

Appendix L1

Outlier Characterization for the 3T3 and NHK NRU Test Methods with the RC Millimole Regression

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L.1 Outlier Analysis for the 3T3 and NHK NRU Test Methods and RC Millimole Regression

The RC millimole regression and each *in vitro* NRU test method were used to identify outlier substances among the reference substances tested in the validation study (i.e., those for which the rodent LD₅₀ was not accurately predicted by the *in vitro* NRU IC₅₀) (see **Section 6.2**).

The outliers, identified for each test method in **Table 6-3**, were evaluated for common characteristics that may assist in determining the types of chemicals that are not suited for use in the 3T3 and NHK NRU test methods to determine starting doses for acute systemic toxicity test methods.

A number of physico-chemical characteristics were evaluated for their frequency of occurrence among the 28 outlier substances for the 3T3 NRU test method and 31 outlier substances for the NHK NRU test method versus the entire set of reference substances. The frequency of occurrence of outliers versus the total number of reference substances for each category of each characteristic examined is shown in **Table L1-1**.

Table L1-1 Outliers per Category and NRU Test Method

Category	3T3 NRU Test Method ¹		NHK NRU Test Method ²	
	Number of Outliers	Total Substances in Category	Number of Outliers	Total Substances in Category
Boiling Point (BP) [in degrees C]				
No information	13	34	13	34
< 100	1	6	2	7
100-200	1	5	2	5
200-300	3	4	3	4
300-400	5	6	4	6
465	1	1	1	1
960	0	1	0	1
1500	0	1	0	1
decompose, sublime, or BPs were provided at less than atmospheric pressure	4	12	6	12
Molecular Weight (g/mol)				
< 100	3	14	4	15
100-200	6	18	9	18
200-300	12	20	12	20
300-400	3	11	3	11
400-500	2	4	3	4
500-600	1	1	0	1
600-700	0	1	0	1
700-800	1	1	0	1
IC₅₀ (mM)				
≤ 0.0001	0	3	0	4
0.0001 – 0.001	1	1	1	2
0.001 – 0.01	1	4	3	7
0.01 – 0.1	8	14	5	8
0.1 – 1	13	21	12	19
1 – 10	3	13	7	19
10 – 100	1	9	2	7
> 100	1	5	1	5
pH				
< 7.1	0	0	0	6
7.1	0	0	0	0
7.2	0	0	1	1
7.3	0	0	0	0
7.4	0	0	1	4
7.5	0	0	4	7
< 7.6	0	9	0	0
7.6	0	0	4	7
7.7	1	1	8	22
7.8	0	1	11	17
7.9	2	6	0	3
8.0	5	11	0	1
8.1	10	18	0	0
8.2	3	6	1	1
8.3	3	8	0	0

Table L1-1 Outliers per Category and NRU Test Method

Category	3T3 NRU Test Method ¹		NHK NRU Test Method ²	
	Number of Outliers	Total Substances in Category	Number of Outliers	Total Substances in Category
8.4	1	5	0	0
8.5	0	1	1	1
> 8.5	3	4	0	1
log K _{ow}				
< -4	0	1	1	1
> -4 to < -3	0	1	0	1
> -3 to < -2	0	0	0	0
-2 to -1	1	5	1	5
-1 to 0	3	6	5	7
0 to 1	4	7	3	7
1 to 2	5	13	5	13
2 to 3	1	4	1	4
3 to 4	5	8	5	8
4 to 5	2	2	2	2
5 to 6	1	2	1	2
6 to 7	0	1	0	1
No information	6	20	7	20
Chemical Class				
<i>Organic Compounds</i>				
Acyclic hydrocarbon	1	1	1	1
Alcohol	3	9	4	10
Alkalies	0	1	0	1
Amide	1	3	0	3
Amine	2	3	2	3
Carbohydrate	1	1	0	1
Carboxylic acid	4	14	6	14
Cyclic hydrocarbon	0	3	1	3
Ester	1	1	1	1
Ether	1	1	1	1
Halogenated hydrocarbon	1	3	0	3
Heterocyclic compound	7	14	10	14
Ketone	0	1	0	1
Lipids	0	1	0	1
Nitrile	1	2	1	2
Nitro compound	0	1	0	1
Sodium compound	0	1	1	1
Sulfur compound	5	5	5	5
Organometallic compound	0	1	0	1
Organophosphorous compound	3	3	3	5
Phenol	1	5	2	5
Polycyclic compound	1	5	0	5
Urea	1	1	1	1
<i>Inorganic Compounds</i>				
Arsenical	1	2	1	2
Boron compound	0	1	0	1
Cadmium compound	0	1	0	1
Chlorine compound	2	5	2	5

Table L1-1 Outliers per Category and NRU Test Method

Category	3T3 NRU Test Method ¹		NHK NRU Test Method ²	
	Number of Outliers	Total Substances in Category	Number of Outliers	Total Substances in Category
Chromium compound	0	1	0	1
Fluorine compound	0	1	0	1
Inorganic acid	0	1	0	1
Inorganic carbon compound	0	1	0	1
Lithium compound	0	1	0	1
Mercury compound	1	1	1	1
Metal	1	2	0	2
Nitrogen compound	1	1	1	1
Oxygen compound	1	1	1	1
Potassium compound	1	2	1	2
Selenium compound	1	1	1	1
Sodium compound	2	6	2	6
Sulfur compound	1	2	0	2
Substance Physical Form				
Solid	21	54	22	54
Liquid	7	16	9	17

Abbreviations: NRU=Neutral red uptake; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; BP=Boiling point; K_{ow} = Octanol:water partition coefficient.

¹28 discordant chemicals (i.e., outliers) are characterized for the 3T3 NRU test method by counting the number of outliers in each category and comparing to the total number of chemicals in the category. Analysis excludes carbon tetrachloride and methanol since no IC_{50} values were obtained. Total chemicals = 70.

²31 discordant chemicals (i.e., outliers) are characterized for the NHK NRU test method by counting the number of outliers in each category and comparing to the total number of chemicals in the category. Analysis excludes carbon tetrachloride since no IC_{50} values were obtained. Total chemicals = 71.