MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Uranium (soluble forms)
CAS Numbers: Multiple
Date: July 2012
Profile Status: Draft 2, Postpublic Comment
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 14
Species: Mouse

Minimal Risk Level: 0.002 [X] mg/kg/day [ ] ppm [ ] mg/m^3

Reference: Domingo JL, Paternain JL, Llobet JM, et al. 1989c. The developmental toxicity of uranium in mice. Toxicology 55:143-152.

Experimental design: Groups of 20 pregnant Swiss mice were administered via gavage 0, 5, 10, 25, or 50 mg/kg/day uranyl acetate dihydrate (0, 2.8, 5.6, 14, or 28 mg U/kg/day) on gestation days 6–15. Body weights, food consumption, and general appearance were monitored daily. At termination, maternal liver and kidney weights were measured and uterine contents (number of implantation sites, resorptions, dead fetuses, and live fetuses) were evaluated. Live fetuses were evaluated for body weight, body length, sex, gross morphological abnormalities, visceral malformations, visceral anomalies (evaluated in 1/3 of fetuses), and skeletal defects (evaluated in 2/3 of fetuses).

Effect noted in study and corresponding doses: Significant decreases in maternal body weight were observed in all uranium groups; during the exposure period, the dams in the 2.6, 5.6, 14, and 28 mg U/kg/day groups weighed 33, 53, 75, and 88% less than controls, respectively. Significant decreases in food intake were also observed in the dams exposed to ≥5.6 mg U/kg/day. A significant decrease in the number of live fetuses was observed at 5.6 mg U/kg/day, but was not observed at the two higher dose levels. No significant alterations in the number of early or late resorptions, number of dead fetuses, or sex ratio were observed. Significant decreases in fetal body weight were observed at ≥2.8 mg U/kg/day and decreases in fetal length were observed at ≥5.6 mg U/kg/day. Significant increases in the incidences of external defects were observed at 2.8 mg U/kg/day. The alterations included cleft palate (significant at ≥5.6 mg U/kg/day) and hematomas (significant at 2.8 and 28 mg U/kg/day). The total number of skeletal defects was significantly increased at 14 and 28 mg U/kg/day; skeletal defects included bipartite sternebrae (significant at 2.8, 14, and 28 mg U/kg/day), some metatarsal of hindlimb poorly ossified (significant at 14 and 28 mg U/kg/day), delayed ossification of skull (significant at 14 and 28 mg U/kg/day), and caudal reduced ossification (significant at 14 and 28 mg U/kg/day).

Dose and end point used for MRL derivation:

[ ] NOAEL [ ] LOAEL [X] BMDL 0.2 mg U/kg/day for developmental toxicity

The results of the Domingo et al. (1989c) study suggest maternal body weight gain and fetal body weight and external and skeletal alterations as sensitive end points of uranium toxicity. It is possible that the developmental effects were secondary to the maternal toxicity; however, some of these effects may also be primary effects of uranium on the developing fetus. BMD modeling was used to identify potential points of departure for maternal and fetal end points. The maternal end point was decreased maternal body weight gain and the fetal end points included decreased fetal body weights and external and skeletal alterations. As summarized in Table A-2, there were significant increases in the incidence of litters with a particular types of external defect or skeletal defect and increases in the total number of litters with

external or skeletal defects. At all but the lowest dose tested, the increase in the incidence of external defects was primarily due to increases in the incidence of cleft palate. The incidence of hematomas does not appear to be dose-related. Thus, only the incidence of cleft palate was considered for BMD modeling. The skeletal defects consisted of increases in the incidence of bipartite sternebrae and reduced or delayed ossification in several locations (skull, caudal, hindlimb metatarsals, and proximal phalanges). Unfortunately, the investigators did not provide the information on the total number of litters with reduced or delayed ossification. To estimate potential points of departure for skeletal defects, the incidence data for bipartite sternebrae and the total incidence of skeletal defects were modeled.

Table A-2. Incidence of Litters with External or Skeletal Defects

Dose level (mg U/kg/day)	0	2.8	5.6	14	28
Number of litters	18	17	18	18	18
Cleft palate	0	2 (12%)	13[a] (72%)	13[a] (72%)	16[a] (89%)
Hematomas (dorsal or in facial area)	0	6[b] (35%)	2 (11%)	4 (22%)	8[b] (44%)
Total external defects	0	8[c] (47%)	14[a] (78%)	14[a] (78%)	17[b] (94%)
Bipartite sternebrae	0	6[c] (35%)	3 (17%)	9[a] (50%)	13[a] (72%)
Poor ossification of hindlimb metatarsal	4 (22%)	9 (53%)	15 (83%)	18[b] (100%)	18[a] (100%)
Poor ossification of proximal phalanges	2 (11%)	0	6 (33%)	13[b] (72%)	14[b] (78%)
Delayed skull ossification	0	0	3 (17%)	9[c] (50%)	12[c] (67%)
Reduced caudal ossification	4 (22%)	9 (53%)	12 (67%)	18[b] (100%)	18[b] (100%)
Total skeletal defects	4 (22%)	11 (65%)	15 (83%)	18[c] (100%)	18[c] (100%)

[a]Significantly different from controls ($p<0.01$).
[b]Significantly different from controls ($p<0.001$).
[c]Significantly different from controls ($p<0.05$).

Source: Domingo et al. 1989c

Data for the number of litters with cleft palate, bipartite sternebrae, and total skeletal defects (summarized in Table A-2) were analyzed using all available dichotomous models in the EPA BMDS (version 2.1.2) using the extra risk option. The multistage model was run for all polynomial degrees up to n-1 (where n is the number of dose groups including control). Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. For a given end point, the BMDL from the model with the lowest AIC (among all of the models meeting adequate fit criteria) was chosen. BMDs and lower bounds on the BMD (BMDLs) associated with a BMR of 5% extra risk for dichotomous data were calculated for all models and are presented in Table A-3.

Table A-3. Model Predictions for Developmental Effects in the Offspring of Mice Administered Uranyl Acetate via Gavage on Gestation Days 6–15 (Domingo et al. 1989c)

Model	AIC	χ^2 Goodness-of-fit	p-value[a]	BMD_{05} (mg U/kg/day)	$BMDL_{05}$ (mg U/kg/day)
Cleft palate					
Gamma[b]	78.21	8.69	0.0693	ND (GF)	ND (GF)
Logistic	92.12	18.44	0.0004	ND (GF)	ND (GF)
Log Logistic	**77.98**	**6.15**	**0.1047**	**0.75**	**0.20**
Log Probit	76.56	7.06	0.133	ND (LSR)	ND (LSR)
Multistage (1 degree polynomial)	78.21	8.69	0.0693	ND (GF)	ND (GF)
Multistage (2 degree polynomial)	78.21	8.69	0.0693	ND (GF)	ND (GF)
Multistage (3 degree polynomial)	78.21	8.69	0.0693	ND (GF)	ND (GF)
Multistage (4 degree polynomial)	78.21	8.69	0.0693	ND (GF)	ND (GF)
Probit	92.80	18.94	0.0003	ND (GF)	ND (GF)
Weibull[b]	78.21	8.69	0.0693	ND (GF)	ND (GF)
Quantal-Linear	78.58	8.4	0.078	ND (GF)	ND (GF)
Total skeletal defects					
Gamma[b]	63.69	0.24	0.889	0.39	0.12
Logistic	**61.85**	**0.46**	0.9275	**0.37**	**0.25**
Log Logistic	64.42	0.77	0.6808	0.84	0.12
Log Probit	64.02	0.49	0.7828	0.85	0.30
Multistage (1 degree polynomial)	61.87	0.29	0.9617	0.17	0.12
Multistage (2 degree polynomial)	63.54	0.14	0.9326	0.23	0.12
Multistage (3 degree polynomial)	63.45	0.07	0.9653	0.20	0.12
Multistage (4 degree polynomial)	63.40	0.03	0.9848	0.19	0.12
Probit	61.86	0.49	0.9205	0.36	0.26
Weibull[b]	63.64	0.21	0.902	0.33	0.12
Quantal-Linear	61.87	0.29	0.9617	0.17	0.12

Table A-3. Model Predictions for Developmental Effects in the Offspring of Mice Administered Uranyl Acetate via Gavage on Gestation Days 6–15 (Domingo et al. 1989c)

Model	AIC	χ^2 Goodness-of-fit	p-value[a]	BMD_{05} (mg U/kg/day)	$BMDL_{05}$ (mg U/kg/day)
Bipartite sternebrae					
Gamma[b]	94.61	7.49	0.0578	ND (GF)	ND (GF)
Logistic	97.86	7.44	0.0591	ND (GF)	ND (GF)
Log Logistic	**93.02**	**4.37**	**0.224**	**0.64**	**0.42**
Log Probit	97.82	8.65	0.0343	ND (GF)	ND (GF)
Multistage (1 degree polynomial)	94.61	7.49	0.0578	ND (GF)	ND (GF)
Multistage (2 degree polynomial)	94.61	7.49	0.0578	ND (GF)	ND (GF)
Multistage (3 degree polynomial)	94.61	7.49	0.0578	ND (GF)	ND (GF)
Multistage (4 degree polynomial)	94.61	7.49	0.0578	ND (GF)	ND (GF)
Probit	97.69	7.43	0.0595	2.80	2.11
Weibull[b]	94.61	7.49	0.0578	ND (GF)	ND (GF)
Quantal-Linear	94.61	7.49	0.0578	ND (GF)	ND (GF)

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.

AIC = Akaike Information Criteria; BMD = benchmark dose associated with the selected benchmark response of 5% extra risk; BMDL = 95% lower confidence limit on the BMC ND (GF) = not determined, goodness-of-fit criteria <0.10; ND (LSR) = not determined, largest scaled residual >2

The fetal body weight data and the maternal body weight gain data, summarized in Table A-4 were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS, version 2.1.2). The following procedure for fitting continuous data was used: the simplest model (linear) was first applied to the data while assuming constant variance; if the data were consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means was evaluated and the polynomial, power, and Hill models were fit to the data while assuming constant variance. Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the BMDL from the model with the lowest AIC was chosen. If the test for constant variance was negative, then the linear model was run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provided an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means was evaluated and the polynomial, power, and Hill models were fit to the data and evaluated while the variance model was applied. Model fit and point of departure selection proceeded as described earlier. If the test for constant variance was negative and the nonhomogenous variance model did not provide an adequate fit to the variance data, then the data set was considered unsuitable for modeling. For all fetal body weight models, a BMR of 5% relative deviation was used; a BMR of 10% was used for all maternal body weight gain models. Although the Hill model with constant variance or nonconstant variance

provided an adequate fit to means for the fetal body weight data, the models did not provide adequate fit to the variance and were not considered suitable for identifying a point of departure for an MRL. None of the available models provided adequate fit for the maternal body weight gain data.

Table A-4. Maternal Body Weight Gain and Fetal Body Weights

Dose level (mg U/kg/day)	0	2.8	5.6	14	28
Maternal body weight gain on gestation days 6-15 (g) ±standard deviation	14.5±6.6	9.7±1.8[a]	6.8±9.5[a]	3.6±8.4[b]	1.8±6.2[c]
Fetal body weight (g) ±standard deviation	1.40±0.15	1.04±0.25[a]	0.93±0.24[a]	0.84±0.11[a]	0.77±0.17[a]

[a]Significantly different from controls ($p<0.001$).
[b]Significantly different from controls ($p<0.05$).
[c]Significantly different from controls ($p<0.01$).

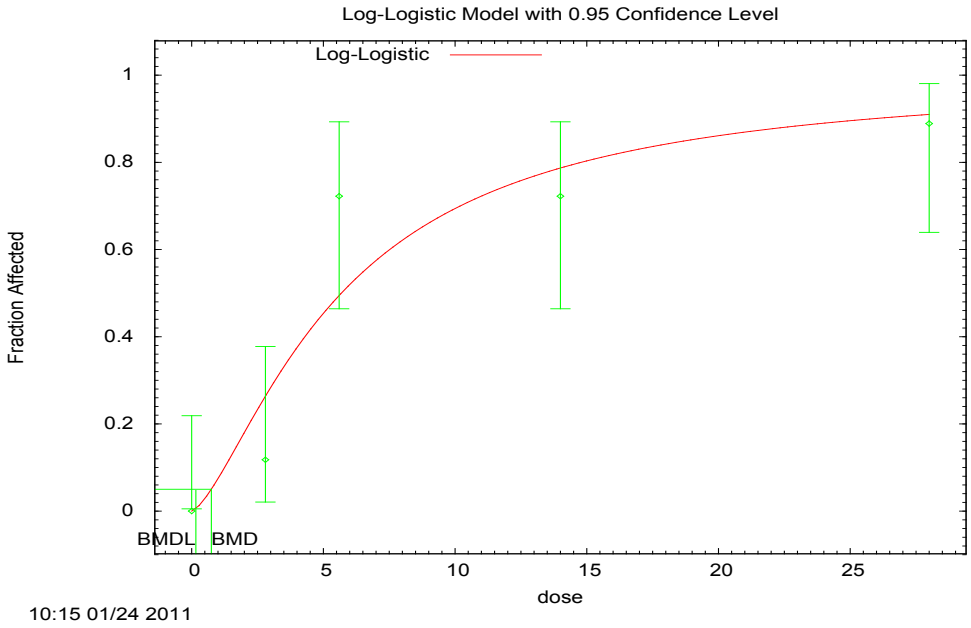
Source: Domingo et al. 1989c

The potential points of departure for the acute-duration oral MRL are summarized in Table A-5. The $BMDL_{05}$ values for external and skeletal defects ranged from 0.20 to 0.42 mg U/kg/day and the LOAEL value for the maternal and fetal body weight effects was 2.8 mg U/kg/day. The $BMDL_{05}$ of 0.20 mg U/kg/day for cleft palate was selected as the basis of the MRL. Because this value is lower than the other potential points of departure, it is likely to be protective for these effects. The fit of the log logistics model to the cleft palate data is presented in Figure A-2.

Table A-5. Summary of Potential Points of Departure for an Acute-Duration Oral MRL

Effect	Point of departure (mg U/kg/day)	Source
Cleft palate	0.20	$BMDL_{05}$ (log logistic model)
Total skeletal defects	0.25	$BMDL_{05}$ (logistic model)
Bipartite sternebrae	0.42	$BMDL_{05}$ (log logistic model)
Fetal body weight	0.28	LOAEL/uncertainty factor of 10
Maternal body weight gain	0.28	LOAEL/uncertainty factor of 10

Figure A-2. Predicted (Log Logistic Model) and Observed Incidence of Cleft Palate*



*BMD and BMDL indicated are associated with 5% extra risk and are in units of mg U/kg/day.

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[x] 10 for extrapolation from animals to humans
[x] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: There are limited human data on the oral toxicity of uranium. Signs of gastrointestinal irritation (nausea, vomiting, diarrhea) were observed in a subject ingesting 14.3 mg U/kg as uranyl nitrate in drinking water (Butterworth 1955); other potential targets of toxicity were not examined. Acute oral exposure studies in rats and mice have examined the lethality, systemic toxicity, neurotoxicity, and developmental toxicity of uranium. Information on the systemic toxicity is limited to two single-exposure toxicity study in rats (Domingo et al. 1987) and mice (Martinez et al. 2003) administered lethal doses and a repeated exposure study in mice (Ozmen and Yurekli 1998). In the 2 weeks following administration of a single gavage dose of 118 mg U/kg as uranyl acetate to rats, significant increases in urine volume (in the absence of changes in water consumption), plasma creatinine and urea, and urinary total protein and creatinine were observed; hyperemia and microhemorrhagic foci were also observed in the liver and kidneys at the end of the 2-week observation period (Domingo et al. 1987). In mice, administration of 166 mg U/kg as uranyl nitrate resulted in increases in blood urea and creatinine levels and proximal tubular necrosis (Martinez et

al. 2003). Similarly, significant increases in BUN and creatinine levels were observed in mice exposed to 508 mg U/kg/day as uranyl acetate in the diet for 5 days (Ozmen and Yurekli 1998); the study did not include a histological examination of the kidney or other tissues. Neurological effects consisted of increased motor activity (Briner and Murray 2005) and increased open field activity (Briner 2009) in mice administered 28 or 6 mg U/kg/day, respectively, as depleted uranyl acetate in drinking water for 2 weeks; exposure to 28 mg U/kg/day also resulted in a 53% decrease in body weight gain. Gestational exposure to ≥2.8 mg U/kg/day as uranyl acetate resulted in significant decreases in fetal body weights and increases in the occurrence hematomas in the fetuses of mice exposed on gestation days 6–15 (Domingo et al. 1989a); increases in the incidence of cleft palate were observed at ≥5.6 mg U/kg/day. Decreases in maternal body weight gain were observed at ≥2.8 mg U/kg/day. Exposure of neonatal rats (1 or 7 days of age) to 42.7 mg U/kg/day as uranyl nitrate administered via gavage in water, resulted in significant reductions in bone formation, increases in bone resorption, and diminished tooth development (Pujadas Bigi et al. 2003).

Agency Contacts (Chemical Managers): Sam Keith, Obaid Faroon, Nickolette Roney, Franco Scinicariello, Sharon Wilbur