



October 29, 2010

Via E-Mail

Thomas Armitage, Ph.D.
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EPA Science Advisory Board Staff Office
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Dear Dr. Armitage:

On behalf of the American Chemistry Council (ACC), I am writing to alert you to persistent errors in the revised version of Table 5-21 from EPA's *Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments* (EPA's Reanalysis). EPA revised the table to address errors previously identified by Dr. Lesa Aylward and discussed in ACC's September 20, 2010, comments to EPA. These errors were also referenced by Dr. Glenn Rice during his October 27, 2010, remarks to the SAB Dioxin Review Panel. Dr. Aylward has now reviewed the revised table and, as indicated in the appended document, has identified persistent errors and discrepancies.

Importantly, these errors and discrepancies raise significant data quality issues. EPA's Reanalysis simply cannot represent a rigorous standard of quality if the underlying scientific information is inaccurate. Ms. Becki Clark noted in her opening remarks on October 27, 2010, that the outcome of the SAB review is expected to be a scientifically justified document. That outcome, however, is unachievable if the data underlying EPA's Reanalysis falls short of Data Quality Act guidelines.

Thank you for your attention to this important issue.

Sincerely,

David B. Fischer
Assistant General Counsel

cc: Dr. Vanessa Vu
Dr. Timothy Buckley



Comments on Revised Table 5-21

Additional comments based on revised Table 5-21 (REVISED 10-1-2010).

Oxidative stress, Cytochrome C reductase, 90-days

The identification of NOEL and LOEL dose rates in Appendix H, p. H-1, do not correspond to those presented in Hassoun et al. 2000. Following is the Hassoun data table:

Table 1
Effects of TCDD, PeCDF and PCB126 on the production of superoxide anion by hepatic and brain tissues of rats*

Compound	Dose ng/kg/day	nMoles cytochrome c reduced/ mg protein/min	
		Liver	Brain
TCDD			
	Control	0.146 ± 0.027 ^a	0.128 ± 0.027 ^a
	3	0.177 ± 0.022 ^a	0.165 ± 0.024 ^a
	10	0.191 ± 0.023 ^a	0.243 ± 0.041 ^b
	22	0.271 ± 0.023 ^b	0.289 ± 0.034 ^b
	46	0.388 ± 0.026 ^c	0.255 ± 0.024 ^b
	100	0.444 ± 0.045 ^c	0.224 ± 0.026 ^b
PeCDF			
	Control	0.133 ± 0.008 ^a	0.126 ± 0.017 ^a
	6	0.240 ± 0.010 ^b	0.239 ± 0.021 ^b
	20	0.306 ± 0.021 ^c	0.347 ± 0.012 ^c
	44	0.328 ± 0.015 ^c	0.324 ± 0.023 ^c
	92	0.436 ± 0.023 ^d	0.313 ± 0.017 ^c
	200	0.450 ± 0.027 ^d	0.328 ± 0.024 ^c
PCB126			
	Control	0.150 ± 0.017 ^a	0.114 ± 0.010 ^a
	10	0.149 ± 0.013 ^a	0.144 ± 0.018 ^a
	30	0.178 ± 0.020 ^a	0.178 ± 0.014 ^b
	100	0.195 ± 0.020 ^a	0.263 ± 0.032 ^c
	175	0.218 ± 0.004 ^a	0.257 ± 0.019 ^c
	300	0.243 ± 0.009 ^b	0.367 ± 0.021 ^d
	550	0.274 ± 0.024 ^b	0.692 ± 0.037 ^e
	1000	0.231 ± 0.023 ^b	0.647 ± 0.028 ^e

* Animals were treated with various doses of TCDD, PeCDF or PCB126 for 13 weeks, and they were terminated at the end of this period. Hepatic and brain tissues were collected and production of superoxide anion by those tissues was determined using the cytochrome c reduction assay. Each value represents the mean ± SD of six samples from six animals. Values with non identical superscripts within the columns presenting the effects in each tissue from each treatment group are significantly different ($P \leq 0.05$).

The NOEL is the 10 ng/kg-d group, the LOEL exposure group is the 22 ng/kg-d group.

In contrast, Appendix H-1 designates as a LOEL the 3 ng/kg-d group (footnote b):

5 H.1.1. Hassoun et al. (2000)

Endpoint	Administered Dose (ng/kg-day)					
	0	3	10	22	46	100
	Internal Dose (ng/kg blood) ^a					
	0 n = 6	1.94 n = 6	4.61 n = 6	8.15 n = 6	14.01 n = 6	25.34 n = 6
Cytochrome C reductase ^d	0.15 ± 0.07	0.18 ± 0.05 ^b	0.19 ± 0.06 ^c	0.27 ± 0.06 ^c	0.39 ± 0.06 ^c	0.44 ± 0.11 ^c
DNA single-strand breaks ^f	7.41 ± 1.54	10.78 ± 1.25 ^{b,c}	13.6 ± 1.69 ^c	15.3 ± 1.71 ^c	20.4 ± 2.25 ^c	23.5 ± 1.37 ^c
TBARs ^e	1.47 ± 0.29	1.55 ± 0.54 ^b	2.15 ± 0.36 ^c	2.28 ± 0.25 ^c	2.62 ± 0.52 ^c	2.29 ± 0.49 ^c

^aFrom the Emond PBPK model described in 3.3.

^bLOEL for selected endpoint.

^cStatistically significant as compared to control ($p < 0.05$).

^dValues are the mean ± SD. Data obtained from Table 1 in Hassoun et al. 2000.

^eValues are the mean ± SD. Data obtained from Table 2 in Hassoun et al. 2000.

^fValues are the mean ± SD. Data obtained from Table 3 in Hassoun et al. 2000.

As noted in my previous comments, the “SD” values presented here do not correspond to those presented in Hassoun et al. (see above).

Based on the Hassoun et al. analysis of their data and the modeled blood concentrations presented in Appendix H, the NOEL and LOEL rat whole blood concentrations should be 4.61 and 8.15, respectively. Using Appendix C.4.2 (5 yr model results), these whole blood concentrations correspond to HEDs of 2.7E-01 and 6.3E-01, respectively. In contrast, the Revised Table 5-21 entry is as follows:

Cytochrome C reductase, 90 days	7.0E-02	2.7E-01	1.2E-01 (Appendix H)	4E-09 ^e	Hassoun et al. (2000, 197431)
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These are not used as the basis for the RfD calculation, currently; however, the revised values would place the BMDL below the NOEL and therefore would necessitate a decision on whether to use the NOEL or the BMDL.

Hepatocellular Proliferation, Hepatocyte hypertrophy, “31 weeks”

Following is the entry from the Revised Table 5-21 for this endpoint:

Hepatocyte hypertrophy, 31 weeks	none	9.3E-02	1.7E-02 ^c (Appendix E)	6E-10 ^e	NTP (2006, 197605)
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The Appendix E-10 input dataset reflects the hypertrophy data from the NTP bioassay at the 2 year time point, not the 31 week time point. Here is the Appendix E report of the data, from p. E-10, note footnote e:

E.1.17. National Toxicology Program (2006)

Endpoint *	Administered Dose (ng/kg-day)					
	0	2.14 ^a	7.14	15.7	32.9	71.4
	Internal Dose (ng/kg blood) ^b					
	0	2.56	5.69	9.79	16.57	29.70
	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Gingival squamous hyperplasia	1/53 (2%)	7/54 (13%) ^d	14/53 (26%) ^e	13/53 (25%) ^e	15/53 (28%) ^e	16/53 (30%) ^e
Liver, hepatocyte hypertrophy	0/53 (0%)	19/54 (40%) ^e	19/53 (40%) ^e	42/53 (80%) ^e	41/53 (80%) ^e	52/53 (100%) ^e
Heart, cardiomyopathy	10/53 (19%)	12/54 (22%)	22/53 ^e (42%)	25/52 ^e (48%)	32/53 ^e (60%)	36/52 ^e (69%)
Liver, eosinophilic focus, multiple	3/53 (6%)	8/54 (15%)	14/53 (26%)	17/53 (32%)	22/53 (42%)	42/53 (79%)
Liver, fatty change, diffuse	0/53 (0%)	2/54 (4%)	12/53 ^e (23%)	17/53 ^e (32%)	30/53 ^e (57%)	48/53 ^e (91%)
Liver, necrosis	1/53 (2%)	4/54 (7%)	4/53 (8%)	8/53 ^d (15%)	10/53 ^e (19%)	17/53 ^e (32%)
Liver, pigmentation	4/53 (8%)	9/54 (17%)	34/53 ^e (64%)	48/53 ^e (91%)	52/53 ^e (98%)	53/53 ^e (100%)
Liver, toxic hepatopathy	0/53 (0%)	2/54 (4%)	8/53 (15%)	30/53 (57%)	45/50 (85%)	53/53 (100%)
Oval cell hyperplasia	0/53 (0%)	4/54 (10%) ^d	3/53 (10%)	20/53 (40%) ^e	38/53 (70%) ^d	53/53 (100%) ^e
Lung, alveolar to bronchiolar epithelial metaplasia (Alveolar epithelium, metaplasia, bronchiolar)	2/53 (4%)	19/54 ^e (35%)	33/53 ^e (62%)	35/52 ^e (67%)	45/53 ^e (85%)	46/52 ^e (89%)

^a LOAEL identified.

^b From the Emond PBPK model described in 3.3.

^c Statistically significant as compared to control ($p < 0.01$).

^d Statistically significant as compared to control ($p < 0.05$).

^e Data are for female rats in 2-year gavage study. Data for all endpoints obtained from Table A5b in NTP 2006.

I have confirmed that the numbers reported for hepatocyte hypertrophy here match the 2 year data in the NTP dataset.

Several issues are raised by this:

1. In the Revised Table 5-21, these data are designated as “31 week” timepoint, and as a result, the 5 yr model rather than lifetime model is applied to estimate HEDs corresponding to the LOEL and benchmark dose.

2. There is no NOEL (all dose groups are significant). So, the LOEL, based on Table E.1.17 above, is 2.56 ng/kg in blood. Using Appendix C.4.1, nongestational lifetime model tables, this corresponds to an HED of 1.4E-01 ng/kg-d.
3. The benchmark dose whole blood concentration modeling for this endpoint is presented on p. E-181:

1 **E.2.35. National Toxicology Program, 2006: Hepatocyte Hypertrophy, 2 Years**

2 **E.2.35.1. Summary Table of BMDs Modeling Results**

Model	Degrees of Freedom	χ^2 p-Value	AIC	BMD (ng/kg)	BMDL (ng/kg)	Notes
gamma	5	0.034	273.875	9.091E-01	7.868E-01	power bound hit (power = 1)
logistic	4	<0.001	297.895	2.475E+00	2.122E+00	negative intercept (intercept = -1.685)
log-logistic	4	0.006	279.210	1.137E+00	6.491E-01	
log-probit	5	0.006	277.800	1.530E+00	1.321E+00	
multistage, 5-degree ^a	4	0.018	275.693	9.272E-01	7.906E-01	
probit	4	<0.001	299.731	2.453E+00	2.137E+00	negative intercept (intercept = -0.985)
Weibull	5	0.034	273.875	9.091E-01	7.868E-01	power bound hit (power = 1)
gamma, unrestricted	4	0.027	275.270	error	error	unrestricted (power = 0.844)
log-probit, unrestricted	4	0.008	278.360	1.191E+00	7.038E-01	unrestricted (slope = 0.864)
Weibull, unrestricted	4	0.024	275.439	7.345E-01	3.588E-01	unrestricted (power = 0.92)

^a Best-fitting model, BMDs output presented in this appendix

The BMDL is 7.9E-01 ng/kg whole blood concentration (note that this is, as above, for the 2 yr endpoint).

Using Appendix C.4.1, nongestational lifetime, the corresponding HED is 2.3E-02 ng/kg-d. Presumably this would be selected as the POD. If the composite UF selected is the same as indicated in the Revised Table 5-21, the following results:

EPA Revised Table 5-21 entry (10-1-2010) reads:

Hepatocyte hypertrophy, 31 weeks	none	9.3E-02	1.7E-02 ^c (Appendix E)	6E-10 ^e	NTP (2006, 197605)
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Based on the corrections identified above, it would read:

Hepatocyte hypertrophy, 2 yrs	none	1.4E-01	2.3E-02 ^c	7E-09 ^e	NTP (2006)
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Hepatotoxicity, Labeling index, 31 weeks

Here is the entry from the Revised Table 5-21 (10-1-2010):

Labeling index, 31 weeks	none	9.3E-02	1.5E-01 ^c (Appendix H)	3E-10 ^e	NTP (2006, 197605)
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The LOEL HED of 9.3E-02 is selected as the POD, and a UF of 30 is designated (footnote e).

However, $9.3E-02 \text{ ng/kg-d}/30 = 3E-03 \text{ ng/kg-d}$, which is $3E-09 \text{ mg/kg-d}$, rather than $3E-10$ as included in the table.