Summary of Animals Used and Animals Dead for ATC Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
			Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
NHK	0.12	Cyto	0.297	9.75	0.91	0.0025	0.280	2.67	0.48	0.0999	8.6%	15.2%
		Default	0.169	10.67			0.334	3.15				
	0.25	Cyto	0.274	9.77	0.98	0.0015	0.276	2.88	0.50	0.3451	9.1%	14.7%
		Default	0.149	10.75			0.324	3.38				
	0.50	Cyto	0.242	9.95	0.96	0.0000	0.254	3.21	0.52	0.0030	8.8%	13.8%
		Default	0.114	10.91			0.297	3.72				
	1.25	Cyto	0.180	10.24	0.86	0.0005	0.193	3.86	0.54	0.0000	7.8%	12.3%
		Default	0.068	11.10			0.228	4.40				
	2.00	Cyto	0.152	10.39	0.86	0.0000	0.160	4.19	0.53	0.0001	7.6%	11.3%
		Default	0.050	11.25			0.189	4.73				
			Average Difference		0.91		Average Difference		0.51			
3T3	0.12	Cyto	0.293	9.55	1.09	0.0006	0.283	2.73	0.47	0.0001	10.2%	14.6%
		Default	0.170	10.64			0.335	3.20				
	0.25	Cyto	0.273	9.61	1.11	0.0002	0.275	2.95	0.48	0.0024	10.4%	14.1%
		Default	0.151	10.73			0.325	3.43				
	0.50	Cyto	0.244	9.85	1.04	0.0001	0.251	3.27	0.50	0.0007	9.6%	13.2%
		Default	0.115	10.89			0.299	3.77				
	1.25	Cyto	0.187	10.22	0.88	0.0000	0.192	3.93	0.51	0.0000	7.9%	11.4%
		Default	0.067	11.10			0.228	4.43				
	2.00	Cyto	0.153	10.43	0.81	0.0000	0.160	4.27	0.49	0.0000	7.2%	10.3%
		Default	0.049	11.25			0.190	4.76				
			Average Difference		0.99		Average Difference		0.49			

Abbreviations: ATC=Acute Toxic Class method (OECD 2001d); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [□g/mL] + 2.024); Default=Default starting dose of 300 mg/kg; Std. Error=Standard error for number of animals; NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 ATC simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]Mean number of animals for 2000 simulations.

[3]Difference between mean number of animals for the default starting dose and mean number of animals for the NRU-based starting dose.

[4]P-value is from one-sided Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
1	NHK	0.12	Cyto	1.195	6.18	2.91	0.1250	1.180	5.96	2.91	0.1250	32.0%	32.8%
			Default	0.083	9.09			0.080	8.86				
		0.25	Cyto	1.250	6.45	2.91	0.2500	1.156	5.77	2.90	0.2500	31.1%	33.5%
			Default	0.178	9.35			0.070	8.67				
		0.50	Cyto	1.277	6.87	2.87	0.2500	1.157	5.61	2.89	0.1250	29.4%	34.0%
			Default	0.158	9.74			0.060	8.50				
		1.25	Cyto	1.215	7.52	2.87	0.2500	1.033	5.26	2.66	0.1250	27.6%	33.6%
			Default	0.111	10.39			0.066	7.92				
		2.00	Cyto	1.225	7.94	2.90	0.2500	0.940	5.10	2.41	0.1250	26.8%	32.1%
			Default	0.066	10.85			0.052	7.51				
				Average Difference		2.89		Average Difference		2.75			
	3T3	0.12	Cyto	0.987	6.85	2.24	0.1250	1.057	6.62	2.24	0.1250	24.6%	25.3%
			Default	0.081	9.08			0.081	8.86				
		0.25	Cyto	0.980	7.12	2.24	0.1250	1.008	6.44	2.23	0.1250	23.9%	25.7%
			Default	0.174	9.36			0.073	8.67				
		0.50	Cyto	1.029	7.56	2.21	0.1250	0.998	6.28	2.22	0.1250	22.6%	26.1%
			Default	0.170	9.77			0.049	8.50				
		1.25	Cyto	1.011	8.16	2.26	0.1250	0.911	5.89	2.04	0.1250	21.7%	25.7%
			Default	0.093	10.42			0.081	7.93				
		2.00	Cyto	1.017	8.61	2.22	0.1250	0.841	5.68	1.86	0.1250	20.5%	24.7%
			Default	0.060	10.84			0.058	7.54				
				Average Difference		2.23		Average Difference		2.12			
2	NHK	0.12	Cyto	0.333	10.40	1.36	0.0049	0.618	5.20	1.36	0.0144	11.5%	20.7%
			Default	0.165	11.76			0.256	6.55				
		0.25	Cyto	0.266	10.31	1.33	0.0034	0.690	5.38	1.33	0.0046	11.5%	19.8%
			Default	0.180	11.64			0.313	6.71				
		0.50	Cyto	0.192	10.31	1.25	0.0061	0.668	5.66	1.22	0.0134	10.8%	17.8%
			Default	0.212	11.56			0.312	6.88				
		1.25	Cyto	0.261	10.21	1.33	0.0105	0.504	5.82	1.09	0.0046	11.5%	15.8%
			Default	0.156	11.54			0.191	6.91				
		2.00	Cyto	0.344	10.21	1.41	0.0034	0.438	5.74	1.03	0.0012	12.1%	15.3%
			Default	0.089	11.62			0.142	6.77				
				Average Difference		1.33		Average Difference		1.21			
	3T3	0.12	Cyto	0.329	10.27	1.48	0.0061	0.597	5.06	1.49	0.0024	12.6%	22.8%
			Default	0.163	11.75			0.255	6.56				
		0.25	Cyto	0.350	10.13	1.51	0.0024	0.645	5.19	1.53	0.0024	13.0%	22.8%
			Default	0.189	11.64			0.314	6.72				
		0.50	Cyto	0.384	10.06	1.51	0.0061	0.630	5.41	1.48	0.0022	13.0%	21.5%
			Default	0.214	11.56			0.313	6.89				
		1.25	Cyto	0.425	9.98	1.52	0.0061	0.486	5.64	1.25	0.0061	13.2%	18.2%
			Default	0.148	11.51			0.194	6.89				
		2.00	Cyto	0.445	10.03	1.55	0.0046	0.426	5.60	1.17	0.0024	13.4%	17.3%
			Default	0.083	11.58			0.146	6.77				
				Average Difference		1.51		Average Difference		1.39			

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
3	NHK	0.12	Cyto	0.489	9.63	-0.20	0.4688	0.073	3.12	0.28	0.0713	-2.1%	8.3%
			Default	0.264	9.44			0.217	3.41				
		0.25	Cyto	0.407	9.86	0.04	0.3013	0.139	3.35	0.45	0.0210	0.4%	11.8%
			Default	0.275	9.89			0.237	3.80				
		0.50	Cyto	0.279	10.41	0.42	0.1099	0.155	3.62	0.64	0.0093	3.8%	15.1%
			Default	0.207	10.83			0.171	4.26				
		1.25	Cyto	0.248	10.91	0.82	0.0342	0.193	4.27	0.76	0.0210	7.0%	15.1%
			Default	0.083	11.73			0.119	5.03				
		2.00	Cyto	0.302	10.74	1.09	0.0034	0.218	4.60	0.81	0.0342	9.2%	15.1%
			Default	0.038	11.82			0.091	5.41				
				Average Difference		0.43		Average Difference		0.59			
	3T3	0.12	Cyto	0.099	9.20	0.22	0.2647	0.104	3.17	0.23	0.1294	2.4%	6.7%
			Default	0.258	9.42			0.213	3.40				
		0.25	Cyto	0.155	9.60	0.31	0.0449	0.165	3.50	0.30	0.1060	3.1%	7.9%
			Default	0.271	9.90			0.237	3.80				
		0.50	Cyto	0.176	10.35	0.47	0.0225	0.160	3.83	0.43	0.0522	4.3%	10.1%
			Default	0.202	10.81			0.169	4.26				
		1.25	Cyto	0.228	11.11	0.62	0.0210	0.180	4.51	0.52	0.0210	5.3%	10.4%
			Default	0.084	11.73			0.119	5.04				
		2.00	Cyto	0.253	11.09	0.71	0.0449	0.186	4.88	0.53	0.0640	6.0%	9.8%
			Default	0.037	11.80			0.095	5.41				
				Average Difference		0.47		Average Difference		0.40			
4	NHK	0.12	Cyto	0.645	10.23	-0.97	0.1445	0.068	3.045	-0.005	0.2444	-10.4%	-0.1%
			Default	0.098	9.26			0.067	3.040				
		0.25	Cyto	0.565	10.23	-0.80	0.1089	0.093	3.13	-0.01	0.0229	-8.5%	-0.4%
			Default	0.095	9.43			0.092	3.12				
		0.50	Cyto	0.500	10.46	-0.69	0.6416	0.058	3.20	-0.02	0.0744	-7.1%	-0.6%
			Default	0.062	9.77			0.057	3.18				
		1.25	Cyto	0.296	10.91	-0.34	0.0256	0.057	3.64	0.06	0.3259	-3.3%	1.6%
			Default	0.043	10.57			0.067	3.70				
		2.00	Cyto	0.093	11.12	-0.02	0.4851	0.070	4.04	0.13	0.6791	-0.2%	3.0%
			Default	0.048	11.10			0.080	4.16				
				Average Difference		-0.57		Average Difference		0.03			
	3T3	0.12	Cyto	0.664	10.65	-1.39	0.0762	0.068	3.04	0.00	0.8160	-15.0%	0.0%
			Default	0.102	9.26			0.068	3.04				
		0.25	Cyto	0.586	10.56	-1.14	0.0437	0.094	3.13	0.00	0.5871	-12.1%	0.0%
			Default	0.098	9.42			0.095	3.13				
		0.50	Cyto	0.496	10.67	-0.93	0.0229	0.057	3.18	-0.01	0.4691	-9.5%	-0.2%
			Default	0.065	9.75			0.056	3.18				
		1.25	Cyto	0.279	10.95	-0.38	0.0928	0.053	3.62	0.08	0.1208	-3.6%	2.2%
			Default	0.040	10.57			0.069	3.71				
		2.00	Cyto	0.105	11.12	-0.01	0.4212	0.061	4.00	0.17	0.1089	-0.1%	4.2%
			Default	0.047	11.11			0.077	4.17				
				Average Difference		-0.77		Average Difference		0.05			

Summary of Animals Used and Animals Dead for ATC Simulations by GHS Toxicity Category and NRU Test Method[1]

Toxcat	NRU Test Method	Sigma	Starting Dose	Animals Used				Animals Died				% Savings - Animals Used	% Difference - Animals Died
				Std. Error	Number[2]	Difference[3]	P[4]	Std. Error	Number[2]	Difference[3]	P[4]		
5	NHK	0.12	Cyto	0.604	11.03	0.84	0.8867	0.188	0.393	-0.003	0.1055	7.1%	-0.9%
			Default	0.101	11.87			0.189	0.390				
		0.25	Cyto	0.528	10.88	0.75	0.3223	0.156	1.10	-0.02	0.0098	6.4%	-1.8%
			Default	0.119	11.63			0.157	1.08				
		0.50	Cyto	0.365	10.69	0.53	0.1523	0.098	1.819	-0.002	0.7891	4.7%	-0.1%
			Default	0.083	11.22			0.097	1.817				
		1.25	Cyto	0.175	10.52	0.24	0.1934	0.041	2.87	0.01	0.3223	2.2%	0.5%
			Default	0.038	10.77			0.041	2.89				
		2.00	Cyto	0.094	10.69	0.14	0.1934	0.034	3.37	-0.01	0.7695	1.3%	-0.3%
			Default	0.011	10.83			0.026	3.35				
				Average Difference		0.50		Average Difference		0.00			
	3T3	0.12	Cyto	0.876	9.44	2.43	0.0742	0.184	0.38	0.01	0.1856	20.5%	2.1%
			Default	0.103	11.88			0.188	0.39				
		0.25	Cyto	0.751	9.54	2.08	0.1934	0.150	1.09	-0.01	0.5566	17.9%	-0.5%
			Default	0.117	11.62			0.158	1.09				
		0.50	Cyto	0.514	9.80	1.43	0.0273	0.095	1.80	0.02	0.0488	12.7%	1.1%
			Default	0.083	11.22			0.093	1.82				
		1.25	Cyto	0.260	10.08	0.69	0.0273	0.052	2.87	0.01	0.6953	6.4%	0.4%
			Default	0.037	10.77			0.041	2.88				
		2.00	Cyto	0.127	10.49	0.36	0.0273	0.046	3.41	-0.06	0.1055	3.3%	-1.6%
			Default	0.010	10.85			0.027	3.35				
				Average Difference		1.40		Average Difference		0.00			
6	NHK	0.12	Cyto	0.853	8.75	3.25	0.0068	0.00022	0.0005	-0.0001	0.5313	27.1%	-27.3%
			Default	0.000	12.00			0.00021	0.0004				
		0.25	Cyto	0.847	8.75	3.25	0.0105	0.033	0.11	-0.0004	0.1099	27.1%	-3.8%
			Default	0.002	11.99			0.033	0.10				
		0.50	Cyto	0.776	8.91	2.94	0.0081	0.099	0.72	0.02	0.0327	24.8%	2.3%
			Default	0.034	11.86			0.099	0.73				
		1.25	Cyto	0.481	9.44	1.78	0.0085	0.106	2.12	0.15	0.0085	15.9%	6.4%
			Default	0.058	11.23			0.086	2.27				
		2.00	Cyto	0.318	9.89	1.09	0.0266	0.090	2.82	0.11	0.0288	9.9%	3.8%
			Default	0.031	10.98			0.053	2.93				
				Average Difference		2.46		Average Difference		0.05			
	3T3	0.12	Cyto	0.912	8.67	3.33	0.0098	0.00020	0.0005	-0.00017	0.2500	27.7%	-50.0%
			Default	0.000	12.00			0.00018	0.003				
		0.25	Cyto	0.912	8.68	3.31	0.0068	0.034	0.11	0.01	0.0210	27.6%	4.4%
			Default	0.002	11.99			0.035	0.12				
		0.50	Cyto	0.833	8.83	3.02	0.0068	0.106	0.74	0.00	0.8057	25.5%	-0.3%
			Default	0.035	11.85			0.106	0.74				
		1.25	Cyto	0.542	9.41	1.81	0.0122	0.117	2.12	0.15	0.0269	16.1%	6.6%
			Default	0.060	11.22			0.096	2.27				
		2.00	Cyto	0.346	9.86	1.12	0.0161	0.095	2.83	0.12	0.0269	10.2%	4.0%
			Default	0.029	10.99			0.057	2.95				
				Average Difference		2.52		Average Difference		0.05			

Abbreviations: ATC=Acute Toxic Class method (OECD 2001d); Toxcat=Category from Globally Harmonized System of Classification and Labeling of Chemicals[5] (GHS; UN 2005); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024); Default=Default starting dose of 300 mg/kg; Std. Error=Standard error for number of animals; NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 ATC simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]Mean number of animals for 2000 simulations.

[3]Difference between mean animals used for the default starting dose and mean animals used for the NRU-based starting dose.

[4]P-value is from one-side Wilcoxon signed rank test for difference in animals between the default and NRU-based starting doses. Significant values at $p < 0.05$.

[5]GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
6	$LD_{50} > 5000$ mg/kg

Concordance of NRU-Based Starting Dose with Default Starting Dose for GHS Acute Oral Toxicity Category Outcome for ATC Simulations[1]

GHS Category Outcome with Default Starting Dose	GHS Category Outcome with NHK NRU-Based Starting Dose									
	1	**2**	**3**	**4**	**5**	**6**	**Total**	**Category Match**	**Higher NRU Category**	**Lower NRU Category**
1	8	0	0	0	0	0	8	100%	0%	0%
2	0	11	0	0	0	0	11	100%	0%	0%
3	0	1	13	0	0	0	14	93%	0%	7%
4	0	0	1	12	0	0	13	92%	0%	8%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	1	1	100%	0%	0%
Total	8	12	14	12	21	1	68	97%	0%	3%

GHS Category Outcome with Default Starting Dose	GHS Category Outcome with 3T3 NRU-Based Starting Dose									
	1	**2**	**3**	**4**	**5**	**6**	**Total**	**Category Match**	**Higher NRU Category**	**Lower NRU Category**
1	8	0	0	0	0	0	8	100%	0%	0%
2	0	11	0	0	0	0	11	100%	0%	0%
3	0	0	15	0	0	0	15	100%	0%	0%
4	0	0	1	11	0	0	12	92%	0%	8%
5	0	0	0	0	20	0	20	100%	0%	0%
6	0	0	0	0	0	1	1	100%	0%	0%
Total	8	11	16	11	20	1	67	99%	0%	1%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); ATC=Acute Toxic Class method (OECD 2001d); NHK=Normal human epidermal keratinocytes; 3T3= BALB/c 3T3 mouse fibroblasts; RC=Registry of Cytotoxicity.

[1]For 2000 UDP simulations at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg. The NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [µg/mL] + 2.024).. The default starting dose = 300 mg/kg. Shaded cells are those containing the correct predictions.

[2]GHS Toxicity Category	Oral LD_{50} Limit
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
6	$LD_{50} > 5000$ mg/kg

Discordant Substances[1] for GHS Category[2] Outcomes of ATC Simulations

NRU Test Method	Substance	NRU-Based Starting Dose[3] Toxicity Category	Default Starting Dose[4] Toxicity Category
3T3	Cupric sulfate pentahydrate	3	4
NHK	Hexachlorophene	2	3
NHK	Propranolol	3	4

Abbreviations: GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); ATC= Acute Toxic Class method (OECD 2001d); 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; NRU=Neutral red uptake.

[1]Substances for which the simulated ATC outcome for the NRU-based starting dose did not match the simulated ATC outcome for the default starting dose. Simulations were performed with 2000 runs at each starting dose and dose-mortality slope for 67 substances in the 3T3 NRU test method and 68 substances in the NHK NRU test method. Upper limit dose =2000 mg/kg.

[2]GHS Toxicity Category	Oral LD_{50} Limits
1	$LD_{50} \leq 5$ mg/kg
2	$5 < LD_{50} \leq 50$ mg/kg
3	$50 < LD_{50} \leq 300$ mg/kg
4	$300 < LD_{50} \leq 2000$ mg/kg
5	$2000 < LD_{50} \leq 5000$ mg/kg
6	$LD_{50} > 5000$ mg/kg

[3]NRU-based starting dose was one dose lower than the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mg/kg] = 0.372 log IC_{50} [μg/mL] + 2.024).

[4]The default starting dose = 300 mg/kg.

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