Toxicity Reference Database (ToxRefDB): Curating legacy *in vivo* toxicity data for the future

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Outline & Disclaimer

- ToxRefDB: Overview, Goals, and History
- Curation example
- Database coverage and accessibility
- Version comparison
- Future efforts

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ToxRefDB:
Overview, Goals, and A Little History

ToxRefDB: Overview

- **Toxicity Reference Database** contains highly-curated legacy information from guideline and guideline-like *in vivo* studies
- Important for many retrospective and predictive toxicology applications, such as:
  - To set benchmarks to predict quantitative points-of-departures and build scientific confidence in the performance of new approach methodologies (NAMs)
  - To inform toxicity predictions as training data ex. GENRA (GENeralized Read-Across)
  - To evaluate reproducibility and variability of observed *in vivo* outcomes

ToxRefDB: Goals

- Aggregate complex and heterogeneous in vivo study data into an interoperable database
- Capture the quantitative dose-response data for each dose treatment group, including control groups, for all observed endpoints
  - Including treatment group size, incidence or effect values, and variance information (e.g., standard deviation, standard error) where provided
- Capture points of departures (PODs) from dose-response data including doses that are deemed ***treatment-related*** (statistically significant from control group) and/or ***critical*** (adverse) within a study
- Employ a controlled vocabulary for accurate data extraction, aggregation, and integration, enhancing data quality at the source
- Distinguish between missing (not tested) or negative (tested with no effect observed) endpoints

ToxRefDB: History

- v1.0 (c. 2009) captured basic study design, dose, and treatment-related effects in Excel format
- **Positives-only** database with only **qualitative** data for treatment related effects (only LELs and LOELs)
- Initially released as a series of spreadsheets, which are still available on EPA's FTP site and referenced in FigShare (https://doi.org/10.23645/epacomptox.6062545.v1)

ToxRefDB v1.0 general schema

Chemical
- CASRN
- Chemical Name

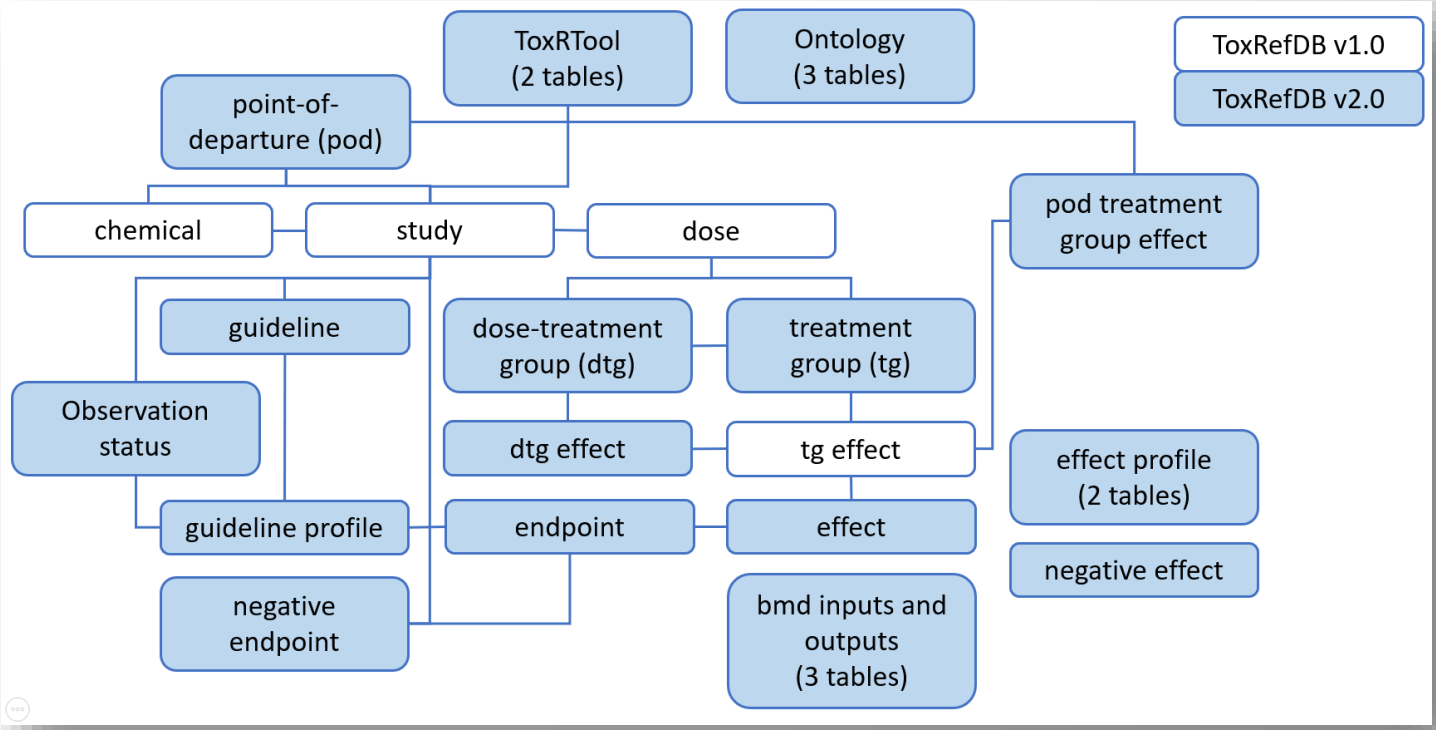
Study
- Source
- Study Type
- Species
- Strain
- Admin Route (oral, dermal, inhalation, injection)
- Admin Method (gavage, feed, i.e. injection)
- Dose start/end
- Etc.

Treatment Group
- Sex
- Dose duration
- Dose period (interim, terminal, recovery, satellite, etc.)
- Generation
- Dose levels
- Concentration (ppm)
- Dose (mg/kg/day)

Effect
Treatment related only
- Effect description
- Effect's endpoint
- Direction of net change across all doses

ToxRefDB v2.0: An Improved Resource…

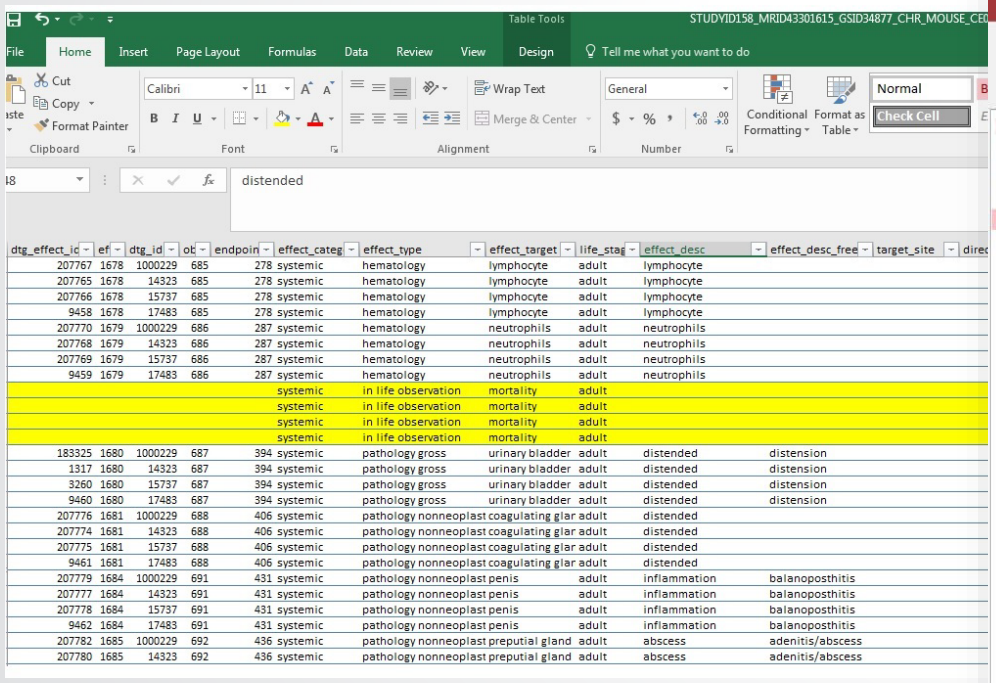
- v2.0 (c. 2018) improved the quantitative value of v1.0 via manual curation effort. Some improvements included:
  - Treatment related effects are denoted
  - Effects that occur at the critical effect level are denoted
  - Large effort to standardize units for effect values
  - Doses converted to mg/kg/day using stored procedures in the database
  - More quantitative value with controls and responses collected *at all doses*
  - Increased accuracy of mapping of dose and effect to each treatment group (e.g., for studies with multiple generations or male and females)
  - Largest implementation of Python-driven BMDS v2.7 to provide BMDL, BMD, and BMDU values from winning models whenever practicable

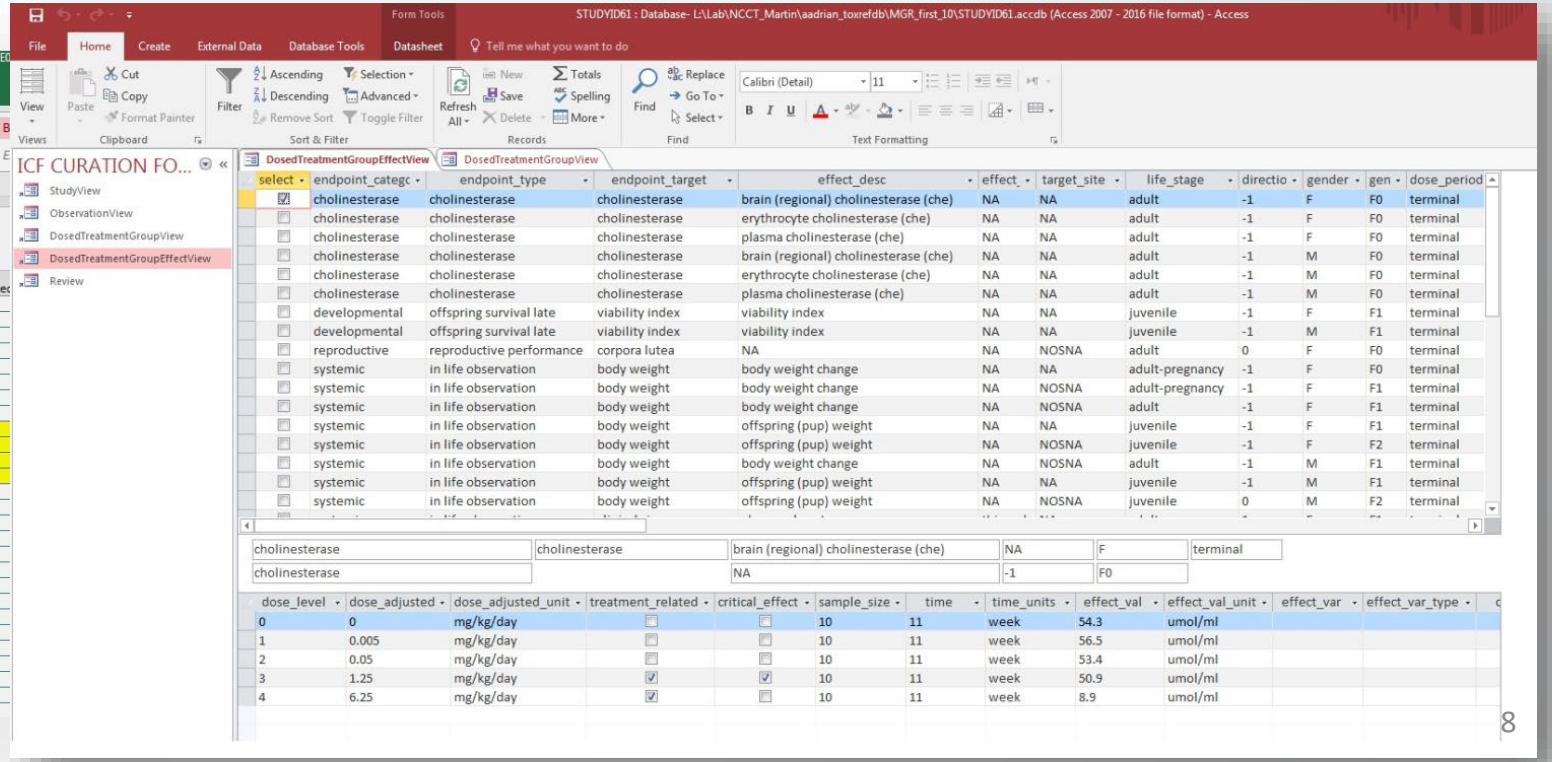


(Watford et al, 2019)

Manual Curation with Excel and Access

- V1.0 to V2.0 switched from Excel sheet entry to Access form entry
- Access form entry enabled complete dose-treatment group-effect quantitative data capture and decreased error rate
  - Only treatment-related effects were entered into ToxRefDB v1.0 (i.e. no control groups)
- Additional QA steps included primary and secondary review of extractions



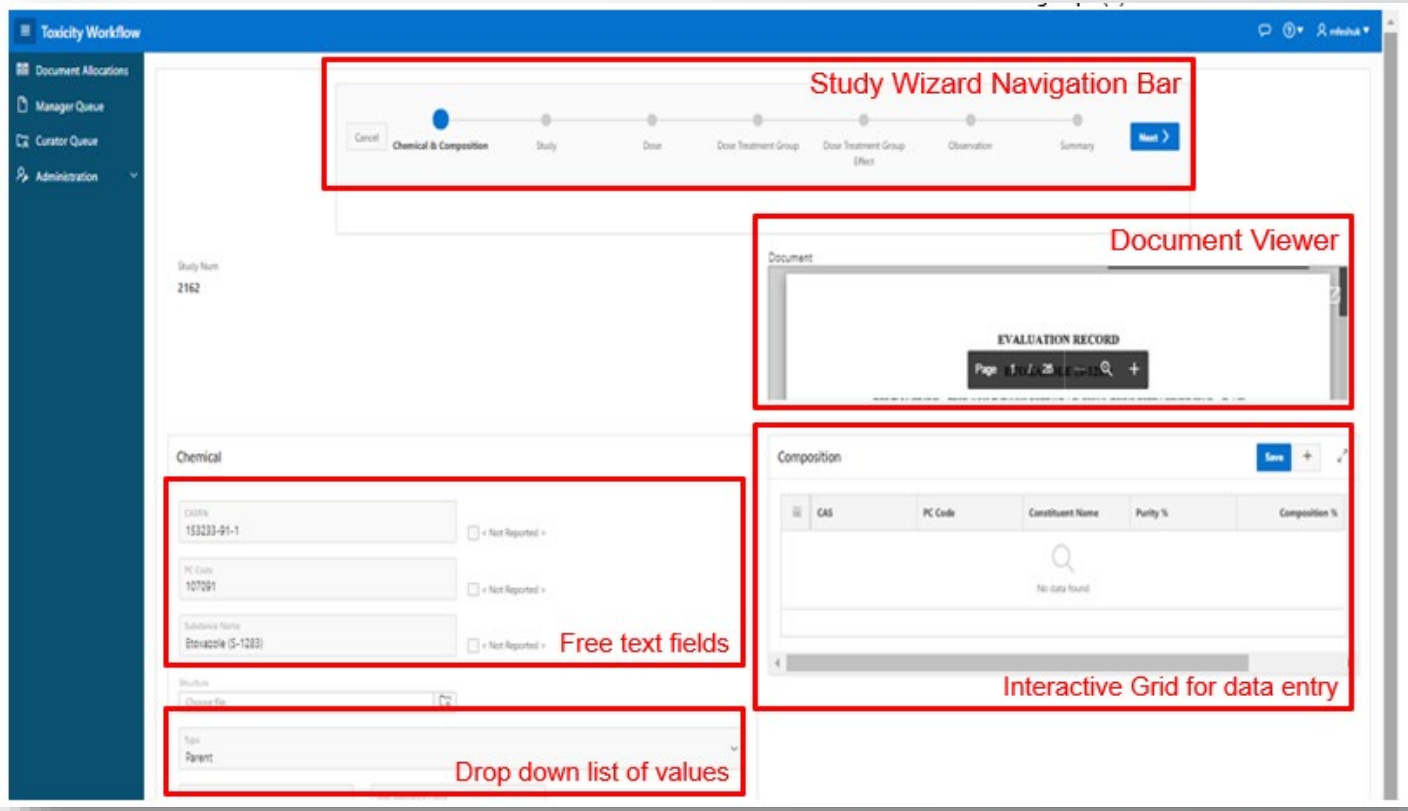


Example Curation with the DCT

DCT: Overview

The Data Collection Tool (DCT) was designed to replace the legacy ToxRefDB workflow and create a more sustainable process for loading curated information to a database.

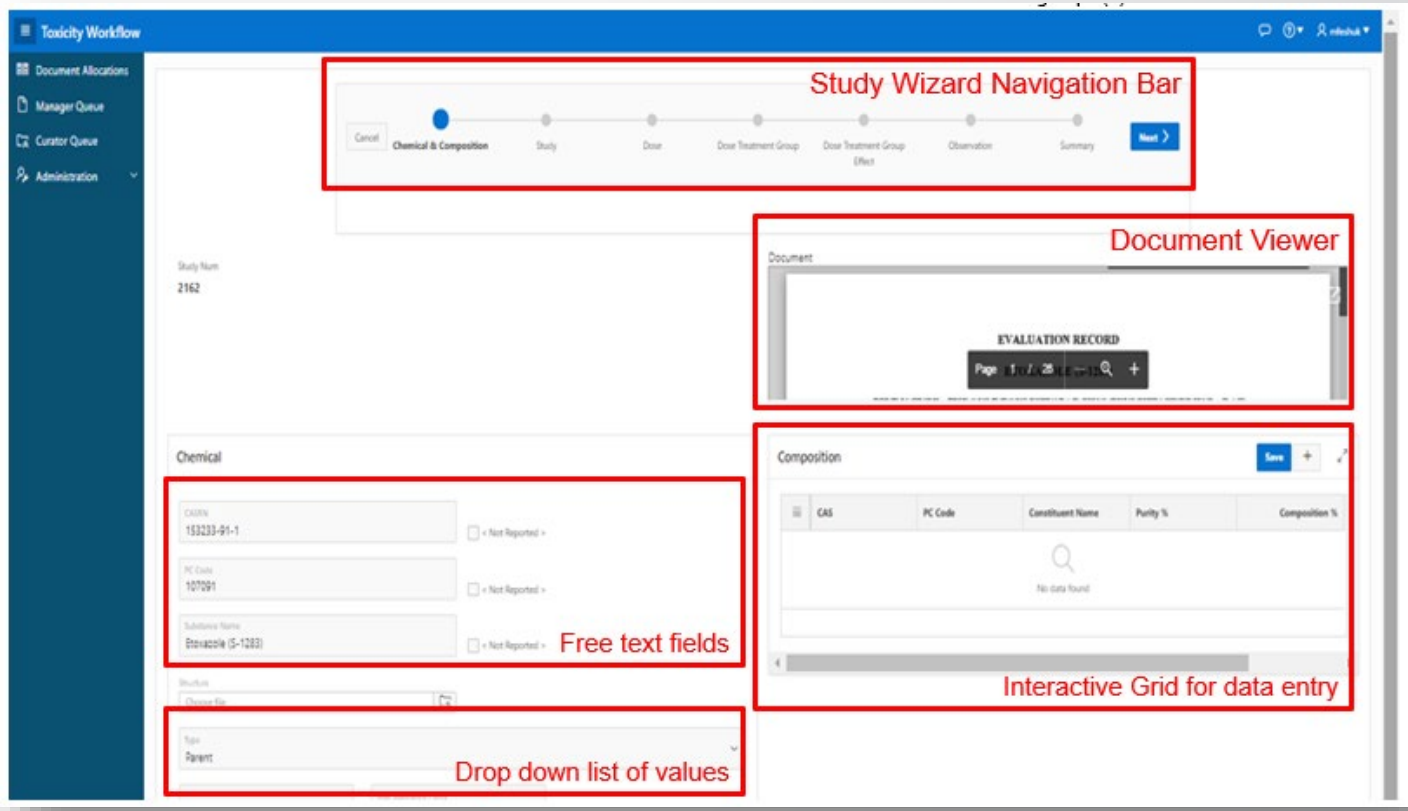
Although the DCT is currently designed to only support ToxRef, the DCT is scalable with minimal developments to support other projects that require similar document management, curation-based extraction, or QA features.



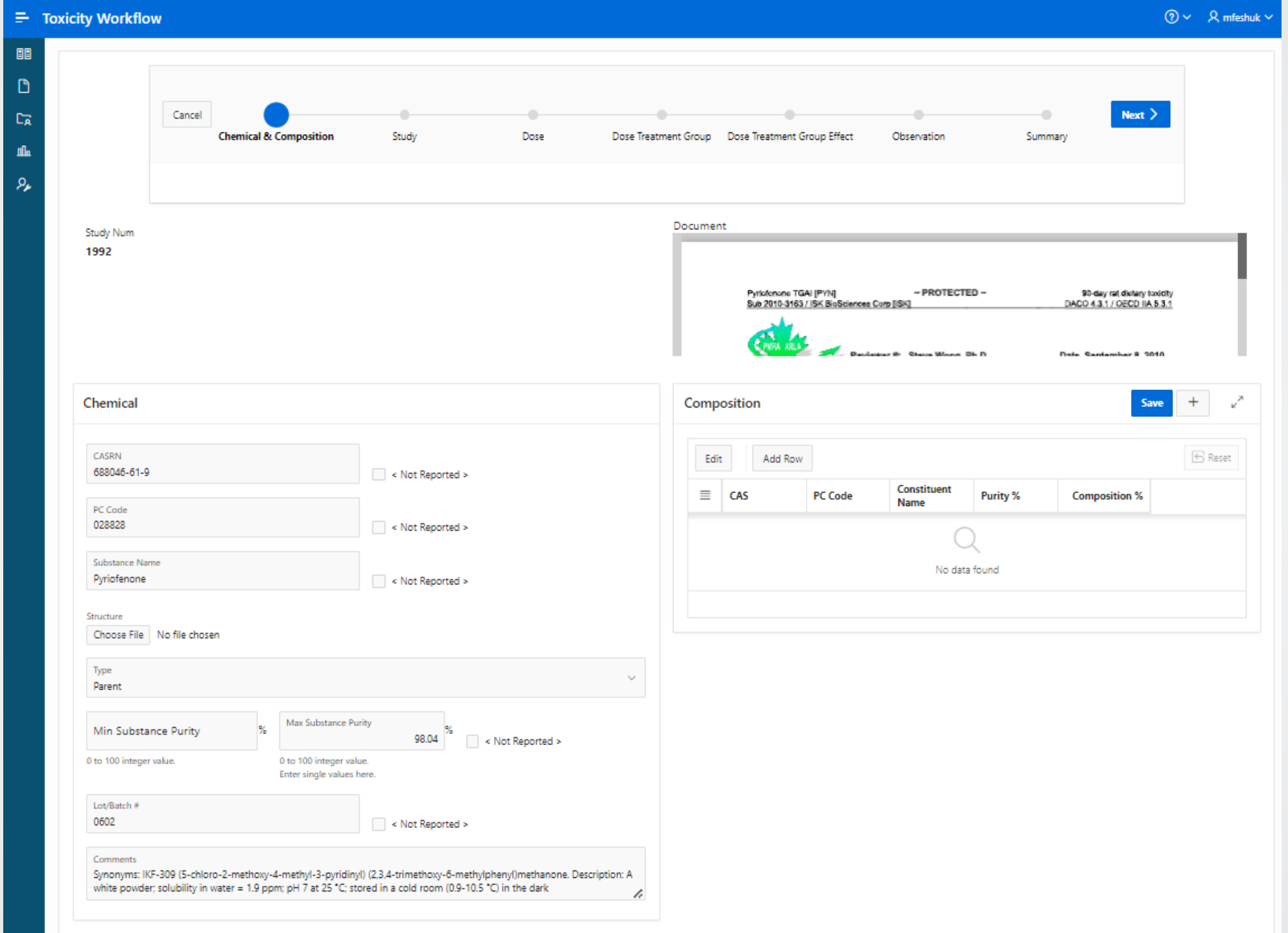
DCT: Overview

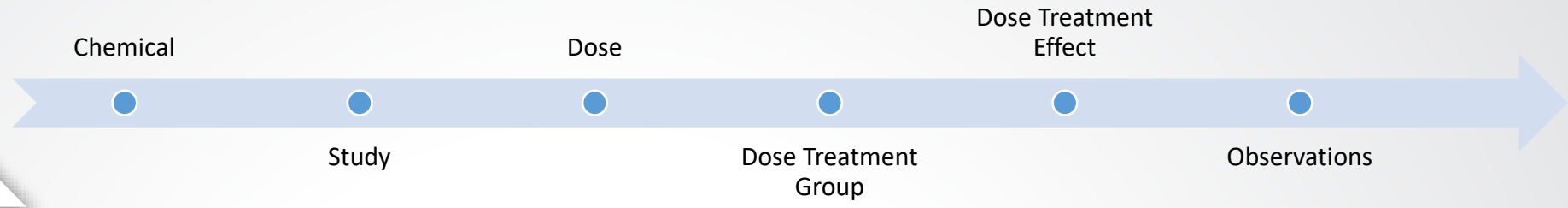
The DCT:

- Captures basic study design metadata, dose-response, treatment-related and critical effects, and endpoint testing status information while employing controlled vocabulary developed for ToxRefDB
- Offers flexibility for curating the heterogeneous and complex in vivo study designs via a modular workflow
- Provides document allocation, curation and workflow management among users (internal and external) with manager review and data conflict resolution
- Links a quality-controlled curation to Clowder source documents
- Creates a sustainable pipeline for data integration.



Our example will walkthrough a document extraction process as curator using the DCT


Toxicity Workflow
mfeshuk
Cancel
Chemical & Composition
Study
Dose
Dose Treatment Group
Dose Treatment Group Effect
Observation
Summary
Next
Study Num
1992
Document
Pyriofenone TGAI [PYN]
– PROTECTED –
90-day rat dietary toxicity
Sub 2010-3163 / ISK BioSciences Corp [ISK]
DACO 4.3.1 / OECD IIA 5.3.1
Chemical
CASRN
688046-61-9
< Not Reported >
PC Code
028828
< Not Reported >
Substance Name
Pyriofenone
< Not Reported >
Structure
Choose File
No file chosen
Type
Parent
Min Substance Purity
%
0 to 100 integer value.
Max Substance Purity
98.04
%
0 to 100 integer value.
Enter single values here.
< Not Reported >
Lot/Batch #
0602
< Not Reported >
Comments
Synonyms: IKF-309 (5-chloro-2-methoxy-4-methyl-3-pyridinyl) (2,3,4-trimethoxy-6-methylphenyl)methanone. Description: A white powder; solubility in water = 1.9 ppm; pH 7 at 25 °C; stored in a cold room (0.9-10.5 °C) in the dark
Composition
Save
Edit
Add Row
Reset
CAS
PC Code
Constituent Name
Purity %
Composition %
No data found



- Review Executive Summary, Author's Conclusions, and/or Reviewer's Comments, if available

EXECUTIVE SUMMARY:

In a 13-week toxicity study, IKF-309 (98.04%) was administered in the diet daily to groups of Fischer (F344/DuCrlCrlj) rats, 10/sex/group, at 0, 300, 1000, 2500, or 5000 ppm (♂ = 0, 18, 61, 150, 305; ♀ = 0, 21, 69, 171, 350 mg/kg bw/d, respectively). During the study, all animals were observed for mortality and clinical signs. Body weight, food consumption, and achieved dosage were determined. Functional observation was carried out at week 11. Ophthalmoscopy and urinalysis were performed at week 13. At study termination on day 93, haematology, blood chemistry, organ weight, gross pathology and histopathology were evaluated.

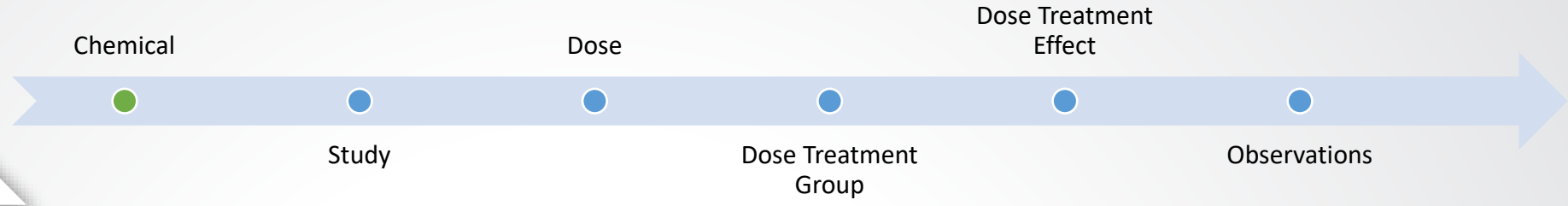
At 1000 ppm, males showed an increase in relative liver weight, while the females showed a prolongation of activated partial thromboplastin time (APTT). The changes were not considered to be toxicologically relevant because of the magnitude and inconsistency of the changes. At 2500 ppm, males exhibited increased absolute weights of the liver, kidneys, and cecum. The female showed prolonged APTT, and increased relative liver weight as well as absolute and relative weights of the cecum. At 5000 ppm, treatment-related toxicological effects in the male included increased motor activity, and urine volume. The high-dose females exhibited yellow/brown-coloured urine and prolonged APTT. At necropsy, distended ceca with contents were observed in high-dose males and females. Histopathological examination revealed diffuse hepatocellular hypertrophy as well as kidney pathology (increased incidences of hyaline droplets in the proximal tubular cells and basophilic change in renal tubular cells) in high-dose males and females. Based on the higher weights of the liver, cecum, and/or kidneys, as well as prolonged APTT at 2500 ppm, the LOAEL of 2500 ppm (♂ = 150; ♀ = 171 mg/kg bw/d) and NOAEL of 1000 ppm (♂ = 61; ♀ = 69 mg/kg bw/d) were established.

This study is acceptable and satisfies the guideline requirement for a 90-day oral toxicity study in rats (OPPTS 870.3100; OECD 408).

Study Design

Effects

Guideline Adherence

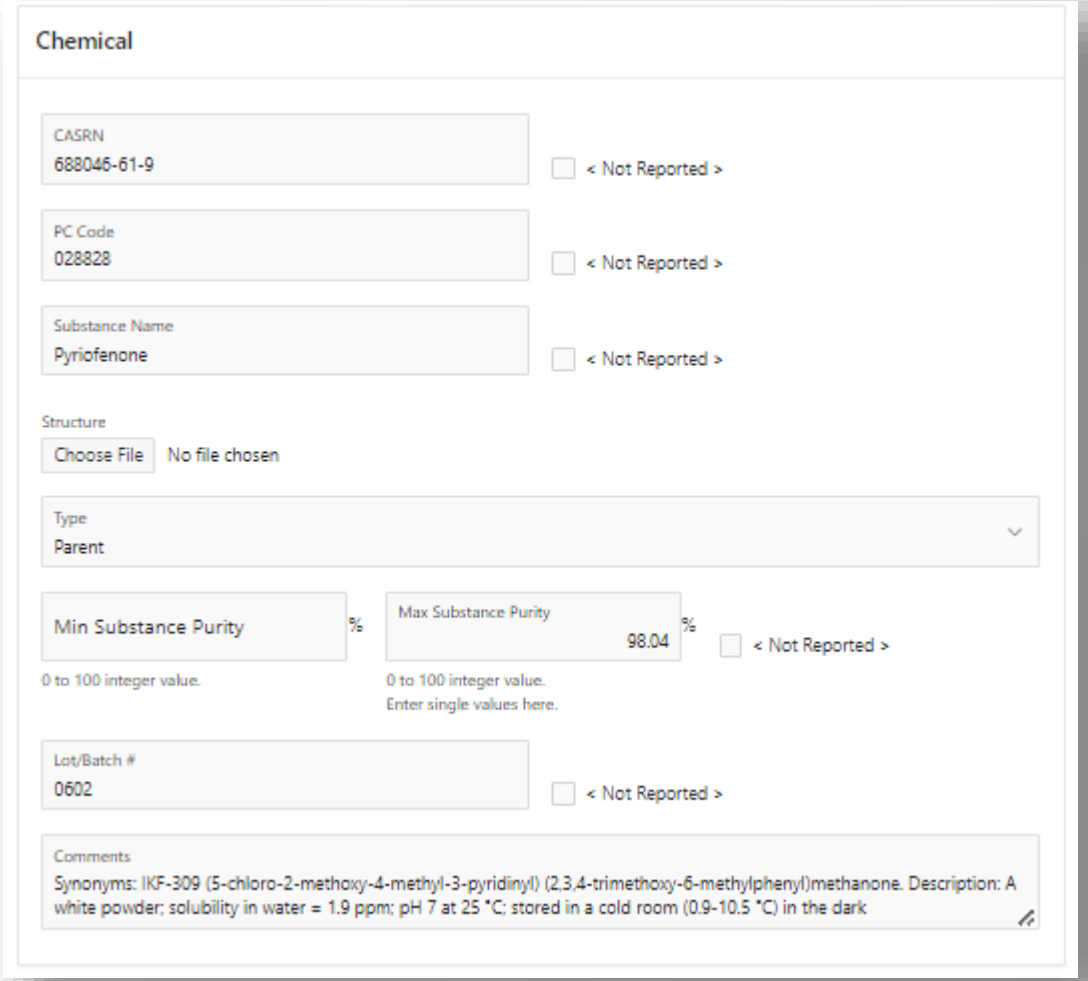


I. MATERIALS AND METHODS

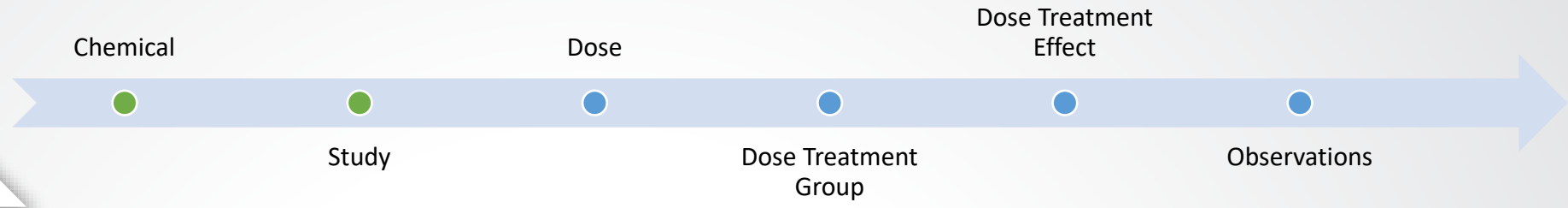
A. MATERIALS:

1.

Test material:	IKF-309 (5-chloro-2-methoxy-4-methyl-3-pyridinyl) (2,3,4-trimethoxy-6-methyl phenyl)methanone
Description:	a white powder; solubility in water = 1.9 ppm; pH 7 at 25 °C; stored in a cold room (0.9-10.5 °C) in the dark
Lot/Batch #:	0602
Purity:	98.04%
CAS #:	688046-61-9



- All source chemical metadata is extracted as reported, then shared with EPA chemical curators to assign DTXSIDs



Study

Study Source ID
48112816;48476202

Source Type
EPA OPP

MRID or other source identifier type
MRID

Study Year
2010

Study Citation
Ohtsuka, R. (2010) IKF-309 Technical: Repeated Dose 90-Day Oral Toxicity Study in Rats. Project Number: IET/06/0015. Unpublished study prepared by Institute of Environmental Toxicology. 423 p., Gelin, M. (2011) Response to Request for Historical Control Data: IKF-309 Technical: Repeated Dose 90-Day Oral Toxicity Study in Rats. Project Number: IB/2011/MG/001/06, IET/06/0015. Unpublished study prepared by ISK Bioscience Corporation. 13 p.

CITATION: PMRA: 1933848 PMRA DER: 1943926 EPA MRID 48112816
Ohtsuka, Ryoichi. January 19, 2010. IKF-309 technical: Repeat dose 90-day oral toxicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Document number IET 06-0015, ISK Biosciences Corporation. Concord, OH, USA. Unpublished.
PMRA: 2055027 PMRA DER: 1943926 EPA MRID 48112816
Gelin, Mark D, May 5, 2011. Response to request for historical control data IKF-309 technical: Repeated dose 90-day oral toxicity study in rats. IB-2011-MG-001-06, ISK Biosciences Corporation, Concord, OH.

STUDY TYPE: 90-day feeding study - rat; OPPTS 870.3100; OECD 408.

Study Type
SUB - Subchronic oral toxicity in rodents

Guideline Name
Subchronic oral toxicity in rodents

Guideline No.
OPPTS 870.3100

Guideline Comment
Acceptable [870.3100, 90-Day oral toxicity in rodents]. OECD 408.

Species
Rat

Strain Group
Fischer rat

Strain
F344/DuCrj

Admin Route
Oral

Admin Method
Diet

Dose Start
0

Dose Start Unit
Day

Dose End
93

Dose End Unit
Day

Observation Type
1 | 870.31 | SUB_oral_rode | 90-day Oral Toxicity in Rodents

3. **Test animals:**

Species:	rat	
Strain:	Specific-pathogen-free (SPF) Fischer (F344/DuCrlCrlj)	
Age/weight at study initiation:	Age: 5 weeks; Mean weight: ♂ = 107-121; ♀ = 91-99 g	
Source:	Atsugi Breeding Center, Charles River Japan, Inc. (Kanagawa, Japan)	
Housing after grouping:	2 of same sex per wire-mesh stainless cage	
Diet:	Certified diet MF Mash (Oriental Yeast Co., Ltd., Tokyo, Japan) *ad libitum*	
Water:	Local tap water *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22±2 °C (21.4-22.9) 50±20% *"Periodic checks were made on the number of air changes in the animal rooms."* 12h dark / 12h light
Acclimation period:	10 days	

B. STUDY DESIGN

1. **In life dates:** Start: August 7, 2006 End: March 30, 2007

EXECUTIVE SUMMARY:

In a 13-week toxicity study, IKF-309 (98.04%) was administered in the diet daily to groups of Fischer (F344/DuCrlCrlj) rats, 10/sex/group, at 0, 300, 1000, 2500, or 5000 ppm (♂ = 0, 18, 61, 150, 305; ♀ = 0, 21, 69, 171, 350 mg/kg bw/d, respectively). During the study, all animals were observed for mortality and clinical signs. Body weight, food consumption, and achieved dosage were determined. Functional observation was carried out at week 11. Ophthalmoscopy and urinalysis were performed at week 13. At study termination on day 93, haematology, blood chemistry, organ weight, gross pathology and histopathology were evaluated.

Comments
Pyriofenone (IKF-309): In a 13-week toxicity study, IKF-309 (98.04%) was administered in the diet daily to groups of Fischer (F344/DuCrlCrlj) rats, 10/sex/group, at 0, 300, 1000, 2500, or 5000 ppm (♂ = 0, 18, 61, 150, 305; ♀ = 0, 21, 69, 171, 350 mg/kg bw/d, respectively). During the study, all animals were observed for mortality and clinical signs. Body weight, food consumption, and achieved dosage were determined. Functional observation was carried out at week 11. Ophthalmoscopy and urinalysis were performed at week 13. At study termination on day 93, haematology, blood chemistry, organ weight, gross pathology and histopathology were evaluated.

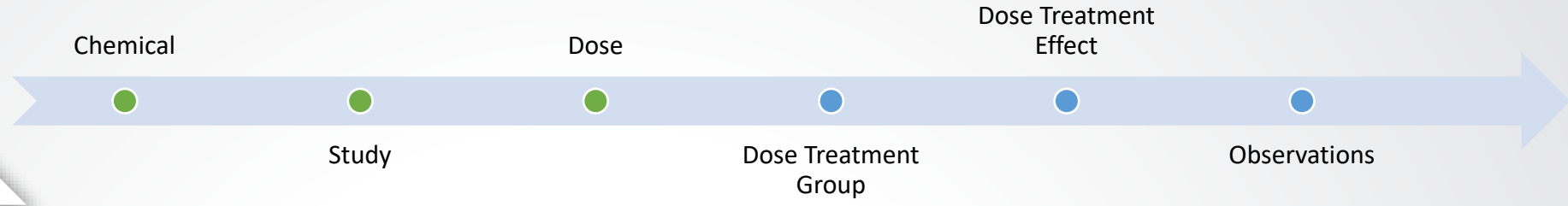


Table 1: Study design

	♂ (N = 10/group)					♀ (N = 10/group)				
ppm	0	300	1000	2500	5000	0	300	1000	2500	5000
mg/kg bw/d	0	18	61	150	305	0	21	69	171	350
	Compound consumption was based on 13 data points for each group									

2. **Vehicle:** IKF-309 was administered in the diet.

Dose

Save

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Dose Level	Conc	Conc Unit	Vehicle	Comment
0	0	mg/kg/day	diet	Target concentration 0 ppm
1	18	mg/kg/day	diet	Target concentration 300 ppm
2	21	mg/kg/day	diet	Target concentration 300 ppm
3	61	mg/kg/day	diet	Target concentration 1000 ppm
4	69	mg/kg/day	diet	Target concentration 1000 ppm
5	150	mg/kg/day	diet	Target concentration 2500 ppm
6	171	mg/kg/day	diet	Target concentration 2500 ppm
7	305	mg/kg/day	diet	Target concentration 5000 ppm
8	350	mg/kg/day	diet	Target concentration 5000 ppm

1 - 9

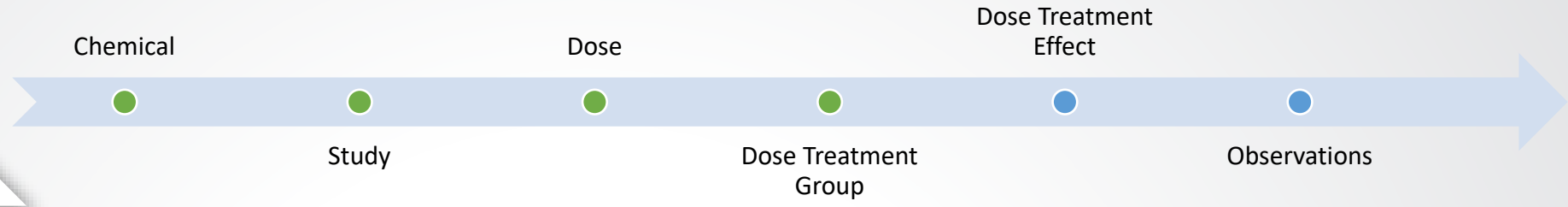


Table 1: Study design

	♂ (N = 10/group)					♀ (N = 10/group)				
ppm	0	300	1000	2500	5000	0	300	1000	2500	5000
mg/kg bw/d	0	18	61	150	305	0	21	69	171	350
	Compound consumption was based on 13 data points for each group									

8. Sacrifice and pathology:

All animals were sacrificed on schedule (day 93 after overnight fasting and by exsanguination under deep ether anaesthesia) were subjected to gross pathological examination. All tissues were collected

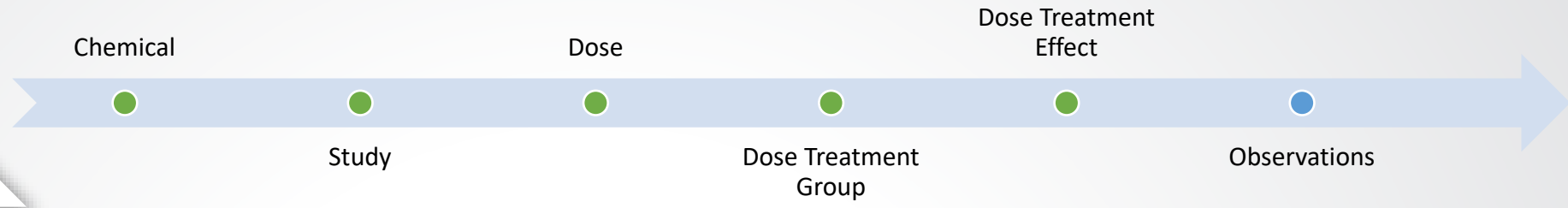
Dose Treatment Group

Save

Edit | Add Row | Reset

Dose	Sex	Gen	Num Animals	Period	Duration	Duration Unit	Comment
0 | 0 | mg/kg/day | diet	F	F0	10	Terminal	93	Day	-
0 | 0 | mg/kg/day | diet	M	F0	10	Terminal	93	Day	-
1 | 18 | mg/kg/day | diet	M	F0	10	Terminal	93	Day	-
2 | 21 | mg/kg/day | diet	F	F0	10	Terminal	93	Day	-
3 | 61 | mg/kg/day | diet	M	F0	10	Terminal	93	Day	-
4 | 69 | mg/kg/day | diet	F	F0	10	Terminal	93	Day	-
5 | 150 | mg/kg/day | diet	M	F0	10	Terminal	93	Day	-
6 | 171 | mg/kg/day | diet	F	F0	10	Terminal	93	Day	-
7 | 305 | mg/kg/day | diet	M	F0	10	Terminal	93	Day	-
8 | 350 | mg/kg/day | diet	F	F0	10	Terminal	93	Day	-

1 - 10



Let's consider changes in "relative liver weight"

Table 8. Selected organ weight values (mg±SD)

		♂ (N = 10/group)					♀ (N = 10/group)				
Ppm		0	300	1000	2500	5000	0	300	1000	2500	5000
mg/kg bw/d		0	18	61	150	305	0	21	69	171	350
body weight		310±16	314±17	313±12	320±20	310±10	173±7	171±10	170±7	169±13	167±6
Liver	g	6.83±0.36	7.01±0.45	7.22±0.33	7.81±0.6**	8.26±0.41**	3.83±0.26	3.74±0.24	3.83±0.21	3.96±0.11	4.31±0.19**
	%	2.21±0.05	2.23±0.05	2.30±0.04**	2.44±0.07**	2.66±0.08**	2.21±0.13	2.19±0.09	2.26±0.06	2.34±0.07**	2.57±0.08**
Kidney	mg	1951±55	1973±107	1966±98	2108±140**	2249±97**	1157±64	1148±55	1132±40	1177±47	1243±68**
	%	0.63±0.03	0.63±0.02	0.63±0.02	0.66±0.02*	0.73±0.04**	0.67±0.02	0.67±0.01	0.67±0.03	0.70±0.03	0.74±0.03**
Cecum	mg	3567±311	3475±518	3332±331	4623±1021	9191±2159**	2724±382	2817±240	2711±332	3775±538**	5544±698**
	%	1.16±0.12	1.11±0.17	1.06±0.09	1.44±0.28	2.97±0.71**	1.58±0.23	1.65±0.13	1.60±0.20	2.23±0.29**	3.30±0.31**

Data taken from Tables 27-28, pages 150-57; * p ≤0.05, ** p ≤0.01; **Bold** values are considered biologically significant

1. Organ weight: Table 8

In the 5000 ppm group, males and females showed significant increases in both absolute and relative (ratio to body weight) weights of liver, kidneys and cecum.

In the 2500 ppm group, males showed significant increases in both absolute and relative weights of liver and kidneys, while females exhibited significant increases in the relative liver weight and both absolute and relative weights of cecum. An increasing trend in both absolute and relative cecum weights was also noted in males.

In the 1000 ppm group, males showed a significant increase in relative weight of liver, while females exhibited no significant changes in organ weights.

In the 300 ppm group, there were no significant changes in organ weights for either sex.

Dose Treatment Group Effect

Save

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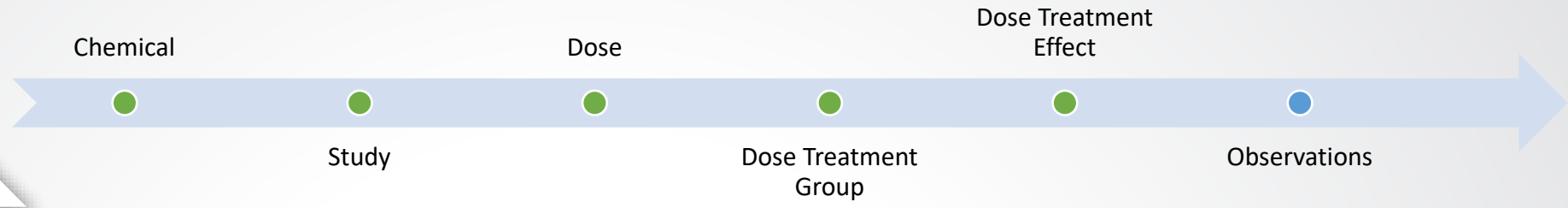
Observation equals systemic | organ weight | liver | Effect Value Unit equals %

Dose Treatment Group	Observation	Effect Desc	Effect Desc Free	Target Site	Life Stage	Direction	Critical Effect	Treatment Related	Sample Size	Effect Value	Effect Value Unit	Effect Variance	Effect Variance Type
F | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	0	0	10	2.21	%	0.13	SD
F | F0 | 10 | Terminal | 93 | Day -- 2 | 21 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	0	0	10	2.19	%	0.09	SD
F | F0 | 10 | Terminal | 93 | Day -- 4 | 69 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	0	0	10	2.26	%	0.06	SD
F | F0 | 10 | Terminal | 93 | Day -- 6 | 171 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	1	1	10	2.34	%	0.07	SD
F | F0 | 10 | Terminal | 93 | Day -- 8 | 350 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	1	1	10	2.57	%	0.08	SD
M | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	0	0	10	2.21	%	0.05	SD
M | F0 | 10 | Terminal | 93 | Day -- 1 | 18 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	0	0	10	2.23	%	0.05	SD
M | F0 | 10 | Terminal | 93 | Day -- 3 | 61 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	0	1	10	2.3	%	0.04	SD
M | F0 | 10 | Terminal | 93 | Day -- 5 | 150 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	1	1	10	2.44	%	0.07	SD
M | F0 | 10 | Terminal | 93 | Day -- 7 | 305 | mg/kg/day | diet	systemic | organ weight | liver	relative to body weight	liver weight ratio...	liver	Adult	Increase	1	1	10	2.66	%	0.08	SD

1 - 10



Dose Treatment Group Effect

Save

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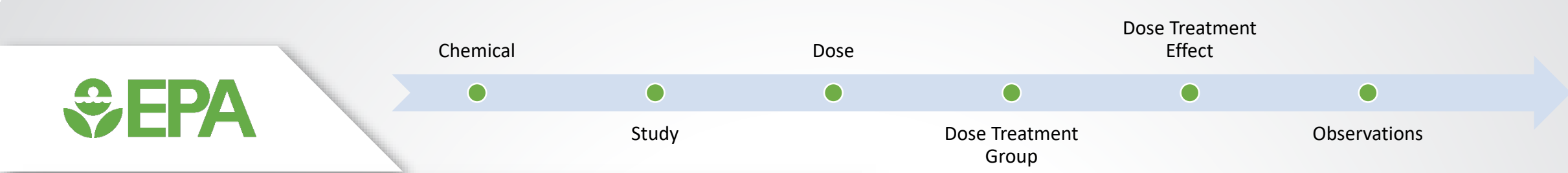
Dose Treatment Group	Observation	Effect Desc	Effect Desc Free	Target Site	Life Stage	Direction	Critical Effect	Treatment Related	Sample Size	Effect Value	Effect Value Unit	Effect Variance
F | F0 | 10 | Terminal | 93 | Day -- 8 | 350 | mg/kg/day | diet	systemic | hematology | blood clotting	blood clotting - activated p...	mean APTT, sec	NA; not reported	Adult	Increase	1	1	10	20	seconds	1
F | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | hematology | blood clotting	blood clotting - activated p...	mean APTT, sec	NA; not reported	Adult	Increase	0	0	10	18.1	seconds	0.8
F | F0 | 10 | Terminal | 93 | Day -- 6 | 171 | mg/kg/day | diet	systemic | hematology | blood clotting	blood clotting - activated p...	mean APTT, sec	NA; not reported	Adult	Increase	1	1	10	19.8	seconds	1.1
F | F0 | 10 | Terminal | 93 | Day -- 2 | 21 | mg/kg/day | diet	systemic | hematology | blood clotting	blood clotting - activated p...	mean APTT, sec	NA; not reported	Adult	Increase	0	0	10	18.2	seconds	0.8
F | F0 | 10 | Terminal | 93 | Day -- 4 | 69 | mg/kg/day | diet	systemic | hematology | blood clotting	blood clotting - activated p...	mean APTT, sec	NA; not reported	Adult	Increase	0	1	10	19.2	seconds	0.9
M | F0 | 10 | Terminal | 93 | Day -- 7 | 305 | mg/kg/day | diet	systemic | in life observation | clinical signs	motor activity	total motor activ...	NA; not reported	Adult	Increase	1	1	10	2865	count	766
M | F0 | 10 | Terminal | 93 | Day -- 1 | 18 | mg/kg/day | diet	systemic | in life observation | clinical signs	motor activity	total motor activ...	NA; not reported	Adult	Increase	0	0	10	2329	count	701
M | F0 | 10 | Terminal | 93 | Day -- 3 | 61 | mg/kg/day | diet	systemic | in life observation | clinical signs	motor activity	total motor activ...	NA; not reported	Adult	Increase	0	0	10	2186	count	704
M | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | in life observation | clinical signs	motor activity	total motor activ...	NA; not reported	Adult	Increase	0	0	10	1923	count	528
M | F0 | 10 | Terminal | 93 | Day -- 5 | 150 | mg/kg/day | diet	systemic | in life observation | clinical signs	motor activity	total motor activ...	NA; not reported	Adult	Increase	1	0	10	1943	count	768
F | F0 | 10 | Terminal | 93 | Day -- 8 | 350 | mg/kg/day | diet	systemic | organ weight | [other]	relative to body weight	cecum weight ra...	colon	Adult	Increase	1	1	10	3.3	%	0.31
F | F0 | 10 | Terminal | 93 | Day -- 6 | 171 | mg/kg/day | diet	systemic | organ weight | [other]	relative to body weight	cecum weight ra...	colon	Adult	Increase	1	1	10	2.23	%	0.29
F | F0 | 10 | Terminal | 93 | Day -- 4 | 69 | mg/kg/day | diet	systemic | organ weight | [other]	relative to body weight	cecum weight ra...	colon	Adult	Increase	0	0	10	1.6	%	0.2
F | F0 | 10 | Terminal | 93 | Day -- 2 | 21 | mg/kg/day | diet	systemic | organ weight | [other]	relative to body weight	cecum weight ra...	colon	Adult	Increase	0	0	10	1.65	%	0.13
F | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | organ weight | [other]	relative to body weight	cecum weight ra...	colon	Adult	Increase	0	0	10	1.58	%	0.23
M | F0 | 10 | Terminal | 93 | Day -- 3 | 61 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	0	0	10	3332	mg	331
M | F0 | 10 | Terminal | 93 | Day -- 1 | 18 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	0	0	10	3475	mg	518
M | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	0	0	10	3567	mg	311
F | F0 | 10 | Terminal | 93 | Day -- 6 | 171 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	1	1	10	3775	mg	538
F | F0 | 10 | Terminal | 93 | Day -- 2 | 21 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	0	0	10	2817	mg	240
F | F0 | 10 | Terminal | 93 | Day -- 4 | 69 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	0	0	10	2711	mg	332
F | F0 | 10 | Terminal | 93 | Day -- 0 | 0 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	0	0	10	2724	mg	382
F | F0 | 10 | Terminal | 93 | Day -- 8 | 350 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	1	1	10	5544	mg	698
M | F0 | 10 | Terminal | 93 | Day -- 7 | 305 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	1	1	10	9191	mg	2159
M | F0 | 10 | Terminal | 93 | Day -- 5 | 150 | mg/kg/day | diet	systemic | organ weight | [other]	absolute	cecum weight	colon	Adult	Increase	1	0	10	4623	mg	1021
M | F0 | 10 | Terminal | 93 | Day -- 5 | 150 | mg/kg/day | diet	systemic | organ weight | kidney	absolute	kidney weight	kidney, right	Adult	Increase	1	1	10	2108	mg	140
M | F0 | 10 | Terminal | 93 | Day -- 3 | 61 | mg/kg/day | diet	systemic | organ weight | kidney	absolute	kidney weight	kidney, right	Adult	Increase	0	0	10	1966	mg	98
M | F0 | 10 | Terminal | 93 | Day -- 1 | 18 | mg/kg/day | diet	systemic | organ weight | kidney	absolute	kidney weight	kidney, right	Adult	Increase	0	0	10	1973	mg	107
M | F0 | 10 | Terminal | 93 | Day -- 7 | 305 | mg/kg/day | diet	systemic | organ weight | kidney	absolute	kidney weight	kidney, right	Adult	Increase	1	1	10	2249	mg	97

1 - 85



Study Num 1992 | Reset Observations to Default | Populate

Observation | Save

Edit | Reset

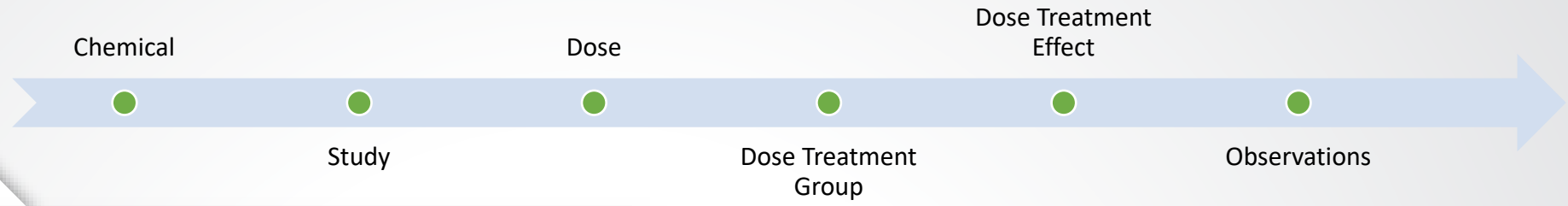
Tested Status | 2 Study Type, Observation Type

Endpoint Category	Endpoint Type	Endpoint Target	Tested Status	Reported Status
Study Type: SUB - Subchronic oral toxicity in rodents, Observation Type: 90-DAY ORAL TOXICITY IN RODENTS[SUB_ORAL_RODE				
systemic	hematology	blood clotting	Yes	Yes
systemic	in life observation	body weight	Yes	No
systemic	in life observation	clinical signs	Yes	Yes
systemic	in life observation	food consumption	Yes	No
systemic	in life observation	mortality	Yes	No
systemic	organ weight	[other]	Yes	Yes
systemic	organ weight	adrenal gland	Yes	No
systemic	organ weight	brain	Yes	No
systemic	organ weight	epididymis	Yes	No
systemic	organ weight	heart	Yes	No
systemic	organ weight	kidney	Yes	Yes
systemic	organ weight	liver	Yes	Yes
systemic	organ weight	ovary	Yes	No
systemic	organ weight	spleen	Yes	No

1 - 89

We distinguish between ***missing*** (meaning not tested) and ***negative*** (meaning tested with no effect observed) by setting tested and reported status using the following assumptions:

Tested Status	Reported Status	Assumption Description
Yes	Yes	The text of the study document explicitly stated the endpoint was measured, or data was presented in tables for the endpoint. **This is the combination if required by the guideline for that study type and data is provided within the document**, even the effects measured were not significant.
No	Yes	**This is the combination if the study document explicitly states the endpoint was not measured or data was not collected, even though the endpoint was required by the study guidelines**. Tested Status should be changed from Yes to No, and Reported Status changed to Yes.
Yes	No	The text of the study document does not state the endpoint was measured and data for the endpoint is not present. However, other evidence suggests that the endpoint was measured. **This is the default for endpoints required by the study guideline and should only be changed in the face of direct evidence from the document.**
No	No	The DCT displays a long table with observations from all study guidelines. **This is the default setting for the endpoints not required by the alternative study guidelines and they should not be changed.**



Study Num **1992** | Reset Observations to Default | Populate

Observation

Save

Edit | Reset

Tested Status | 2 Study Type, Observation Type

Endpoint Category	Endpoint Type	Endpoint Target	Tested Status	Reported Status
Study Type: SUB - Subchronic oral toxicity in rodents, Observation Type: 90-DAY ORAL TOXICITY IN RODENTS|SUB_ORAL_RODE				
systemic	hematology	blood clotting	Yes	Yes
systemic	in life observation	body weight	Yes	No
systemic	in life observation	clinical signs	Yes	Yes
systemic	in life observation	food consumption	Yes	No
systemic	in life observation	mortality	Yes	No
systemic	organ weight	[other]	Yes	Yes
systemic	organ weight	adrenal gland	Yes	No
systemic	organ weight	brain	Yes	No
systemic	organ weight	epididymis	Yes	No
systemic	organ weight	heart	Yes	No
systemic	organ weight	kidney	Yes	Yes
systemic	organ weight	liver	Yes	Yes
systemic	organ weight	ovary	Yes	No
systemic	organ weight	spleen	Yes	No

1 - 89

6. Haematology & clinical chemistry:

At termination, blood samples were collected from all animals after an overnight fasting. The animals were under ether anesthesia and blood was collected from the posterior vena cava using untreated syringes. Blood samples were collected into tubes containing EDTA or heparin anticoagulant. The blood samples were process and the checked (X) parameters were examined.

a. **Haematology:**

x	hematocrit (Hct)*	x	hemoglobin (Hb)*	x	leukocyte differential count*
x	leukocyte count (WBC)*			x	mean corpuscular HGB (MCH)*
x	erythrocyte count (RBC)*			x	mean corpuscular Hb concentration (MCHC)*
x	platelet count			x	mean corpuscular volume (MCV)*
x	red blood cell distribution width (RDW)			x	hemoglobin distribution width (HDW)
x x	blood clotting measurements* (activated thromboplastin time, APTT; prothrombin time, PT)			x	reticulocyte count
					* Recommended for subchronic rodent studies based on Guideline 870.3100

b. **Clinical chemistry:**

	ELECTROLYTES							OTHER		
x	calcium*	x	phosphorus*	x	magnesium	x	albumin*	x	globulins	
x	chloride*	x	potassium*	x	sodium*	x	glucose*	x	total bilirubin*	
	ENZYMES					x	blood urea nitrogen*			
x	alkaline phosphatase (AP)					x	total cholesterol			
x	serum alanine amino-transferase (ALT/also SGPT)*					x	total serum protein (TP)*			
x	serum aspartate amino-transferase (AST/also SGOT)*					x	triglycerides			
x	γ-glutamyl transpeptidase (GGTP)*					x	creatinine			

* Recommended for

7. Urinalysis:

Urinalysis was pe
individually placed
examined include
appearance, urine

8. Sacrifice and pa

All animals were s
deep ether anaes
and fixed. For the
examination. In ac
histopathological

Digestive system		Cardiovasular/Hematologic				Neurologic system			
x	tongue	x	aorta*	xx	spleen*+	xx	brain*+		pituitary*
x	salivary glands*	xx	heart*+	xx	thymus*+	x	peripheral nerve*		
x	esophagus*	x	bone marrow*			x	spinal cord (3 levels)*		
x	stomach*	x	lymph nodes*			x	eyes (optic nerve)*		
x	duodenum*	Urogenital system				Glandular organs			
x	jejunum*	xx	kidneys*+	xx	testes*+	xx	adrenal gland*+		
x	ileum*	xx	uterus*+	x	prostate*	x	lacrimal gland		
xx	cecum*	xx	ovaries*+	x	vagina	x	mammary gland*		
x	colon*	x	urinary bladder*			x	parathyroid*		
x	rectum*	xx	epididymides*+			xx	thyroid*		
xx	liver*+	x	seminal vesicles*			Other			
x	gall bladder*	Respiratory system				x	bone	x	skin
x	pancreas*	x	trachea*	x	nose*	x	skeletal muscle		
		x	lungs*	x	pharynx*	x	all gross lesions and masses*		
		x	larynx*			x	target organs*		

* Recommended for subchronic rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies; T = required only when toxicity or target organ

Database Coverage & Accessibility

Coverage

- ToxRefDB contains summary information from 5986 studies for 1143 chemicals.
- As part of ToxRefDB v2.0 curation effort, complete dose-response data and observations were extracted for 3871 studies (as indicated with a 'processed' flag within the study table.)
- There are plans to extract and update the remaining studies in subsequent data releases, but *no additional curation was performed for the v2.1 update.*
- Many of the studies (over 3,000) come from registrant-submitted toxicity studies in data evaluation records (DERs) from the U.S. EPA's Office of Pesticide Programs (OPP).
  - 90% of the studies with completed curation correspond to pesticide actives and inerts
  - Other sources include NTP reports, Pharma, and OpenLit

Figure 1: Study-Level Data Landscape

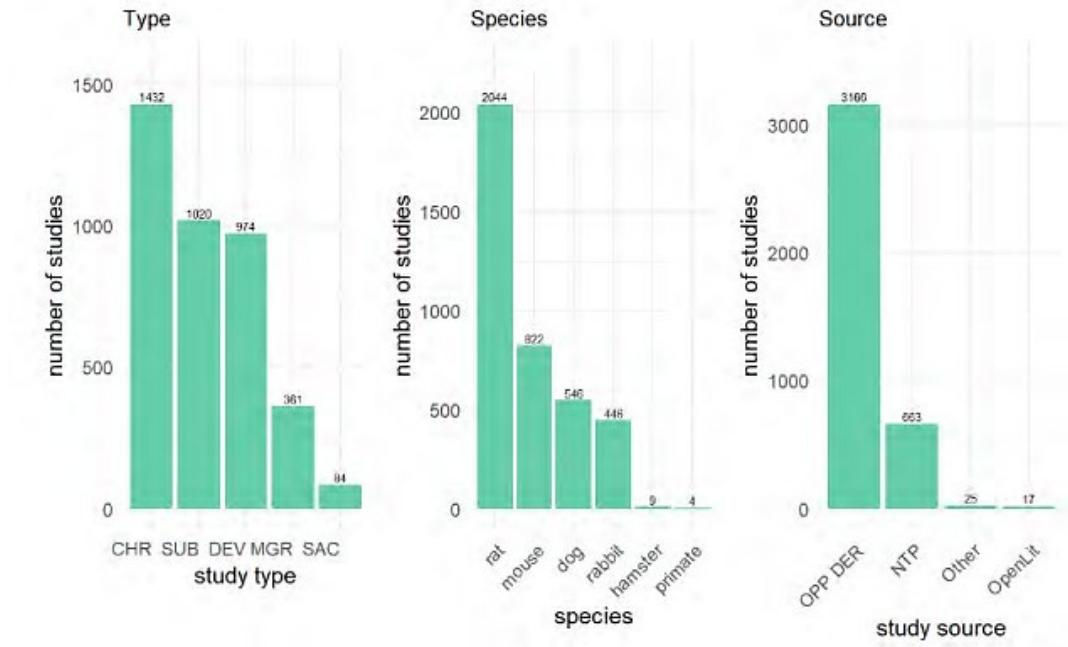
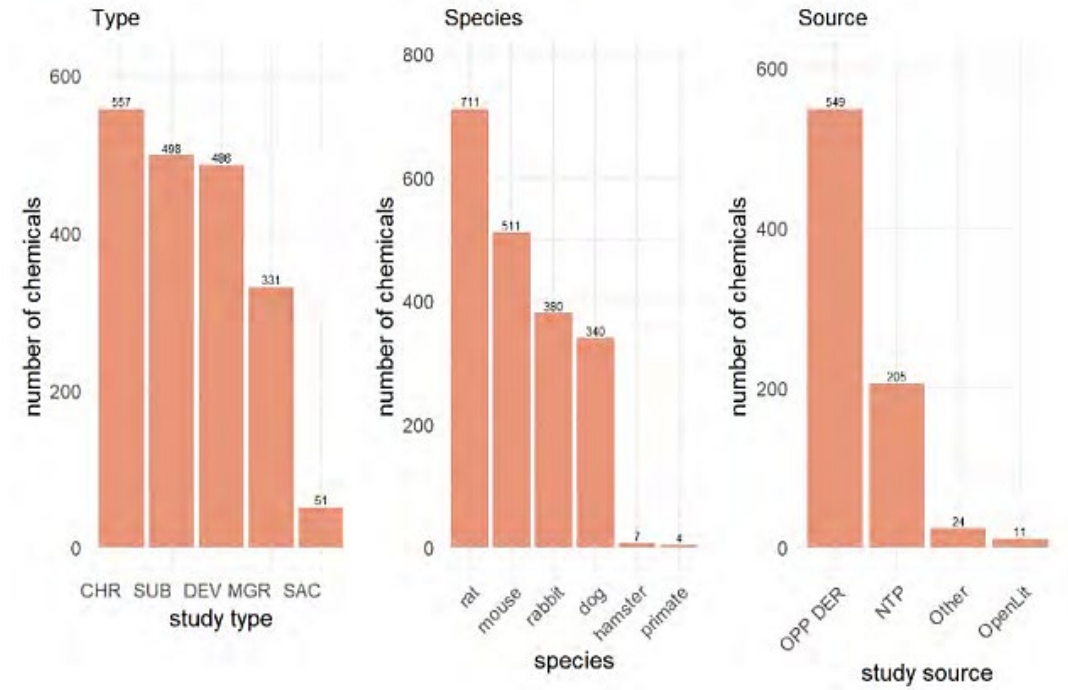


Figure 2: Chemical-Level Data Landscape



Coverage

- The study types covered include the following repeat dose study designs utilizing various administration routes (predominantly oral):
  - **Chronic** (CHR; 1-2 year exposures depending on species and study design) conducted in rats, mice, and dogs
  - **Subchronic** (SUB; 90 day exposures) conducted in rats, mice, and dogs
  - **Subacute** (SAC; 14-28 day exposures depending on the source and guideline) conducted in rats, mice, and dogs
  - **Prenatal developmental** (DEV) conducted in rats and rabbits
  - **Multigeneration reproductive** (MGR) conducted in rats
  - Reproductive (REP) conducted in rats
  - *Developmental neurotoxicity* (DNT) conducted in rats
  - Small number of studies with designs characterized as acute (ACU), neurological (NEU), or "*other*"(OTH)
    - ToxRefDB includes this guideline profile **currently** or *planned for FY23*

Figure 1: Study-Level Data Landscape

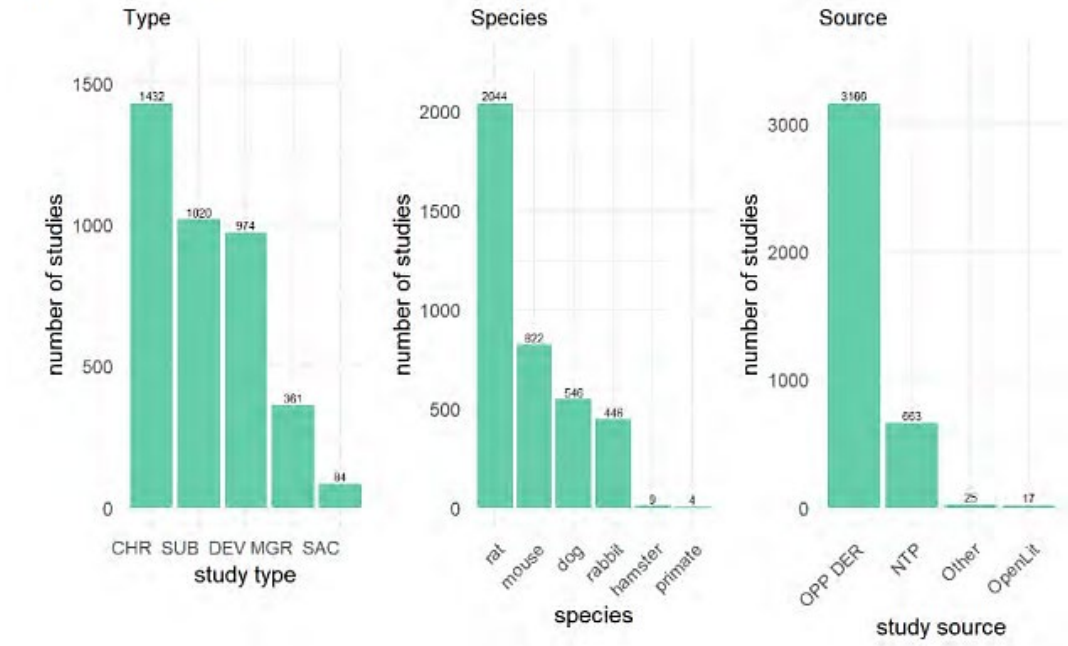
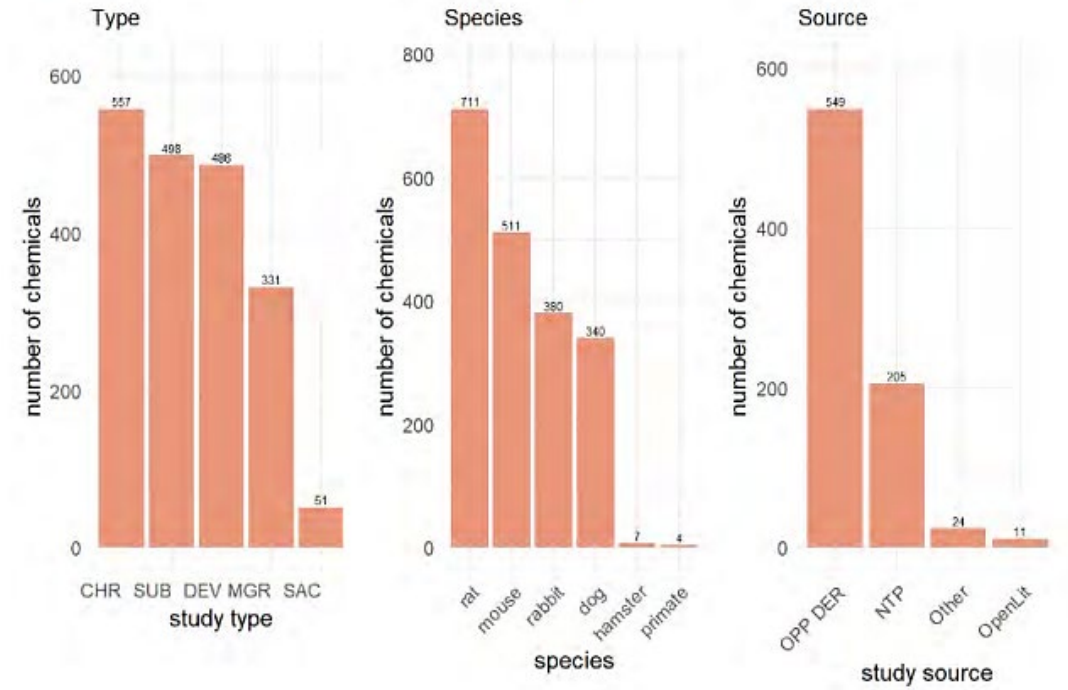


Figure 2: Chemical-Level Data Landscape



ToxRefDB v2.1: Even Better!

- ToxRefDB v2.1 was released in August 2022

- ToxRefDB v2.1 is a **minor data update** to ToxRefDB v2.0 to correct issues discovered with the compilation script which caused some extracted values to not import properly from AccessDB curation files, such as failure to import some effects.

ToxRefDB: Accessibility

Reproductive Toxicology 89 (2019) 145–158

Contents lists available at ScienceDirect

Reproductive Toxicology

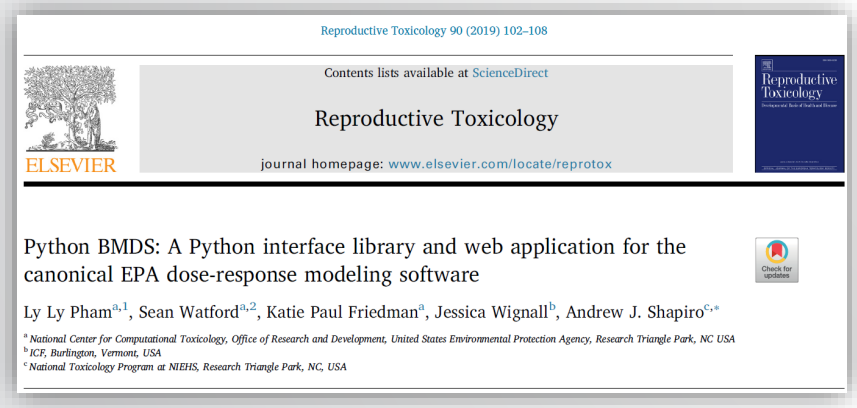
journal homepage: www.elsevier.com/locate/reprotox

ToxRefDB version 2.0: Improved utility for predictive and retrospective toxicology analyses

Sean Watford[a,b], Ly Ly Pham[a,c], Jessica Wignall[d], Robert Shin[d], Matthew T. Martin[a,e], Katie Paul Friedman[b,*]

[a] ORAU, Contractor to U.S. Environmental Protection Agency through the National Student Services Contract, United States
[b] National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, United States
[c] ORISE Postdoctoral Research Participant, United States
[d] ICF, Burlington, VT, United States
[e] Currently at Drug Safety Research and Development, Global Investigative Toxicology, Pfizer, Groton, CT, United States

Watford S, Ly Pham L, Wignall J, Shin R, Martin MT, Friedman KP. ToxRefDB version 2.0: Improved utility for predictive and retrospective toxicology analyses. Reprod Toxicol. 2019 Oct;89:145-158. doi: 10.1016/j.reprotox.2019.07.012. Epub 2019 Jul 21. PMID: 31340180; PMCID: PMC6944327.



Reproductive Toxicology 90 (2019) 102–108

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Python BMDS: A Python interface library and web application for the canonical EPA dose-response modeling software

Ly Ly Pham[a,1], Sean Watford[a,2], Katie Paul Friedman[a], Jessica Wignall[b], Andrew J. Shapiro[c,*]

[a] National Center for Computational Toxicology, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, NC USA
[b] ICF, Burlington, Vermont, USA
[c] National Toxicology Program at NIEHS, Research Triangle Park, NC, USA

Pham LL, Watford S, Friedman KP, Wignall J, Shapiro AJ. Python BMDS: A Python interface library and web application for the canonical EPA dose-response modeling software. Reprod Toxicol. 2019 Dec;90:102-108. doi: 10.1016/j.reprotox.2019.07.013. Epub 2019 Aug 12. PMID: 31415808; PMCID: PMC7169420.

- *Note: ToxRefDB v2.0's BMDS tables will be discontinued in future instances to prioritize curation*

Visit https://www.epa.gov/chemical-research/downloadable-computational-toxicology-data to download v2.1 database package and user guide

If you have trouble getting access or find a curation error, please let us know! Happy to troubleshoot your connection or inspect the source documents

Email: Feshuk.Madison@epa.gov
Watford.Sean@epa.gov

Differences between v2.0 and v2.1

Output	v2.0	v2.1	Change
Total number of studies with complete curation	3882	3871	-11
Number of studies with extracted effects	3068	3662	**594**
Total number of chemicals	748	748	0
Total database rows, including studies with no extracted effects	328623	344868	16245
Total effects extracted	313525	335281	**21756**
Dose treatment groups with effects	35679	40905	**5226**
Unique effects: Cholinesterase endpoint category	5323	6008	685
Unique effects: Developmental endpoint category	8502	9640	1138
Unique effects: Reproductive endpoint category	4691	5775	1084
Unique effects: Systemic endpoint category	284352	302674	18322
Unique critical effects: Cholinesterase endpoint category	713	796	83
Unique critical effects: Developmental endpoint category	1118	1276	158
Unique critical effects: Reproductive endpoint category	488	645	157
Unique critical effects: Systemic endpoint category	18757	20989	2232

- The overall number of studies and chemical remains unchanged.
- The v2.1 update includes additional data from previously curated studies (+594 studies with extracted effects) with extracted dose treatment groups (+5226 dose treatment groups with effects) and effects (+21756 effects) are now fully accessible.

Summary

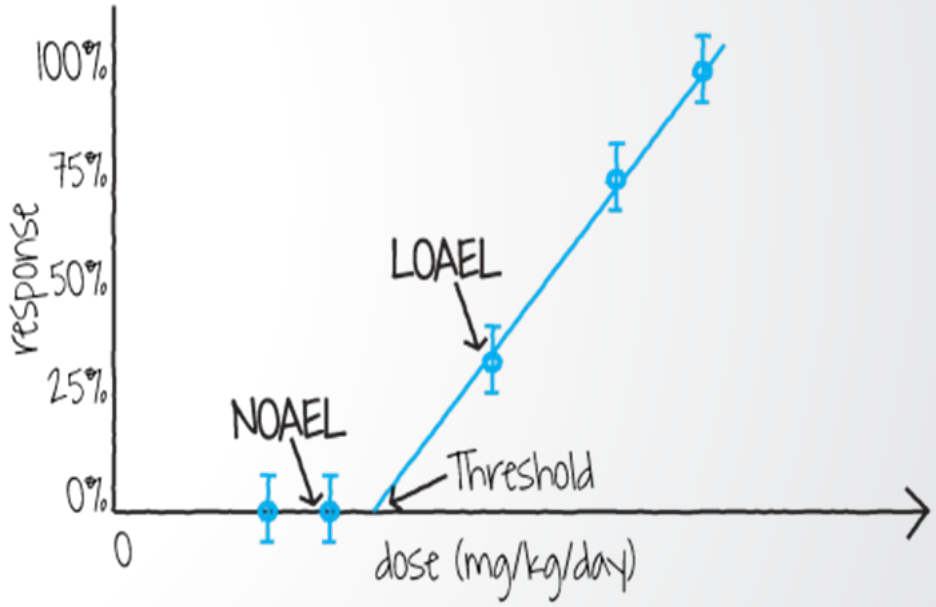
- This added data can improve the utility of ToxRefDB as a resource for curated legacy in vivo information by providing more complete information of the past animal studies conducted.

- **But how impactful were these added data, particularly in relation to calculated points of departure?**

Version Comparison

Points-of-Departure (PODs)

- For each animal toxicity study, data on multiple endpoint targets is collected at each dose level.
- PODs correspond with the lowest dose levels at which effects are observed, which are important for extrapolating to a reference dose (RfD) in risk assessments
- ToxRefDB's pod table derives POD values for each effect profile, by study and chemical.
- 4 POD Types:
  - **LEL:** Lowest Effect Level
  - **NEL:** No Effect Level
  - **LOAEL:** Lowest Observed Adverse Effect Level
  - **NOAEL:** No Observed Adverse Effect Level



Describing POD Logic

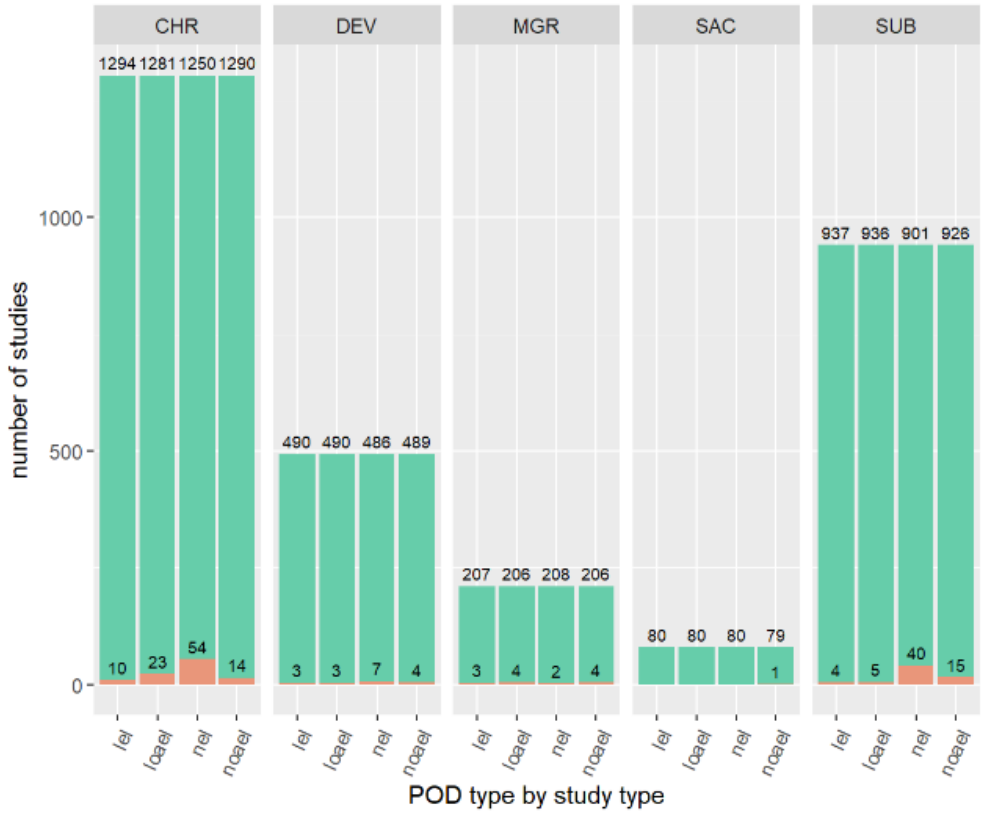
- In the ToxRefDB pod table, effects are grouped together within “effect profiles” for the purposes of POD derivation.
  - The first effect profile calculates POD values for each study’s sex, life stage, and endpoint category combination.
  - A second effect profile calculates POD values for each study’s sex, life stage, endpoint category-endpoint type pairing, except for the systemic endpoint category, which looks at endpoint target (e.g., organs).
- Select **Lowest Effect Level (LEL)** as the lowest dose with observed treatment-related effects and **Lowest Observed Adverse Effect Level (LOAEL)** as the lowest dose with observed critical effects
- Infer NEL and NOAEL as the next lowest dose level from LEL and LOAEL, respectively. **No Effect Level (NEL)** is the highest dose with no observed effect whereas the **No Observed Adverse Effect Level (NOAEL)** is the highest dose with no observed critical effect
- Derive POD values for when no effects were observed in the study using special qualifiers. For all POD types, a **qualifier** (<, >, or =) is given to more precisely describe the observed dose-effect relationships.
  - For instance, if no adverse effects were observed even at highest dose tested, LOAEL > highest dose tested while NOAEL => highest dose tested

chemica	study_i	sex	species	admin_	pod_typ	qualifie	pod_va	pod_un	mg_kg_	dose_le	max_do	effect_	group_i
58779	63	F	dog	Oral	nel	<	0.0015	mg/kg/day	0.0015	1	4	1	0
58779	63	F	dog	Oral	lel	'='	0.0015	mg/kg/day	0.0015	1	4	1	0
58779	63	F	dog	Oral	noael	>=	1.9006	mg/kg/day	1.9006	4	4	1	0
58779	63	F	dog	Oral	loael	>	1.9006	mg/kg/day	1.9006	4	4	1	0
58779	63	M	dog	Oral	nel	<	0.0018	mg/kg/day	0.0018	1	4	1	0
58779	63	M	dog	Oral	lel	'='	0.0018	mg/kg/day	0.0018	1	4	1	0
58779	63	M	dog	Oral	noael	>=	1.6102	mg/kg/day	1.6102	4	4	1	0
58779	63	M	dog	Oral	loael	>	1.6102	mg/kg/day	1.6102	4	4	1	0

Reviewing PODs Using Added Data

- To do the comparison, the v2.1 POD calculation was rerun against v2.0 schema since calculation now includes sex stratification. See “toxrefdb_2_0_recalc_pod.csv” for updated v2.0 POD values.
- For these release note visuals, one set of “extreme” POD values (lowest loael/lel and highest noael/nel mg/kg/day value) are selected for each study id at the study-level, and for each chemical id at the chemical-level, regardless of effect profile. This allowed for a more straightforward 1:1 comparison.

Study-Level Changes in v2.0 to v2.1 PODs



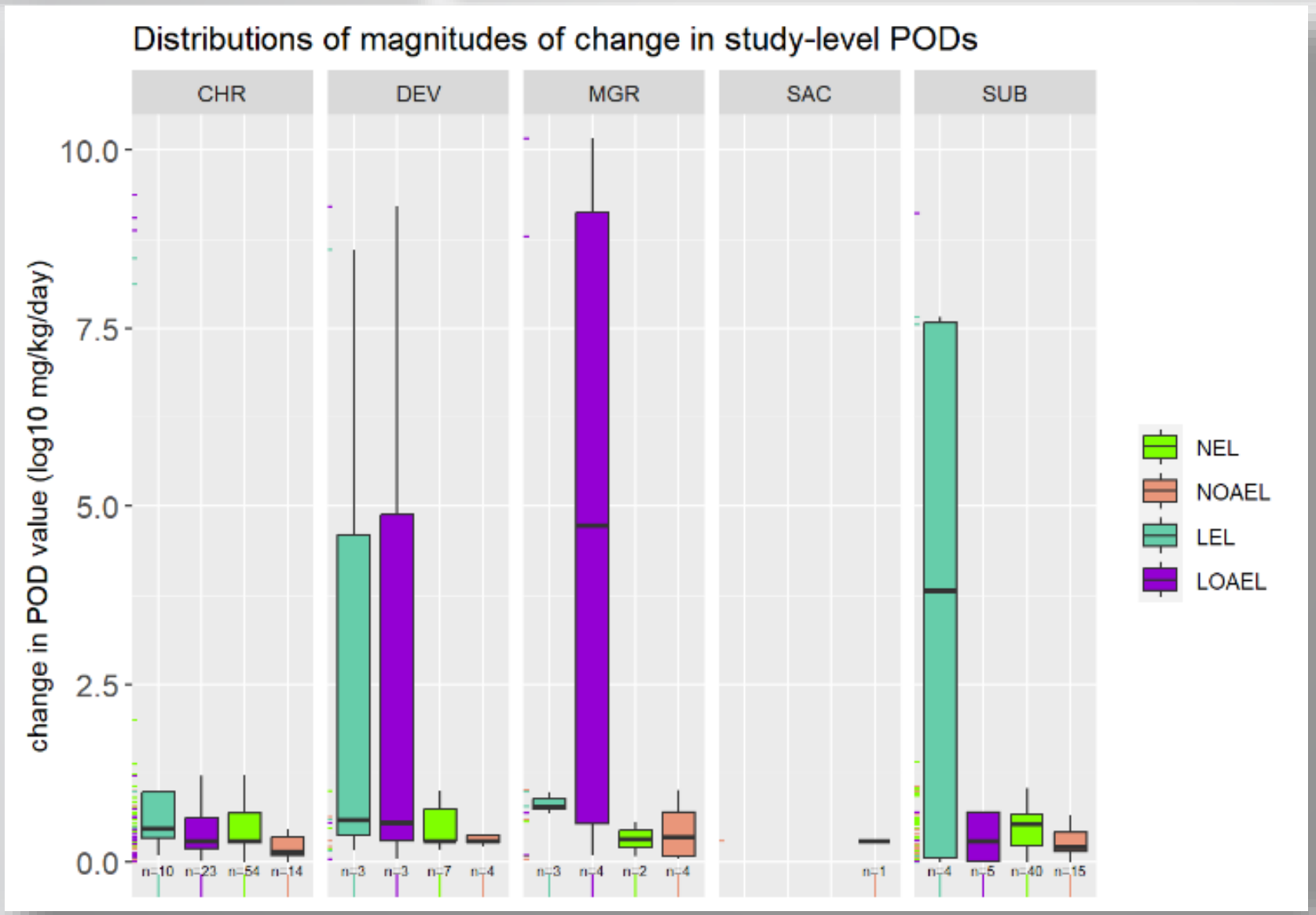
Overall, only 5% of all studies had a change in 1 or more PODs
Most change in CHR & SUB; Least change in SAC
Most change in NEL; Least change in LEL

	Number of POD types changed	No change	One or more	Two or more	Three or more	All four
	All	All	All	All	All	All
1	All studies	95%	5%	1%	0%	0%
2	CHR	95%	5%	2%	1%	0%
3	DEV	98%	2%	1%	0%	0%
4	MGR	96%	4%	2%	0%	0%
5	SAC	99%	1%	0%	0%	0%
6	SUB	95%	5%	1%	0%	0%

POD unchanged
POD changed

Note: These study-level comparison does not consider any new PODs added.
v2.0 had PODs for 3038 studies for comparison; v2.1 includes PODs for 3632.

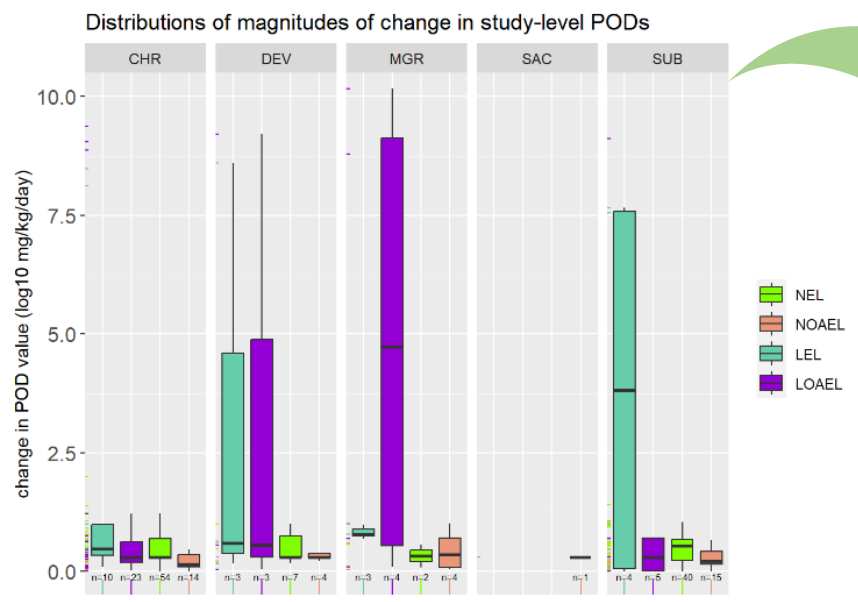
Study-Level Changes in v2.0 to v2.1 PODs

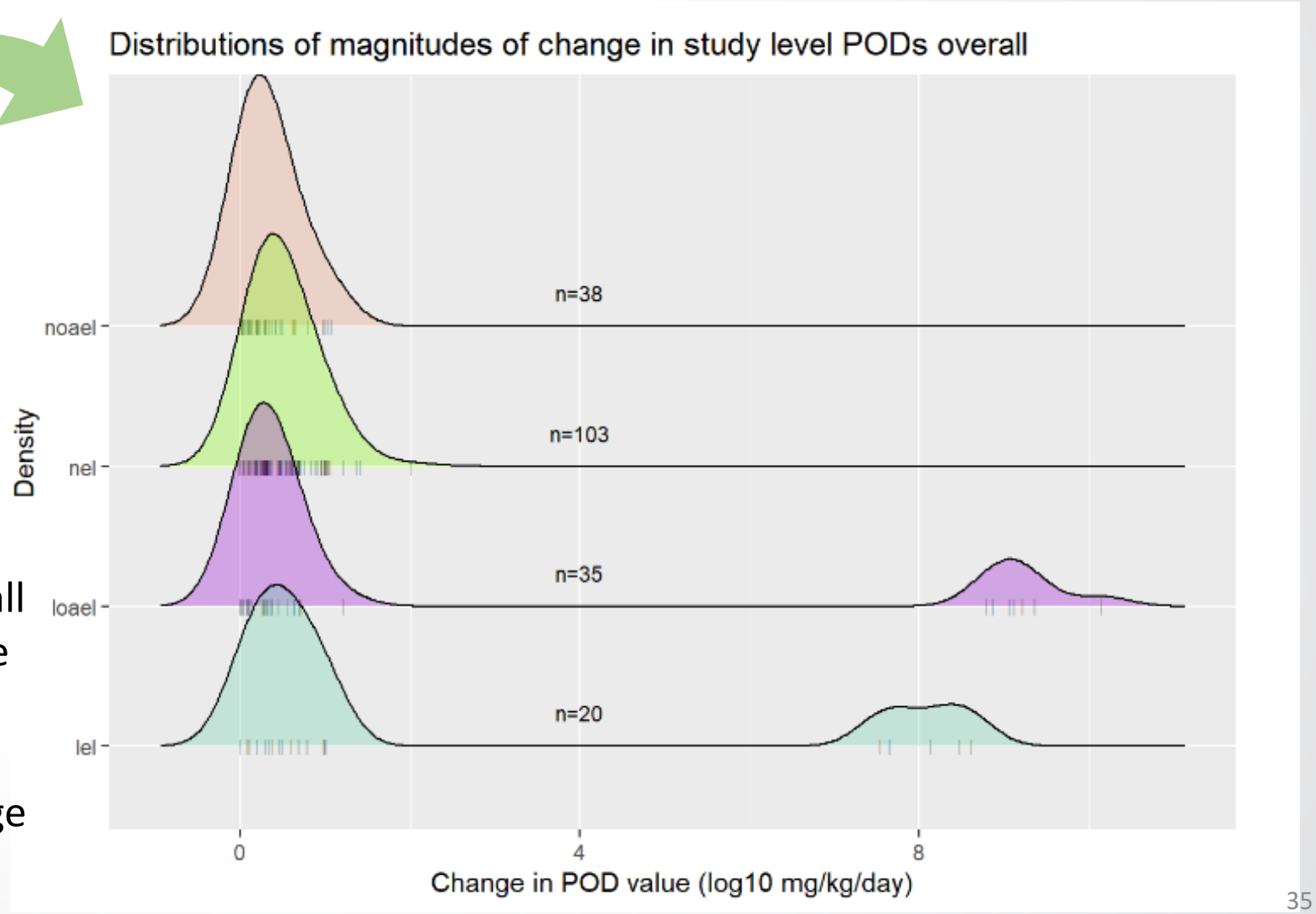


Magnitude of change can be examined within the subset of PODs which changed by study type.

IQR is skewed by a few outlying datapoints where ‘n’ is low.

Study-Level Changes in v2.0 to v2.1 PODs

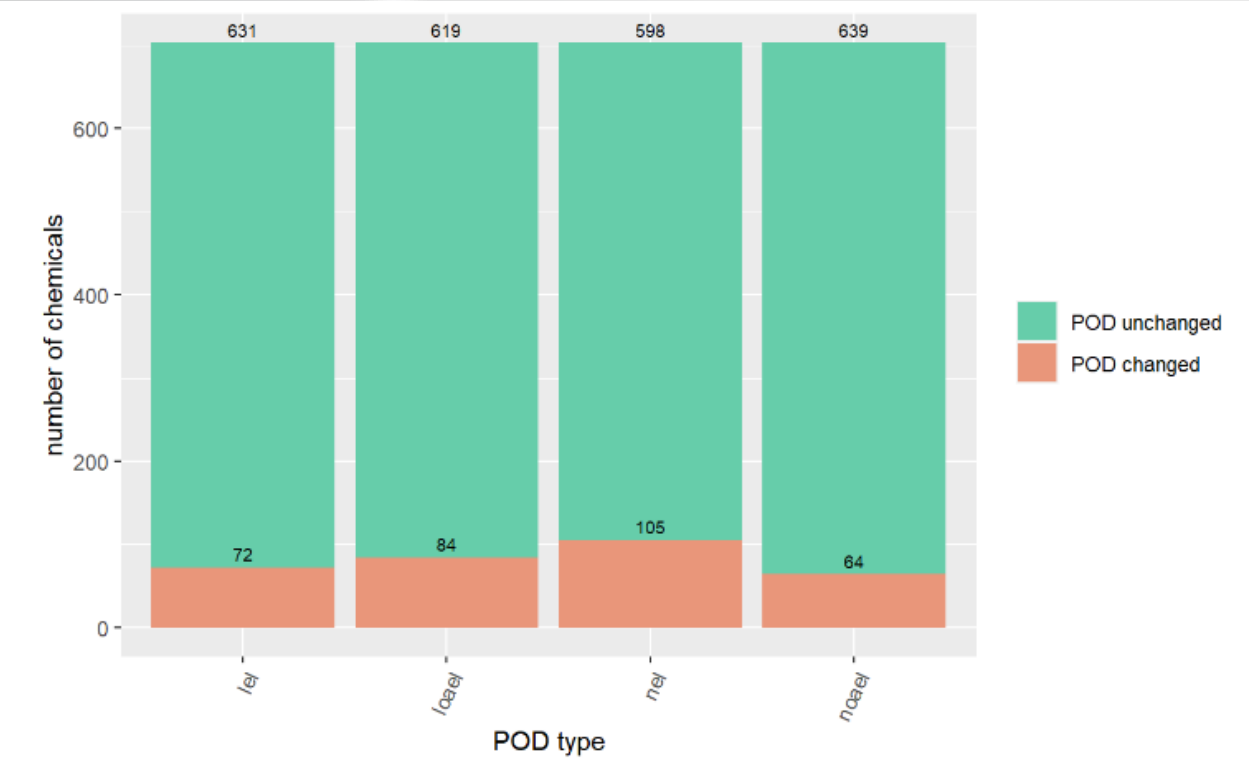




Magnitude of change distributions across all study types could be examined to increase sample size.

Majority of study-level magnitude of change values fall under 1log10 mg/kg/day.

Chemical-Level Changes in v2.0 to v2.1 PODs

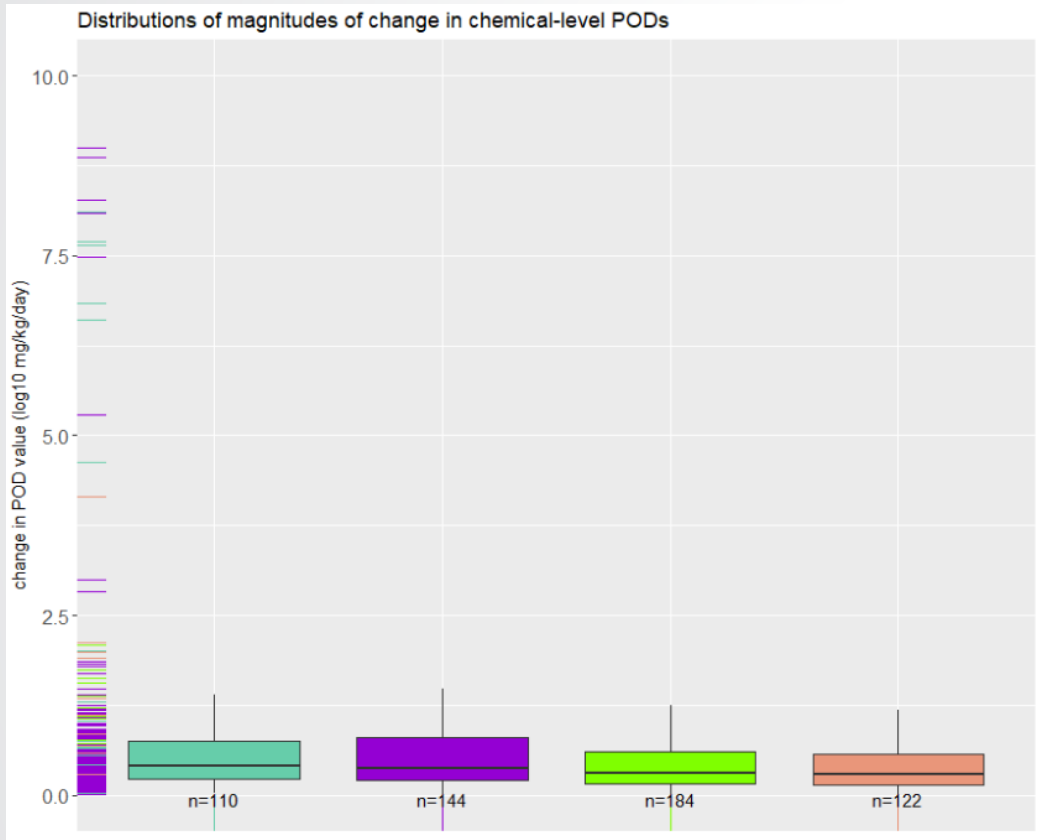


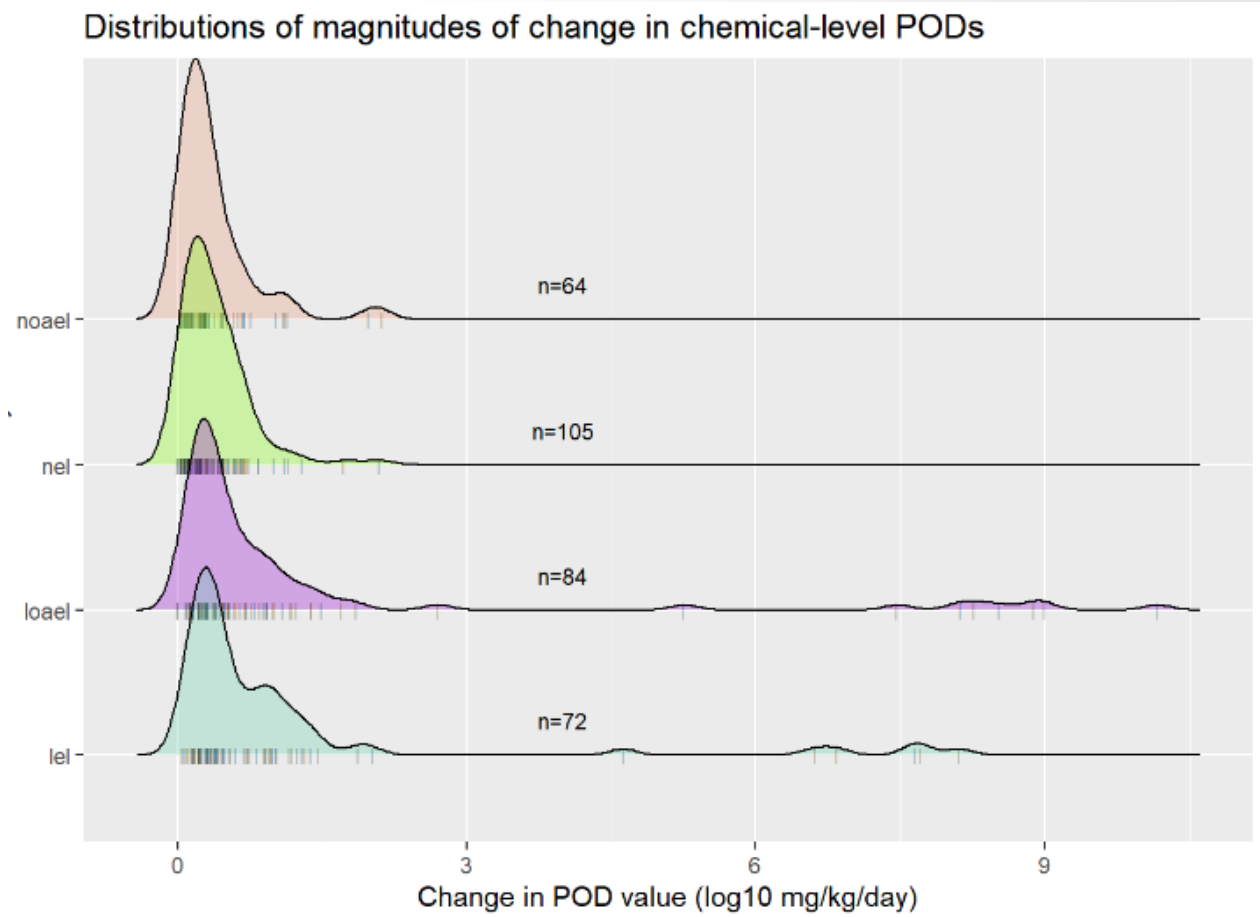
Overall, 29% of chemicals *across all study types* had a change in 1 or more POD types,
with only 2% showing change in 3 or more.

Contributing to this change, new POD values were included for 594 studies.

	Number of POD types changed	No change	One or more	Two or more	Three or more	All four
	All	All	All	All	All	All
1	All chemicals	71%	29%	14%	2%	1%

Chemical-Level Changes in v2.0 to v2.1 PODs

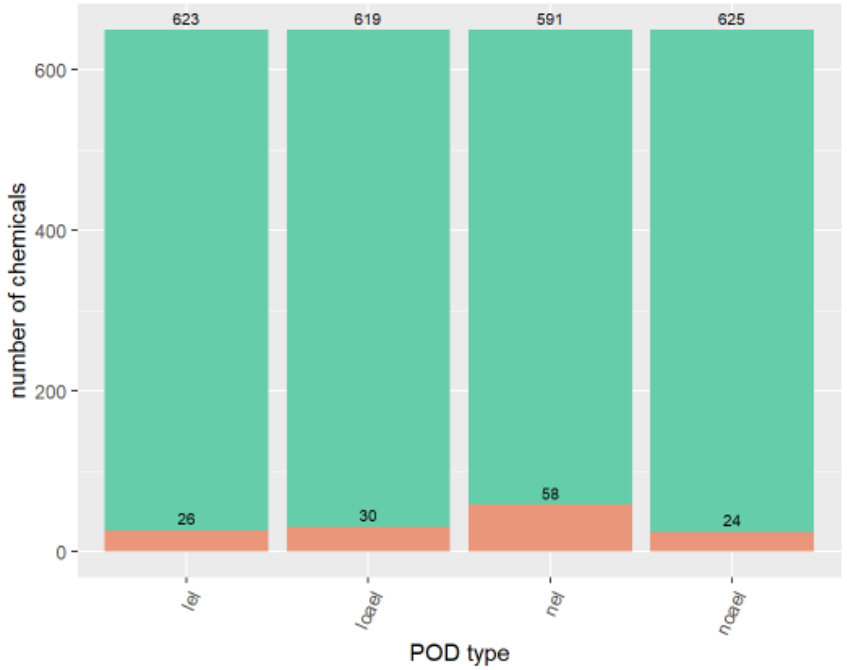




Majority of chemical-level magnitude of change values fall under 1log10 mg/kg/day.

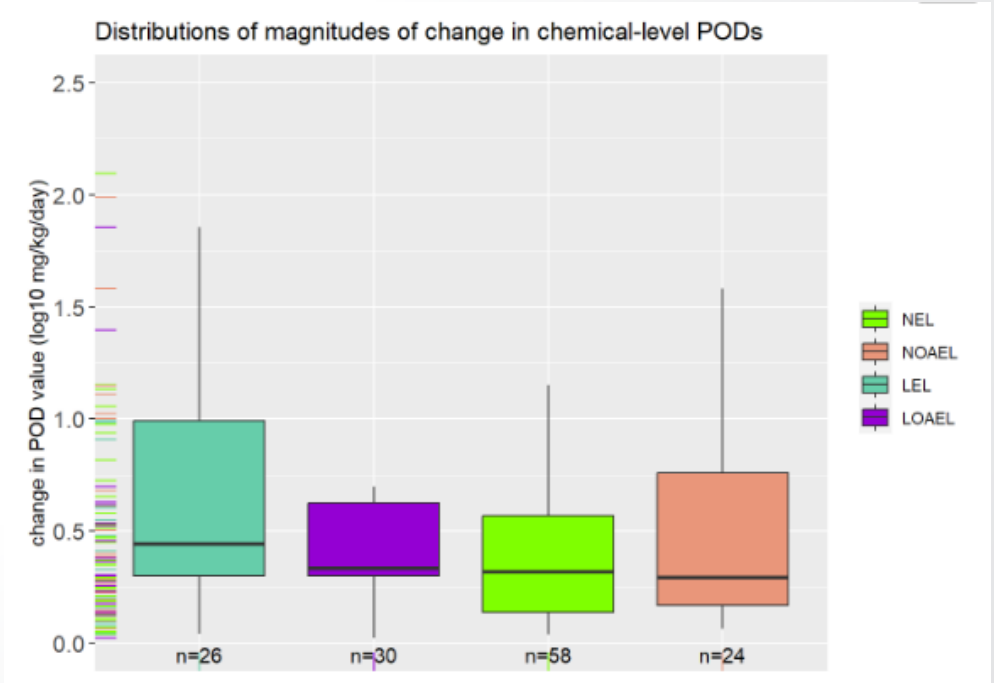
Chemical-Level Changes in v2.0 to v2.1 PODs

How does chemical-level change look for a subset of repeat dose studies (SAC, SUB, CHR)? MGR and DEV studies excluded



Number of POD types changed	No change	One or more	Two or more	Three or more	All four
	All	All	All	All	All	All
1	All chemicals	85%	15%	5%	1%	0%

15% of chemicals *across SAC, SUB, CHR studies* had a change in 1 or more POD types, with only 5% showing change in 2 or more.



Future Updates

Looking ahead

- Migrate from MySQL to PostgreSQL
- Expand chemical and study coverage
  - New study curations from DCT
    - As of November 2022, completed curations for 260 new studies (DEV, SUB, MGR) in the DCT
  - Extractions for a new guideline profiles (ex. DNT, "non-guideline")
    - DNT focus this year
  - New document types, e.g. TSCA reports
- Finalize ETL for loading new DCT curations into ToxRefDB
- Review chemical source metadata-DTXSID mappings

Next Steps

- Systematic QC to identify and correct curation errors
- HERO interoperability for citation management
  - NTP report pilot; ToxRefDB metadata tags will be added to increase utility
- IUCLID interoperability
  - ECHA created Knime workflows to convert ToxRef to IUCLID using Data Uploader
- HAWC interoperability for curation and public interface
  - We will harmonize the ToxRef and HAWC data models and investigate HAWC features to develop to manage all curations and ToxRef data in HAWC
- Ability to “Batch Download” ToxRefDB data on CompTox Chemical Dashboard

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