Summary of Animals Used and Animals Dead for UDP 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]		
3T3	0.12	Cyto	0.210	7.33	0.66	0.0013	0.224	3.60	-0.15	0.2888	8.2%	-4.2%
		Default	0.177	7.98			0.201	3.46				
	0.25	Cyto	0.202	8.03	0.66	0.0015	0.221	3.94	-0.16	0.1284	7.6%	-4.3%
		Default	0.174	8.70			0.196	3.78				
	0.50	Cyto	0.184	8.67	0.68	0.0023	0.211	4.28	-0.16	0.2071	7.2%	-3.9%
		Default	0.160	9.35			0.182	4.12				
	1.25	Cyto	0.159	9.24	0.71	0.0009	0.187	4.65	-0.10	0.9458	7.1%	-2.2%
		Default	0.130	9.95			0.149	4.55				
	2.00	Cyto	0.137	9.39	0.63	0.0005	0.163	4.75	-0.07	0.8240	6.2%	-1.4%
		Default	0.095	10.02			0.127	4.68				
			Average Difference		0.66		Average Difference		-0.13			
NHK	0.12	Cyto	0.216	7.37	0.59	0.0021	0.230	3.55	-0.15	0.1185	7.4%	-4.3%
		Default	0.175	7.96			0.203	3.41				
	0.25	Cyto	0.209	8.07	0.61	0.0017	0.227	3.90	-0.16	0.2017	7.0%	-4.3%
		Default	0.169	8.68			0.197	3.74				
	0.50	Cyto	0.189	8.73	0.62	0.0019	0.215	4.26	-0.17	0.1974	6.6%	-4.2%
		Default	0.153	9.35			0.181	4.08				
	1.25	Cyto	0.161	9.28	0.68	0.0004	0.190	4.63	-0.10	0.8704	6.8%	-2.3%
		Default	0.124	9.96			0.148	4.53				
	2.00	Cyto	0.139	9.43	0.60	0.0004	0.167	4.74	-0.07	0.9230	6.0%	-1.5%
		Default	0.092	10.03			0.125	4.66				
			Average Difference		0.62		Average Difference		-0.13			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., 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} [mg/mL] + 2.024); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 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 =5000 mg/kg.

[2]Mean number of animals for 10,000 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 Stopping Rules Used for the UDP Simulations by NRU Test Method[1]

NRU Test Method	Sigma	Starting Dose	3 Animals at Limit Dose[2]	5 Reversals[2]	Likelihood Ratio[2]	Maximum Number of Animals Used[2]
3T3	0.12	Cyto	15.8%	60.2%	22.9%	1.1%
		Default	15.4%	57.4%	24.8%	2.4%
	0.25	Cyto	15.1%	34.2%	48.1%	2.6%
		Default	14.6%	34.3%	45.9%	5.2%
	0.5	Cyto	13.7%	19.6%	60.8%	5.8%
		Default	12.9%	20.1%	57.5%	9.5%
	1.25	Cyto	10.4%	13.3%	65.1%	11.2%
		Default	9.1%	13.6%	61.0%	16.3%
	2	Cyto	9.3%	12.1%	65.7%	12.9%
		Default	7.4%	12.5%	62.5%	17.6%
NHK	0.12	Cyto	17.0%	56.2%	25.5%	1.2%
		Default	16.6%	56.4%	24.6%	2.3%
	0.25	Cyto	16.2%	33.1%	47.8%	2.8%
		Default	15.8%	33.8%	45.4%	5.1%
	0.5	Cyto	14.5%	19.3%	60.0%	6.2%
		Default	13.8%	19.9%	56.8%	9.5%
	1.25	Cyto	10.5%	13.2%	64.7%	11.6%
		Default	9.6%	13.6%	60.4%	16.4%
	2	Cyto	9.2%	12.0%	65.5%	13.2%
		Default	7.6%	12.5%	62.1%	17.7%

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; Sigma=Reciprocal of dose-mortality slope; Cyto=NRU-determined starting dose (i.e., 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 175 mg/kg; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 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 =5000 mg/kg.

[2]Percentage of the 10,000 test simulations that satisfied the specified condition for completion of testing (see OECD [2001a]; EPA [2002a]).

Summary of Animals Used and Animals Dead for UDP 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	3T3	0.12	Cyto	0.581	9.85	-0.21	0.6250	0.532	6.49	-0.12	0.6250	-2.2%	-1.9%
			Default	0.263	9.64			0.167	6.36				
		0.25	Cyto	0.560	10.45	-0.03	1.0000	0.515	6.87	-0.05	1.0000	-0.3%	-0.7%
			Default	0.188	10.42			0.163	6.82				
		0.50	Cyto	0.582	11.06	0.12	0.8750	0.541	7.30	-0.01	1.0000	1.1%	-0.1%
			Default	0.202	11.18			0.198	7.29				
		1.25	Cyto	0.559	11.45	0.20	0.6250	0.535	7.47	0.05	1.0000	1.7%	0.6%
			Default	0.141	11.65			0.161	7.51				
		2.00	Cyto	0.513	11.31	0.09	0.6250	0.488	7.19	0.02	1.0000	0.8%	0.3%
			Default	0.116	11.40			0.136	7.21				
				Average Difference		0.03		Average Difference		-0.02			
	NHK	0.12	Cyto	0.773	10.35	-0.80	0.6250	0.632	6.77	-0.44	0.6250	-8.3%	-7.0%
			Default	0.284	9.56			0.176	6.33				
		0.25	Cyto	0.614	10.66	-0.30	0.8750	0.538	7.02	-0.22	0.8750	-2.9%	-3.2%
			Default	0.259	10.36			0.190	6.80				
		0.50	Cyto	0.550	11.24	-0.13	0.8750	0.512	7.45	-0.18	0.8750	-1.2%	-2.5%
			Default	0.247	11.11			0.226	7.27				
		1.25	Cyto	0.510	11.60	0.03	0.8750	0.506	7.59	-0.08	0.8750	0.2%	-1.0%
			Default	0.174	11.62			0.189	7.51				
		2.00	Cyto	0.493	11.42	-0.02	0.8750	0.479	7.30	-0.09	0.8750	-0.2%	-1.3%
			Default	0.149	11.40			0.150	7.20				
				Average Difference		-0.24		Average Difference		-0.20			
2	3T3	0.12	Cyto	0.423	8.84	-0.35	0.3054	0.396	5.48	-0.36	0.1677	-4.1%	-6.9%
			Default	0.307	8.49			0.250	5.13				
		0.25	Cyto	0.422	9.54	-0.52	0.0942	0.390	5.88	-0.44	0.0942	-5.7%	-8.1%
			Default	0.214	9.02			0.204	5.44				
		0.50	Cyto	0.449	10.13	-0.58	0.1272	0.406	6.21	-0.49	0.1272	-6.1%	-8.6%
			Default	0.218	9.55			0.205	5.72				
		1.25	Cyto	0.416	10.60	-0.54	0.1099	0.390	6.42	-0.50	0.1099	-5.3%	-8.4%
			Default	0.227	10.07			0.213	5.92				
		2.00	Cyto	0.335	10.61	-0.47	0.1272	0.330	6.31	-0.44	0.1272	-4.7%	-7.4%
			Default	0.174	10.13			0.175	5.88				
				Average Difference		-0.49		Average Difference		-0.44			
	NHK	0.12	Cyto	0.423	8.74	-0.23	0.4548	0.434	5.40	-0.27	0.3054	-2.7%	-5.3%
			Default	0.287	8.51			0.239	5.13				
		0.25	Cyto	0.434	9.64	-0.62	0.0803	0.442	5.90	-0.47	0.1677	-6.9%	-8.6%
			Default	0.175	9.02			0.188	5.43				
		0.50	Cyto	0.465	10.25	-0.71	0.1099	0.460	6.25	-0.54	0.1465	-7.4%	-9.4%
			Default	0.158	9.54			0.183	5.71				
		1.25	Cyto	0.445	10.70	-0.61	0.1099	0.447	6.46	-0.53	0.1099	-6.1%	-9.0%
			Default	0.182	10.08			0.194	5.92				
		2.00	Cyto	0.364	10.70	-0.57	0.0681	0.385	6.35	-0.48	0.0803	-5.6%	-8.2%
			Default	0.147	10.13			0.164	5.87				
				Average Difference		-0.55		Average Difference		-0.46			

Summary of Animals Used and Animals Dead for UDP 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	3T3	0.12	Cyto	0.255	7.32	-0.61	0.0522	0.213	4.02	-0.60	0.0161	-9.1%	-17.5%
			Default	0.212	6.71			0.133	3.42				
		0.25	Cyto	0.269	8.07	-0.78	0.0093	0.221	4.42	-0.66	0.0068	-10.8%	-17.4%
			Default	0.138	7.28			0.094	3.76				
		0.50	Cyto	0.274	8.71	-0.94	0.0093	0.220	4.75	-0.70	0.0068	-12.2%	-17.3%
			Default	0.094	7.76			0.077	4.05				
		1.25	Cyto	0.193	9.35	-0.79	0.0049	0.170	5.04	-0.58	0.0068	-9.2%	-13.1%
			Default	0.059	8.56			0.056	4.45				
		2.00	Cyto	0.120	9.54	-0.48	0.0068	0.128	5.10	-0.42	0.0122	-5.3%	-8.9%
			Default	0.038	9.07			0.047	4.69				
				Average Difference		-0.63		Average Difference		-0.53			
	NHK	0.12	Cyto	0.256	7.24	-0.54	0.1514	0.193	3.88	-0.46	0.0923	-8.0%	-13.6%
			Default	0.217	6.70			0.136	3.42				
		0.25	Cyto	0.260	7.77	-0.49	0.0425	0.193	4.16	-0.40	0.0771	-6.7%	-10.6%
			Default	0.165	7.29			0.107	3.76				
		0.50	Cyto	0.228	8.38	-0.58	0.0342	0.178	4.47	-0.41	0.0923	-7.5%	-10.1%
			Default	0.102	7.79			0.080	4.06				
		1.25	Cyto	0.136	9.07	-0.46	0.0342	0.130	4.80	-0.33	0.0771	-5.3%	-7.3%
			Default	0.056	8.62			0.058	4.48				
		2.00	Cyto	0.086	9.40	-0.31	0.0122	0.102	4.94	-0.25	0.1099	-3.4%	-5.3%
			Default	0.035	9.09			0.048	4.69				
				Average Difference		-0.47		Average Difference		-0.37			
4	3T3	0.12	Cyto	0.179	6.73	0.80	0.0092	0.053	3.30	0.15	0.0739	10.7%	4.3%
			Default	0.259	7.53			0.079	3.44				
		0.25	Cyto	0.173	7.34	0.69	0.0092	0.050	3.58	0.09	0.0386	8.6%	2.4%
			Default	0.224	8.03			0.057	3.66				
		0.50	Cyto	0.180	7.86	0.77	0.0092	0.052	3.80	0.12	0.0507	8.9%	3.1%
			Default	0.227	8.63			0.055	3.93				
		1.25	Cyto	0.144	8.64	0.59	0.0092	0.050	4.16	0.02	0.2744	6.4%	0.4%
			Default	0.147	9.23			0.020	4.18				
		2.00	Cyto	0.104	9.03	0.41	0.0052	0.043	4.34	-0.06	0.1167	4.3%	-1.4%
			Default	0.084	9.44			0.018	4.28				
				Average Difference		0.65		Average Difference		0.06			
	NHK	0.12	Cyto	0.202	6.92	0.61	0.0934	0.098	3.41	0.03	0.3484	8.2%	1.0%
			Default	0.256	7.53			0.077	3.44				
		0.25	Cyto	0.189	7.43	0.63	0.0443	0.076	3.64	0.04	0.0833	7.8%	1.0%
			Default	0.216	8.06			0.056	3.68				
		0.50	Cyto	0.201	7.92	0.73	0.0250	0.076	3.86	0.08	0.1046	8.4%	2.0%
			Default	0.226	8.65			0.056	3.94				
		1.25	Cyto	0.168	8.65	0.59	0.0155	0.067	4.20	-0.01	0.3755	6.4%	-0.3%
			Default	0.147	9.24			0.021	4.19				
		2.00	Cyto	0.123	9.02	0.43	0.0155	0.056	4.37	-0.08	0.0934	4.6%	-1.8%
			Default	0.087	9.45			0.017	4.29				
				Average Difference		0.60		Average Difference		0.01			

Summary of Animals Used and Animals Dead for UDP 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	3T3	0.12	Cyto	0.287	7.12	2.07	0.0020	0.039	3.19	0.13	0.0840	22.5%	4.0%
			Default	0.220	9.19			0.042	3.32				
		0.25	Cyto	0.228	8.01	2.39	0.0020	0.038	3.43	0.17	0.0488	23.0%	4.8%
			Default	0.145	10.40			0.074	3.60				
		0.50	Cyto	0.186	8.45	2.36	0.0020	0.047	3.52	0.16	0.0488	21.8%	4.4%
			Default	0.091	10.81			0.071	3.68				
		1.25	Cyto	0.133	8.55	2.21	0.0020	0.035	3.62	0.29	0.0020	20.6%	7.3%
			Default	0.061	10.76			0.034	3.91				
		2.00	Cyto	0.105	8.64	1.81	0.0020	0.027	3.75	0.26	0.0020	17.4%	6.5%
			Default	0.051	10.46			0.019	4.01				
				Average Difference		2.17		Average Difference		0.20			
	NHK	0.12	Cyto	0.335	7.31	1.90	0.0020	0.048	3.22	0.11	0.3223	20.6%	3.3%
			Default	0.219	9.21			0.057	3.33				
		0.25	Cyto	0.301	8.17	2.21	0.0020	0.047	3.44	0.16	0.2324	21.3%	4.4%
			Default	0.114	10.38			0.081	3.60				
		0.50	Cyto	0.224	8.62	2.16	0.0020	0.039	3.56	0.11	0.2754	20.1%	3.1%
			Default	0.065	10.79			0.077	3.67				
		1.25	Cyto	0.148	8.73	2.01	0.0020	0.038	3.67	0.22	0.0039	18.7%	5.6%
			Default	0.051	10.74			0.041	3.89				
		2.00	Cyto	0.114	8.78	1.66	0.0020	0.036	3.79	0.21	0.0020	15.9%	5.3%
			Default	0.039	10.44			0.023	4.00				
				Average Difference		1.99		Average Difference		0.16			
6	3T3	0.12	Cyto	0.596	5.75	1.99	0.0005	0.327	0.91	-0.06	0.0923	25.7%	-7.5%
			Default	0.575	7.74			0.300	0.84				
		0.25	Cyto	0.574	6.61	2.02	0.0005	0.335	1.40	-0.10	0.0015	23.4%	-8.1%
			Default	0.529	8.63			0.305	1.29				
		0.50	Cyto	0.411	7.69	2.15	0.0005	0.258	2.10	-0.08	0.0068	21.8%	-3.7%
			Default	0.335	9.83			0.253	2.02				
		1.25	Cyto	0.241	8.42	2.46	0.0005	0.125	2.98	0.21	0.0010	22.6%	6.6%
			Default	0.062	10.88			0.123	3.19				
		2.00	Cyto	0.194	8.47	2.31	0.0005	0.088	3.29	0.33	0.0005	21.4%	9.0%
			Default	0.021	10.78			0.069	3.62				
				Average Difference		2.19		Average Difference		0.06			
	NHK	0.12	Cyto	0.588	5.79	1.84	0.0002	0.310	0.85	-0.06	0.0327	24.1%	-7.7%
			Default	0.548	7.63			0.285	0.79				
		0.25	Cyto	0.561	6.72	1.87	0.0002	0.318	1.36	-0.11	0.0012	21.8%	-8.9%
			Default	0.486	8.59			0.283	1.25				
		0.50	Cyto	0.413	7.85	1.97	0.0002	0.247	2.11	-0.11	0.0046	20.1%	-5.4%
			Default	0.309	9.83			0.232	2.00				
		1.25	Cyto	0.240	8.56	2.31	0.0002	0.121	3.02	0.16	0.0061	21.2%	5.0%
			Default	0.059	10.87			0.115	3.18				
		2.00	Cyto	0.194	8.57	2.21	0.0002	0.085	3.33	0.30	0.0005	20.5%	8.2%
			Default	0.021	10.78			0.063	3.62				
				Average Difference		2.04		Average Difference		0.03			

Abbreviations: UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); 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., the LD_{50} predicted by the NRU IC_{50} in the RC rat-only weight regression (log LD_{50} [mmol/kg] = 0.372 log IC_{50} [mM] + 2.024); Default=Default starting dose of 175 mg/kg; Std. Error=Standard error for number of animals; 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; RC=Registry of Cytotoxicity.

[1]For 10,000 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 =5000 mg/kg.

[2]Mean number of animals for 10,000 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 Based on Simulated UDP LD_{50}[1]

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with NHK NRU-Based Starting Dose									
	1	**2**	**3**	**4**	**5**	**6**	**Total**	**Category Match**	**Higher NRU Category**	**Lower NRU Category**
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	15	1	0	17	88%	6%	6%
5	0	0	0	0	22	0	22	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	15	23	0	68	96%	1%	3%

GHS Category Based on LD_{50} Outcome with Default Starting Dose	GHS Category Based on LD_{50} Outcome with 3T3 NRU-Based Starting Dose									
	1	**2**	**3**	**4**	**5**	**6**	**Total**	**Category Match**	**Higher NRU Category**	**Lower NRU Category**
1	4	0	0	0	0	0	4	100%	0%	0%
2	0	13	0	0	0	0	13	100%	0%	0%
3	0	1	11	0	0	0	12	92%	0%	8%
4	0	0	1	14	2	0	17	82%	12%	6%
5	0	0	0	0	21	0	21	100%	0%	0%
6	0	0	0	0	0	0	0	NA	0%	NA
Total	4	14	12	14	23	0	67	94%	3%	3%

Abbreviations: NRU=Neutral red uptake; GHS=Globally Harmonized System of Classification and Labelling of Chemicals (UN 2005); UDP=Up-and-Down Procedure (OECD 2001a, EPA 2002a); 3T3= BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes; NA=Not applicable; RC=Registry of Cytotoxicity.

[1]For 10,000 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 =5000 mg/kg. The NRU-based starting dose was 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 = 175 mg/kg. Shaded cells are those containing the correct predictions.

[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

Discordant Substances for GHS Category Outcomes of UDP Simulations[1]

NRU Test Method	Substance	NRU-Based Starting Dose[2]		Default Starting Dose[3]		LD_{50} Difference
		LD_{50}	Toxcat[4]	LD_{50}	Toxcat[4]	
3T3	Acetaminophen	2146.93	5	1768.39	4	-378.54
	Caffeine	297.82	3	342.76	4	44.95
	Procainamide HCl	2000.24	5	1529.98	4	-470.26
	Sodium dichromate dihydrate	44.48	2	52.17	3	7.69
NHK	Acetaminophen	2171.18	5	1755.21	4	-415.96
	Caffeine	292.06	3	353.96	4	61.91
	Sodium dichromate dihydrate	45.85	2	51.91	3	6.06

Abbreviations: Toxcat=Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2005); UDP= Up-and-Down Procedure (OECD 2001a, EPA 2002a); NRU=Neutral red uptake; 3T3=BALB/c 3T3 mouse fibroblasts; NHK=Normal human epidermal keratinocytes.

[1]Substances for which the simulated UDP outcome (in terms of GHS category) at the NRU-based starting dose did not match the simulated UDP outcome at the default starting dose. Simulations were performed with 10,000 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 =5000 mg/kg.

[2]NRU-based starting dose was 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).

[3]The default starting dose = 175 mg/kg. Shaded cells are those containing the correct predictions.

[4]

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