	45.4 ± 2.5	51.2 ± 4.0	51.0 ± 3.2	51.4 ± 7.1
LDL Cholesterol (mg/dL)	23.2 ± 1.3**	22.0 ± 1.2	22.2 ± 1.0	23.8 ± 0.9	26.8 ± 1.0	28.6 ± 0.9*
HDL Cholesterol (mg/dL)	52.0 ± 2.2**	58.4 ± 2.6	54.2 ± 3.0	64.8 ± 0.9**	63.6 ± 1.7**	65.6 ± 2.6**
Alanine Aminotransferase (IU/L)	59.6 ± 3.99**	89.8 ± 28.61	78.0 ± 7.58*	83.8 ± 9.6*	98.0 ± 11.86**	104.6 ± 14.23**
Aspartate Aminotransferase (U/L)	109.8 ± 5.58	181.6 ± 76.9	131.2 ± 11.61	116.4 ± 10.76	116.4 ± 18.12	116.4 ± 17.21
Sorbitol Dehydrogenase (IU/L)	15.0 ± 1.5	30.2 ± 15.7	21.6 ± 3.4	22.8 ± 5.8	25.2 ± 5.7	19.7 ± 6.1
Bile Salts/Acids (µmol/L)	34.9 ± 4.4	49.7 ± 10.8	43.4 ± 6.9	63.2 ± 8.7	40.6 ± 8.6	32.6 ± 6.3

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

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

A/G Ratio = ratio of albumin to globulin; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

[a]Data are displayed as mean ± standard error of the mean.

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

Table B-4. R07: Hormones and Enzymes Summary[a,b]

Endpoint	0 mg/kg n = 5	62 mg/kg n = 5	125 mg/kg n = 5	249 mg/kg n = 5	497 mg/kg n = 5	995 mg/kg n = 5
Total Thyroxine (µg/dL)	3.98 ± 0.40	5.03 ± 0.47	5.09 ± 0.29	4.57 ± 0.15	4.94 ± 0.36	3.42 ± 0.48
Cholinesterase (IU/L)	285.4 ± 16.1**	164.0 ± 10.8**	138.6 ± 6.8**	111.0 ± 5.7**	80.4 ± 8.2**	63.4 ± 7.9**

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
[a]Data are displayed as mean ± standard error of the mean.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Appendix C. Transcriptomic Quality Control and Additional Data Analysis

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C.1. Gene Expression Quality Control

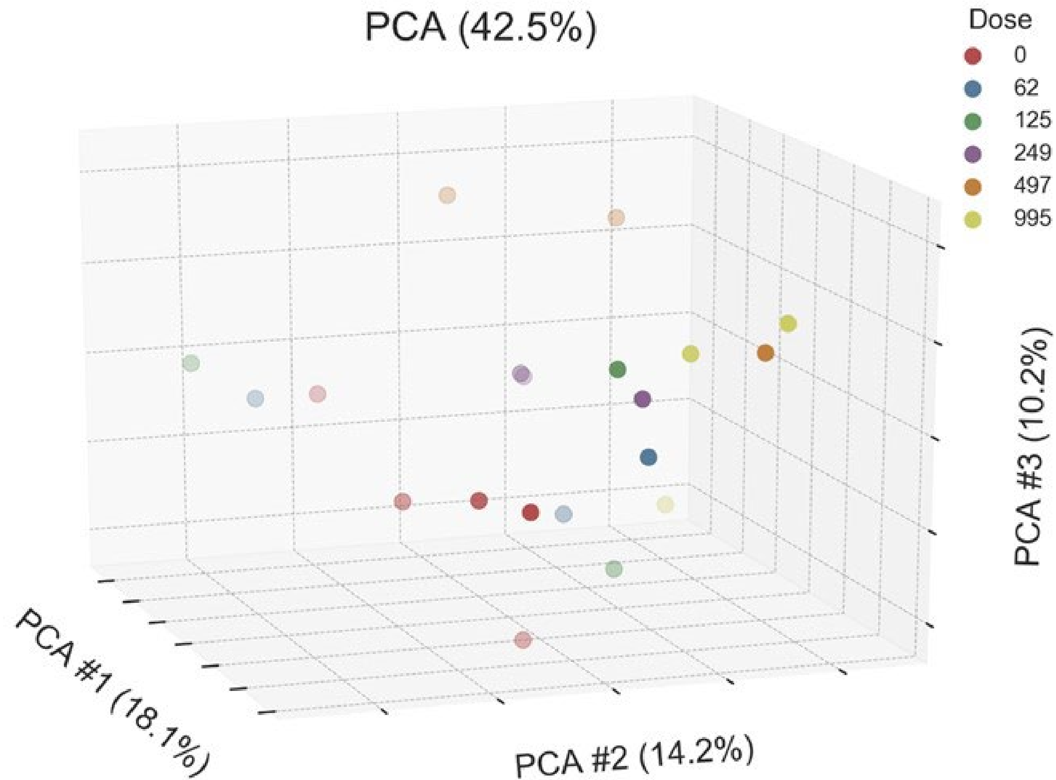

Figure C-1. A Principal Component Analysis of the Robust Multi-array Average-normalized Data

The principal component analysis (PCA) plot enables three-dimensional visualization of global transcriptional changes and the divergence of transcript expression from individual animals (dots) within each dose 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. Lighter color (fogging) indicates a data point is farther back on the z-plane [principal component (PC) #1].

C.2. Additional Data Analysis

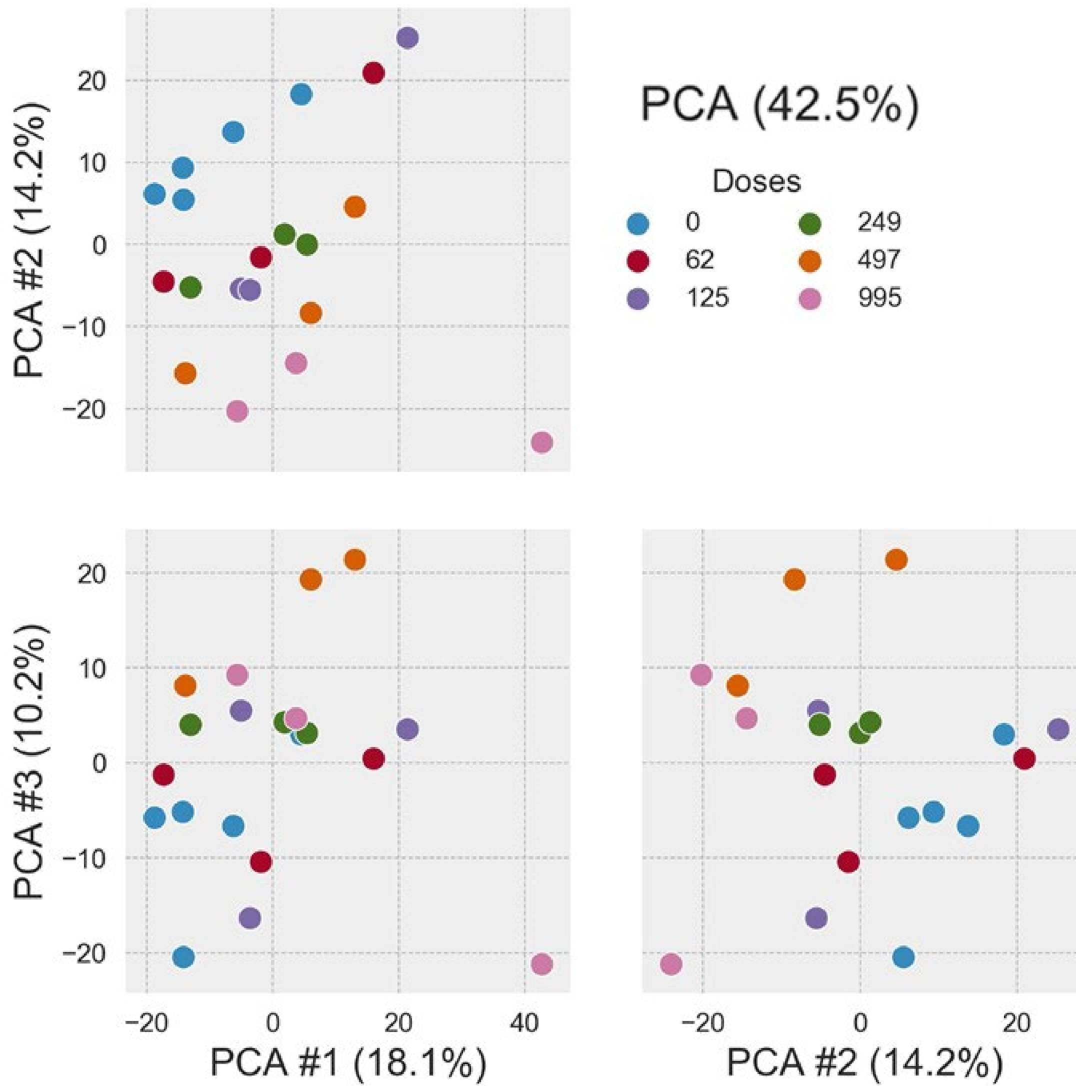

Figure C-2. An Alternative View of the Principal Component Analysis of the Robust Multi-array Average-normalized Data

This alternative view of the 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 dose 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.

Appendix D. Benchmark Dose Model Recommendation and Selection Methodologies

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Figure D-2. Benchmark Dose Model Recommendation/Selection Methodology for Benchmark Dose Execution of Gene Sets with Expression Changes Enacted by Chemical Exposure D-4

Table D-1. Benchmark Dose Model Recommendation/Selection Rules for Apical Endpoints

Rule	Criteria for "Viable"	Numerical Threshold (N)	Bin Placement for Rule Failure
BMD Existence	A BMD exists.	N/A	Failure
BMD_L Existence	A BMD_L exists.	N/A	Failure
AIC Existence	An AIC exists.	N/A	Failure
Residual of Interest Existence	The residual at the dose group closest to the BMD (i.e., the residual of interest) exists.	N/A	Failure
Variance Model Fit	The variance model used fits the data.	N/A	Nonviable
Variance Model Selection	The variance model is appropriate.	N/A	Nonviable
Global Goodness of Fit	The mean model fits the data means sufficiently well (BMDS 2.7.0 Test 4 p value > N).	0.1	Nonviable
Degrees of Freedom	There is at least one degree of freedom (i.e., more dose-groups than model parameters)	N/A	Nonviable
BMD-to-BMD_L Ratio	The ratio of BMD to BMD_L is not large ($BMD/BMD_L < N$).	20	Viable
High BMD_L	The BMD_L is <N times higher than the maximum dose.	1	Viable
High BMD	The BMD is <N times higher than the maximum dose.	1	Viable
Low BMD	The BMD is <N times lower than the minimum nonzero dose.	3	Nonreportable
Control Residual	The residual at control is small (residual < N).	2	Nonviable
Control Standard Deviation	The modeled standard deviation is similar to the actual (<N times different).	1.5	Nonviable
Residual of Interest	The residual at the dose group closest to the BMD (i.e., the residual of interest) is small (residual < N).	2	Nonviable
No Warnings Reported	No warnings in the BMD model system were reported.	N/A	Viable

BMD = benchmark dose; N/A = not applicable; BMD_L = benchmark dose lower confidence limit; AIC = Akaike information criterion; BMDS = Benchmark Dose Software.

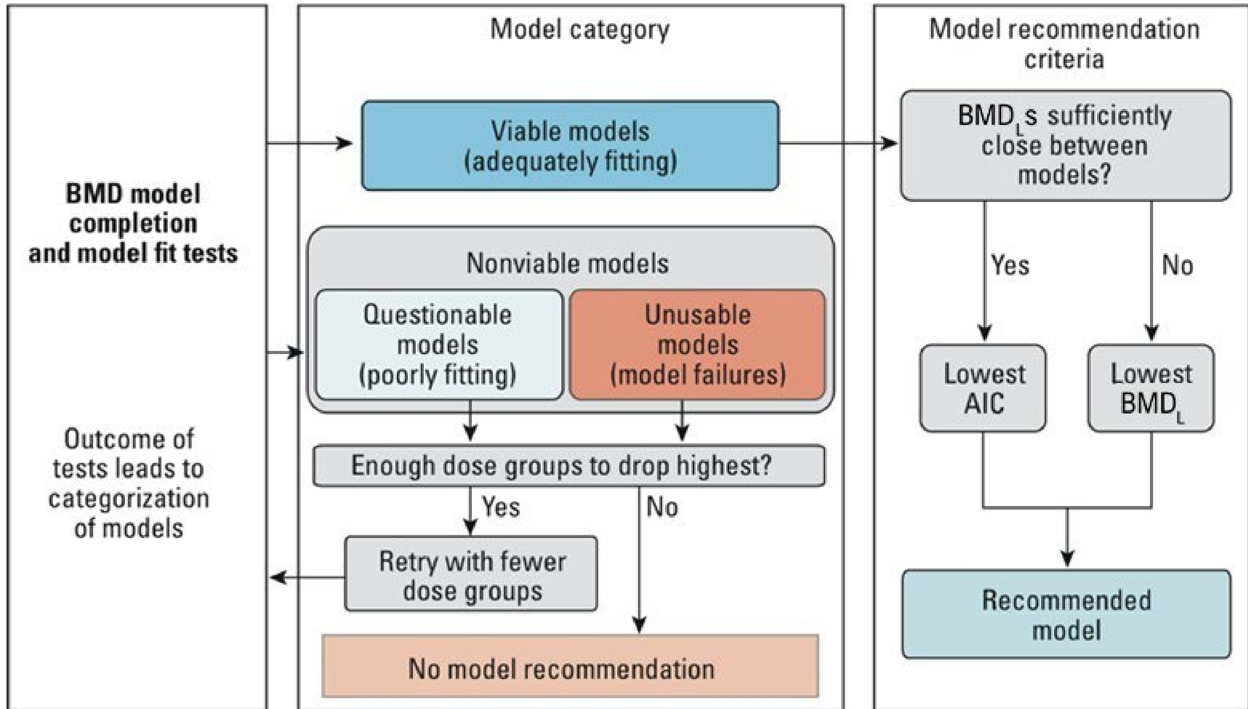

Figure D-1. Benchmark Dose Model Recommendation/Selection Methodology for Automated Benchmark Dose Execution of Apical Endpoints

Source: Figure adapted from Wignall et al. (2014)[18]
BMD = benchmark dose; BMD_L = benchmark dose lower confidence limit; AIC = Akaike information criterion.

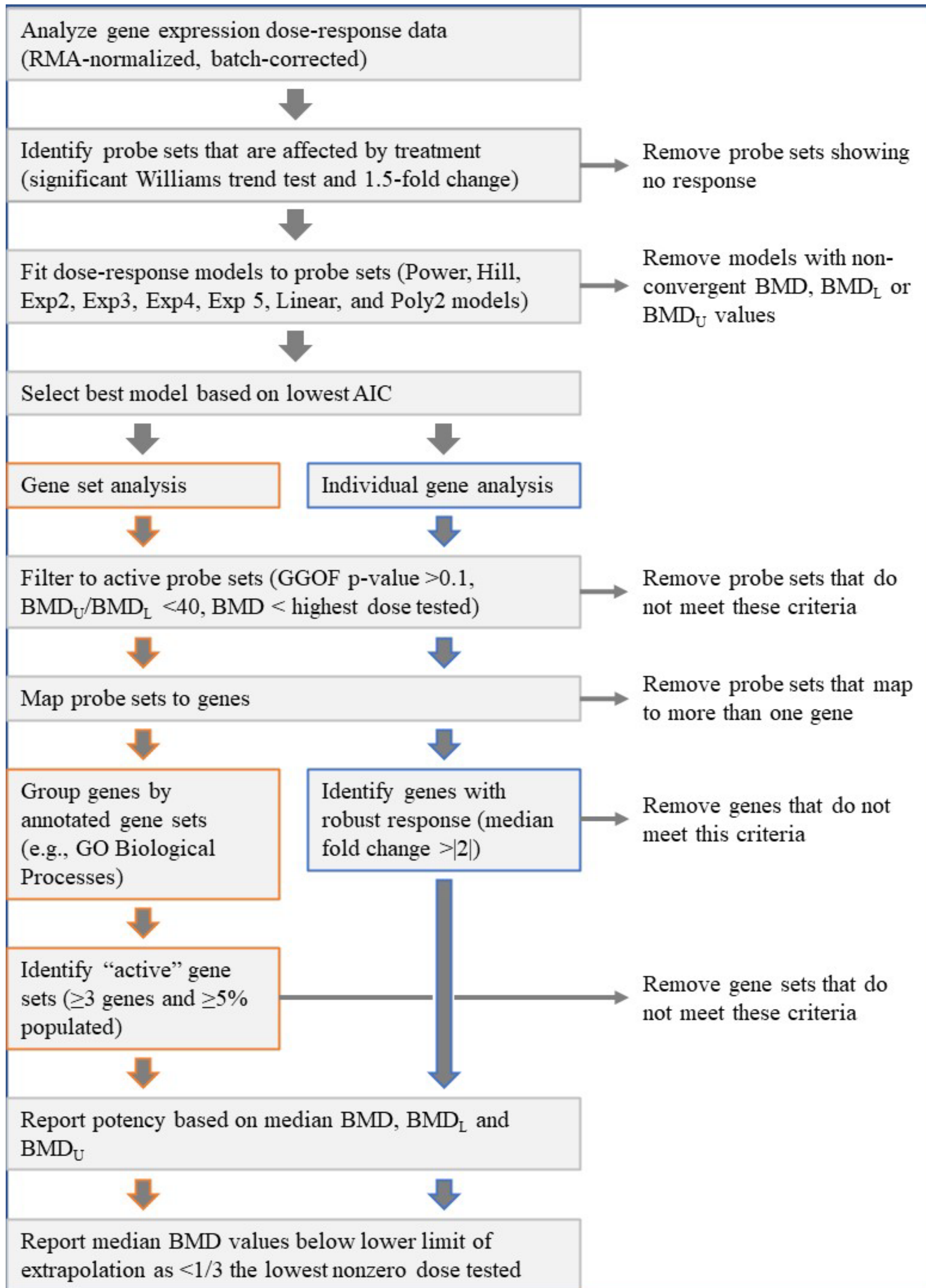

Figure D-2. Benchmark Dose Model Recommendation/Selection Methodology for Benchmark Dose Execution of Gene Sets with Expression Changes Enacted by Chemical Exposure

Adapted from Thomas et al. (2007)[28]
RMA = Robust Multi-array Average; BMD = benchmark dose; BMD$_L$ = benchmark dose lower confidence limit; BMD$_U$ = benchmark dose upper confidence limit; AIC = Akaike information criterion; GGOF = global goodness of fit; GO = Gene Ontology.

Appendix E. Organ Weight Descriptions

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E.1. Organ Weight Descriptions E-2

E.1. Organ Weight Descriptions

Brain: As the principal organ responsible for cognition and control of organ systems and bodily functions, the brain is largely shielded from toxic insults sufficiently severe to affect its weight. Because of this resistance to change, brain weight is often used as a denominator in determinations of other organ weight ratio changes. Other than in cases of grossly observable effects in the brain at necropsy, significant differences in brain weight in subacute toxicity studies are unlikely an effect of chemical exposure. More likely, changes in brain weight are the result of randomization (i.e., sorting of animals into groups for which the mean and standard deviation are significantly different at the outset of study, making it appear that there is an exposure-related difference when it is rather a byproduct of natural variation and chance).

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.

Appendix F. Supplemental Data

The following supplemental files are available at https://doi.org/10.22427/NIEHS-DATA-NIEHS-06.[21]

F.1. Apical Benchmark Dose Analysis

BMD Apical Endpoints Model Fits
BMD_Apical_Endpoints_Model_Fits.docx

BMD Model Recommendation Selection Rules
BMD_Model_Recommendation_Selection_Rules.docx

Read Me
Read_Me.docx

Model Parameters
Model_Parameters.xlsx

BMDs Code Package
BMDs_code_package.zip

F.2. Genomic Benchmark Dose Analysis

BMDExpress Project File (bm2 format)
BMDExpress_Project_File_(bm2_format).bm2

Gene Description
Gene_Description.csv

Top 10 GO Biological Process Gene Sets
Top_10_GO_Biological_Process_Gene_Sets.docx

Top 10 Genes Ranked by Potency of Perturbation
Top_10_Genes_Ranked_by_Potency_of_Perturbation.docx

BMDExpress Project File (JSON format)
BMDExpress_Project_File_(JSON_format).json

GO Biological Process Description
GO_Biological_Process_Description.tsv

BMDExpress Expression Data
BMDExpress_Expression_Data.txt

BMDExpress GO Biological Process Deduplicated BMD Results
BMDExpress_GO_Biological_Process_Deduplicated_BMD_Results.txt

BMDExpress Individual Probe Set BMD Results
BMDExpress_Individual_Probe_Set_BMD_Results.txt

BMDExpress Individual Gene BMD Results
BMDExpress_Individual_Gene_BMD_Results.txt

BMDExpress Prefilter Results
BMDExpress_Prefilter_Results.txt

Animal and Microarray Metadata
Animal_and_Microarray_Metadata.txt

Array Platform Gene and GO Term Annotation File
Array_platform_gene_and_GO_term_annotation_file.zip

BMDExpress Software
BMDExpress_Software.zip

Batch Correction Documentation
Batch_Correction_Documentation.zip

Individual Gene BMD Analysis Results File
Individual_Gene_BMD_Analysis_Results_File.zip

Principal Components Analysis Files
Principal_Components_Analysis_Files.zip

Raw data CEL Files
Raw_data_CEL_files.zip

F.3. Study Tables

I04 - Mean Body Weight Summary
C10277_I04_Mean_Body_Weight_Summary.pdf

I05 - Clinical Observations Summary
C10277_I05_Clinical_Observations_Summary.pdf

PA06 - Organ Weights Summary
C10277_PA06_Organ_Weights_Summary.pdf

PA41 - Clinical Chemistry Summary
C10277_PA41_Clinical_Chemistry_Summary.pdf

R07 - Hormone Summary
C10277_R07_Hormone_Summary.pdf

F.4. Individual Animal Data

Individual Animal Body Weight Data
Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Chemistry Data
Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Clinical Observations Data
Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Hormone Data
Individual_Animal_Hormone_Data.xlsx

Individual Animal Organ Weight Data
Individual_Animal_Organ_Weight_Data.xlsx

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