APPENDIX A

Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake

Animal	Endpoint	Study	Estimated body burden (ng/kg)			Human equiv.[a] intakes (pg/kg/day)
			LOAEL	NOAEL	ED01	
Rats	Cancer	Kociba et al. (1978)[1]	180	18	32	60; 6; 11
Rhesus monkeys	Fetal Mortality	Bowman et al. (1989)[2]	90	21	NC	30; 7
	Developmental Neurotoxicity	Schantz et al. (1992)[3]	21	–	NC	7
	Endometriosis	Rier et al. (1993)[4]	21	–	NC	7
Rats	Reproductive Tox. (multigenerational)	Murray et al. (1979)[5]	180	18	NC	60; 6
Rats	Developmental/ Reproductive Toxicity	Mably et al. (1992a, b, c)[6]	38	–	0.34	13; 0.1
		Gray et al. (1997)[7]	30	–	0.08	10; 0.03
		Faqi et al. (1998)[8]	25	–	0.6	8; 0.2
		Ohsako et al. (2001)[9]	30	8	NC	10; 3
Rats	Developmental Immunotoxicity	Gehrs and Smialowicz (1999)[10]	60	–	NC	20
Rats	Developmental Neurotoxicity	Markowski et al. (2001)[11]	108	36[b]	0.7	36; 12; 0.2
Mice	Immunological Effects (adult)	Burleson et al. (1996)[12]	6	3	NC	2; 1
		Smialowicz et al. (1994)[13]	300	–	2.9	100; 1
		Narasimhan et al. (1994)[14]	100	50[b]	1.5	33; 17; 0.5
		Vecchi et al. (1983)[15]	1200	–	7	401; 2
Rats	Thyroid Effects	Sewall et al. (1995)[16]	76	22	26	25; 7; 8
Mice	CYP1A1/1A2 Enzyme Induction	DeVito et al. (1994)[17]	24	–	22	8; 7
		Diliberto et al. (2001)[18]	2.8	–	67	0.9; 22
		Vogel et al. (1997)[19]	5.1	0.51	0.003	1.6; 0.16; 0.001
		Narasimhan et al (1994)[14]	25	10	3	8; 3; 2; 1
Rats	CYP1A1/1A2 Enzyme Induction	van Birgelen et al. (1995)[20]	243	–	19	81; 6
		Schrenk et al. (1994)[21]	72	–	26	24; 9
		Sewall et al. (1995)[16]	8	2	3.5	3; 0.7; 1
		Walker et al. (1999)[22]	76	–	59	25; 20

Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

[a] Human equivalent intakes were estimated according to the following equation: daily intake (pg/kg/day) = (body burden (ng/kg)*Ln2*1000)/(t½*absorption) where t½ = 2593 days and absorption fraction = 0.8 (Poiger and Schlatter 1986; see Section II). Corresponding human equivalent intake values are arranged in sequence from the previous three columns.

[b] NOAEL values are based on the highest individual dose group in which there are no statistically significant changes. Statistically significant dose response trends plus apparent declines are also evident at all dose levels—20 and 60 ng/kg orally—in all fixed-ratio test groups in Markowski et al. (2001) and in the 50 ng/kg dose group in Narasimhan et al. (1994).

- - = No NOAEL value, as effects seen in the lowest dose group in the study.

NC = Not calculated due to insufficient dose response information (less than three doses and a control) or due to presentation of the data in graphical form without tabulation of mean and variance estimates.

1. **Kociba et al. (1978)**. Increased cancer in female Sprague-Dawley rats exposed for 2 years to TCDD in the food matrix. Statistical LOAEL and NOAEL body burden estimates modeled assuming 50% absorption from the food matrix and a 25-day half-life. Compare to measured lipid levels in the Kociba et al. (1978) rats of 540 and 1700 ppt at 1 and 10 ng/kg/day dose rates and to measured body burdens in the Hurst et al. (2000) subchronic 5/7 day gavage study in female Long-Evans rats of 19 and 120 ng/kg at 1 and 10 ng/kg/day dose rates. ED_{01} calculated for female rat tumors using a multistage formula and EPA Benchmark Dose Software result in an ED_{01} (LED_{01}) of 31.9 (22) ng/kg body burden using Kociba et al. (1978) data and Goodman and Sauer (1992) pathology.

Kociba et al. 1978: Tumors

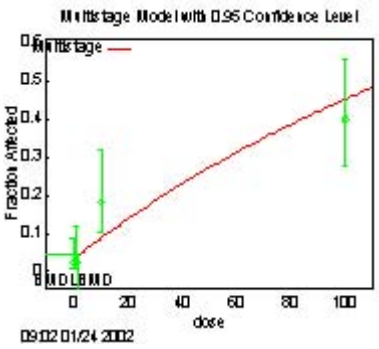
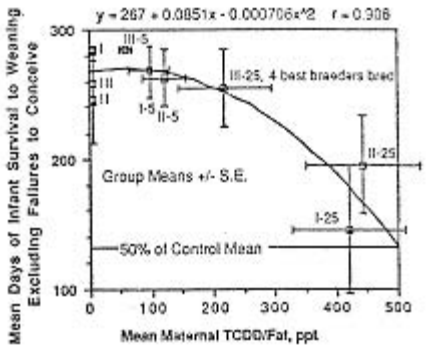


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

2. **Bowman et al. (1989)**. Offspring per cohort significantly reduced at the 25 ppt dose group in cohorts I and II (LOAEL) but not in the 5 ppt dose group (NOAEL; publication Fig. 5 attached). Estimated maternal body burdens are calculated at parturition of the 25 ppt cohort II group for the LOAEL (lowest value of 25 ppt cohorts I and II) and the 5 ppt cohort I for the NOAEL (highest value of 5 ppt cohorts I and II). Maternal TCDD fat levels are estimated according to the empirical formula and data supplied in Bowman et al. (1989; see publication figures 3 and 5): y = 14.9 + 4.29 x (r=0.924), where y=PCDD/fat ppt infant at weaning and x=TCDD/fat ppt mother at parturition. The measured TCDD fat levels in offspring ("y" value) of the 5 ppt cohorts I and II at parturition were 377±141 ppt and 323±70 ppt, respectively, resulting in estimated maternal fat levels at parturition of cohorts I and II of 84 and 72 ppt, respectively. Following the authors' recommendation, the fat level in the 25 ppt dose group is calculated following a 5:1 ratio to the 5 ppt groups, i.e., 420 and 360 ppt for cohorts I and II respectively. Measured maternal data in the 25 ppt dose group at the time of birth of cohort III (488 days post cessation of TCDD dose) were 335±119 ppt (3 non-bred females) and 219±75 ppt (all 7 monkeys) in fat. A 25% body lipid was assumed in converting to human equivalent body burden.

Bowman et al. 1989: Infant Survival



3. **Schantz et al. (1992)**. Increased rough-tumble play (publication Fig. 2 attached), fewer retreats during play bouts, and fewer displacements from preferred positions in the 5 ppt cohort I offspring. Maternal TCDD fat levels are estimated according to the empirical formula and data supplied in Bowman et al. (1989; see Figs. 3 and 5): y = 14.9 + 4.29 x (r=0.924), where y=PCDD/fat ppt infant at weaning and x=TCDD/fat ppt mother at parturition. The measured TCDD fat level in offspring ("y" value) of the 5 ppt cohort I group at parturition was 377±141 ppt, resulting in an estimated maternal fat level at parturition of 5 ppt cohort I of 84 ppt. Fat level converted to body burden by dividing by 4, approximating 25% body fat in a human equivalent comparison.

Schantz et al. 1992: Rough-tumble Play

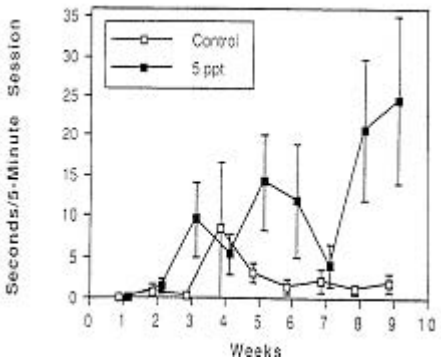
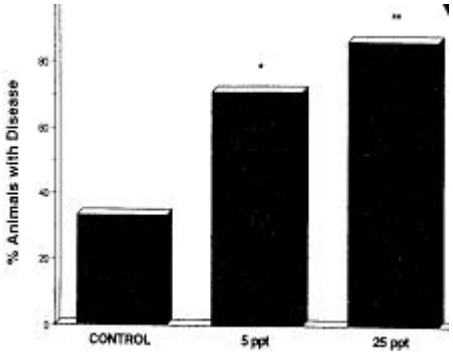


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

4. **Rier et al. (1993)**. Increased incidence, severity and dose-response for rhesus monkeys with endometriosis in the 5 and 25 ppt dose groups (rAFS classification; publication figure 2 attached, * $p<0.17$, ** $p<0.05$). LOAEL (no NOAEL) body burden adopted from the highest maternal fat level calculated according to the formula supplied by Bowman et al. (1989; see footnote 2) of 84 ppt for the 5 ppt dose group occurring at the parturition of cohort I. For comparison, the average of eight measured maternal fat levels at the birth of the 5 ppt cohort III (488 days post cessation of TCDD) was 54±11 ppt fat. A 25% body lipid was assumed in converting to human equivalent body burden.

Rier et al. 1993: Endometriosis



5. **Murray et al. (1979)**. Significant reductions in fertility (graph of publication table 1 data attached), litter size, gestation survival, and neonatal survival and growth in the 10 ng/kg/day food matrix maternal dose group in a three-generation reproduction study in Sprague-Dawley rats. Mathematically estimated body burden of 180 ng/kg at 10 ng/kg/day (half-life = 25 days, 50% absorption from food matrix). Comparison empirical measurements from a similar dose regimen in the related cancer study by Kociba et al. (1978) were 1700 ppt TCDD in lipid in the 10 ng/kg/day dose group, and the measured body burden in Hurst et al. (2000) subchronic 5/7 day gavage study in female Long-Evans rats was 120 ng/kg at the 10 ng/kg/day dose rate. The fertility index in the f_0 generation was so low that further studies with this dose group were discontinued. Thus, the study is essentially two dose levels and a control and was not modeled because of the limited dose response relationship data.

Murray et al. 1979: Rat Fertility Index

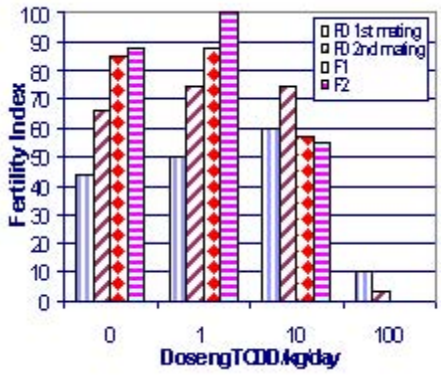
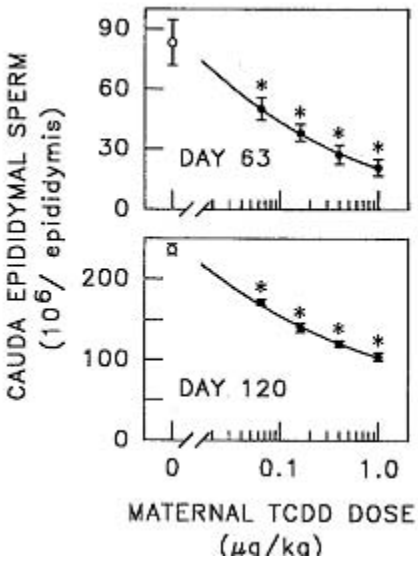


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

6. **Mably et al. (1992a,b,c)**. Decreased daily sperm production (publication Fig. 5 attached), cauda epididymal sperm, epididymis weights and altered sexual behavior in offspring at 64 ng/kg orally to Holtzman rat dams on gestation day 15. LOAEL (no NOAEL) body burden based on Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. ED_{01} value modeled for caudal sperm count of 0.34 ng/kg body burden at day 63 using EPA Benchmark Dose Software Version 1.3, 60% absorption. ED_{01} modeling of Mably et al. (1992) using EPA Benchmark Dose Software Version 1.3 results in a broad range of ED_{01}s, from 0.34 ng/kg for daily sperm production on PND 63 to 461 ng/kg for pinna detachment, with a median value of 3.1 ng/kg for 15 different endpoints.

Mably et al. 1992: Epididymal Sperm



7. **Gray et al. (1997)**. Decrease in ejaculated sperm numbers in male offspring, pooled results from two studies (see publication Fig. 1; results pooled with Gray et al. 1995; attached graph of data from publication text p.15) at 50 ng/kg single dose, day 15 of gestation to female Long-Evans rats. LOAEL (no NOAEL) body burden based on Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. ED_{01} modeling of Gray et al. (1997) using EPA Benchmark Dose Software Version 1.3, 60% absorption, results in a broad range of ED_{01}s from 0.08 ng/kg for epididymal sperm count on D49 to 327 ng/kg for daily sperm production on D49, with a median value of 80 ng/kg for 32 different endpoints.

Gray et al. 1997: Ejaculated Sperm

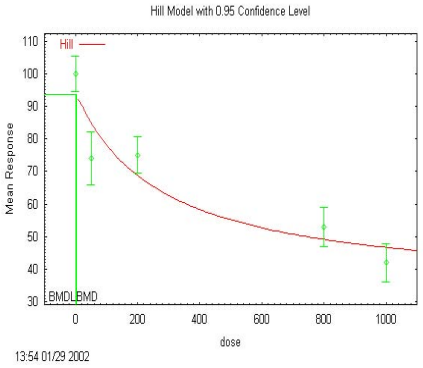
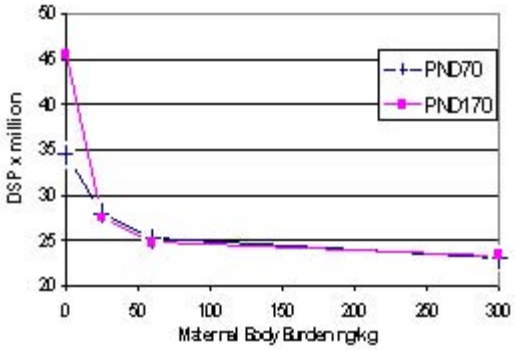


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

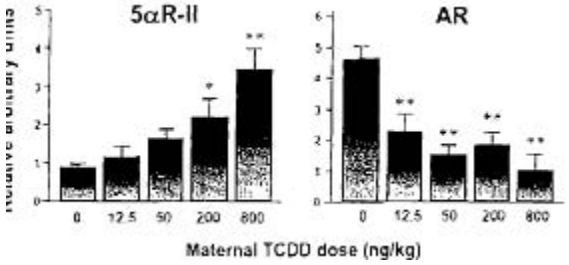
8. **Faqi et al. (1998)**. Decreased daily sperm production (graph attached of data from publication Table 3), cauda epididymus sperm, sperm transit rate, and percent abnormal sperm in offspring of 25/5 ng/kg (loading/weekly maintenance) maternal Wistar rat group. Maintenance dose of 5 ng/kg/week subcutaneous administered to maintain body burden of 25 ng/kg. Additional data on TCDD levels measured in maternal fat at gestation day 21 estimated from publication Figure 1 at 150 ng/kg in 25/5 group. Decreases in cauda epididymal sperm numbers (PND170) and daily sperm production (PND 70 and 170) were observed at all doses. In addition, increases in sperm transit rate and percent abnormal sperm were observed at all dose levels at PND 170. ED_{01} value of 0.6 ng/kg for decreases in daily sperm production, on PND70 and modeled using EPA Benchmark Dose Software Version 1.3.

Faqi et al. 1998; Daily Sperm Production



9. **Ohsako et al. (2001)**. Decreased ano-genital distance in male offspring of Holtzman rat dams receiving 50 ng/kg single dose or greater on gestation day 15 (publication Fig. 7 attached). NOAEL at 12.5 ng/kg single dose. Dose-dependent decreases in androgen receptor mRNA levels in ventral prostate in all dose groups (publication Fig. 8 attached). No changes in daily sperm production or sperm reserve. LOAEL/NOAEL body burdens based on Hurst et al. (2000) gestation day (GD) 16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. ED_{01} values for this study were not calculated because the significant data were not presented in tabular format.

Ohsako et al. 2001: Androgen Receptor



Ohsako et al. 2001: Ano-genital Distance

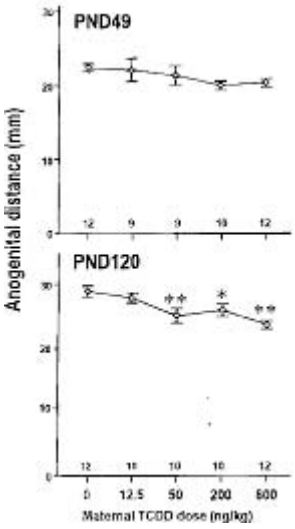
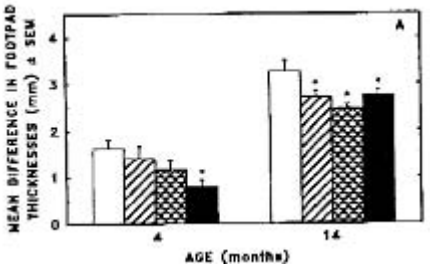


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

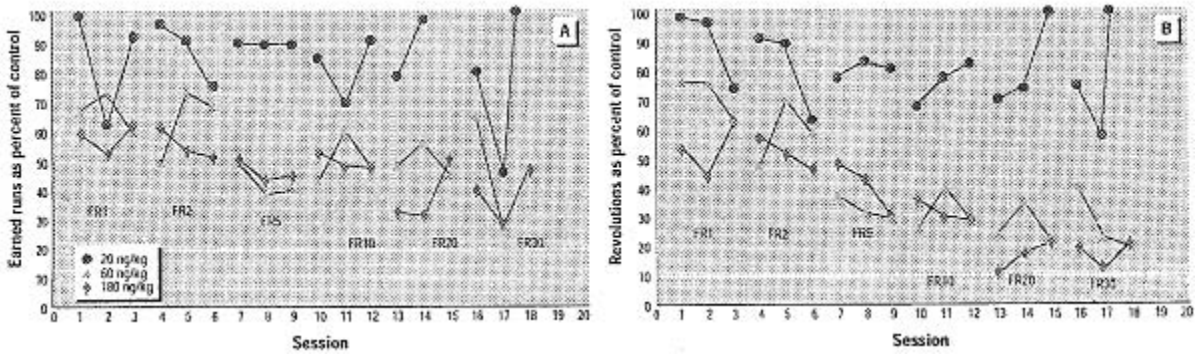
10. **Gehrs and Smialowicz (1999)**. Decreased delayed-type hypersensitivity (DTH; publication Fig. 2a attached; dose units for columns are 0, 100, 300, and 1000 ng/kg) in male offspring following single maternal oral dose of 100 ng/kg on gestation day 14 to F344 rats. LOAEL (no NOAEL) body burden based on Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. Benchmark dose analysis was not performed on this study because the data were presented in graphical format.

Gehrs and Smialowicz 1999: Delayed-Type Hypersensitivity



11 **Markowski et al. (2001)**. Perinatal TCDD exposure produced a significant dose-related reduction in the number of earned opportunities to run, lever response rate, and total number of revolutions in the wheel in offspring of Holtzman rats exposed to single oral TCDD doses on GD18. Statistically significant dose group effects at 180 ng/kg dose (LOAEL). NOAEL at 60 ng/kg dose group, where apparent declines are not statistically significant (see publication Fig. 2 attached; publication Table 3). ED_{01} results modeled by the authors. Table includes result for total wheel revolutions. Body burdens based on 180 and 60 ng/kg single oral doses and Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats.

Markowski et al. 2001: Operant Conditioning



12. **Burleson et al. (1996)**. Increased susceptibility to influenza infection challenge in B6C3F1 mice following 10 ngTCDD/kg (LOAEL) and higher single oral gavage dose to 8-week-old mice (publication Fig. 1 attached). No significant effects seen at 1 and 5 ng/kg doses (NOAEL). Assume 60% absorption. Benchmark dose analysis was not performed on study because the data were not presented in tabular format.

Burleson et al. 1996: Influenza Susceptibility

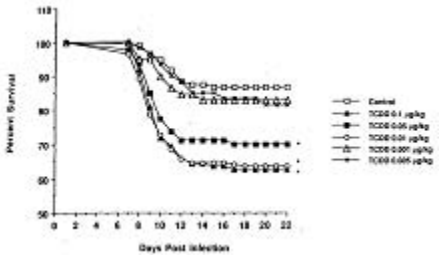
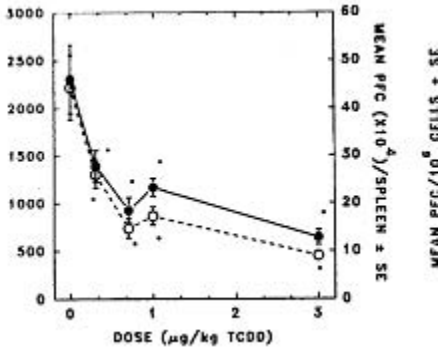


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

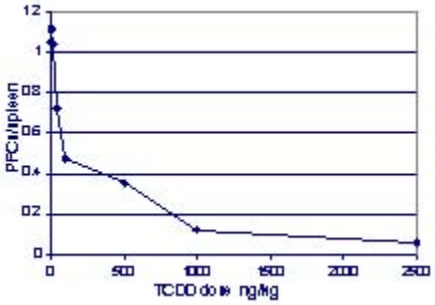
13. **Smialowicz et al. (1994)**. Dose-related suppression of antibody plaque forming cell (PFC; publication Fig. 1 attached) response in adult female B6C3F1 mice at 300 ng/kg single intraperitoneal injection and higher. PFC increases reported in high-dose-group male F344 and female Long-Evans rat species tested, accompanied by alterations to splenic $CD4^{-}CD8^{+}$ lymphocytes. ED_{01} values for mice calculated for plaque forming cells per million cells of 2.9 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

Smialowicz et al. 1994: PFC Immune Response



14. **Narasimhan et al. (1994)**. Decreased splenic antibody plaque-forming cell (PFC; graph of publication Table 5 data attached) response following single intraperitoneal dose administered to female B6C3F1 mice (7–9 weeks old). LOAEL for decreased SRBC and splenic PFC responses at 100 ng/kg, nonstatistically significant decrease evident at 50 ng/kg, NOAEL at 25 ng/kg. CYP1A1 LOAEL (NOAEL) at 25 (10) ng/kg dose (graph of publication table 1 data attached). ED_{01} values calculated for spleen PFC/million cells of 1.5 ng/kg body burden and for CYP1A1 mRNA induction of 3 ng/kg using EPA Benchmark Dose Software Version 1.3.

Narasimhan et al. 1994: PFC Immune Response



CYP1A1 mRNA Induction

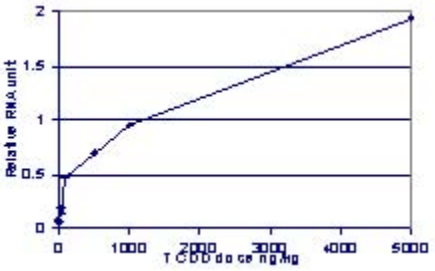
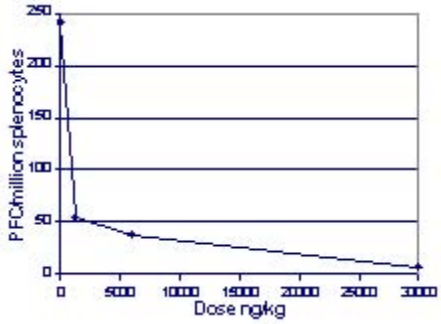


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

15. **Vecchi et al. (1983)**. Decreased plaque-forming cells (PFC) per million and PFC/spleen (graph of publication table 2 data attached) at all doses tested in aryl hydrocarbon hydroxylase sensitive mouse strains (B6, C3) following single intraperitoneal doses. Less sensitivity in other strains (e.g. DBA/2 and AKR). LOAEL (no NOAEL) of 1200 ng/kg. ED_{01} calculated for PFC/million splenocytes of 7 ng/kg for B6 mice using EPA Benchmark Dose Software Version 1.3.

Vecchi et al. 1983: PFC Immune Response



16. **Sewall et al. (1995)**. Statistically significant decreased ratio of thyroid parenchymal area to thyroid follicle area (publication Fig. 6 attached) was reported in female Sprague-Dawley rats following oral gavage biweekly dosing for 30 weeks at daily equivalent doses of 0.1–125 ng/kg/day. LOAEL (NOAEL) of 3.5 (1) ng/kg/day for thyroid parenchyma/follicle ratio, calculating to approximate body burdens of 76 and 22 ng/kg for the LOAEL and NOAEL, respectively. These calculations assume a half-life of 25 days and 60% body burden fraction following gavage dose, based on Hurst et al. (2000). Significant increases were also reported for thyroid stimulating hormone, with a LOAEL of 3.5 ng/kg/d and a NOAEL of 1 ng/kg/d. Serum thyroxine was significantly decreased at 10.5 ng/kg/day and at higher doses. ED_{01} values were not calculated for thyroid parenchymal/follicle ratio. ED_{01} for decreases in serum thyroxin of 43 ng/kg body burden using EPA Benchmark Dose Software Version 1.3. The ED_{01} for increased serum thyroid stimulating hormone is 26 ng/kg. LOAEL(NOAEL) for CYP1A1 mRNA induction of 0.35 (0.1) ng/kg/day, approximating to 8 (2) ng/kg body burden (assuming 60% absorption, 25 day halflife). ED_{01} for increases in CYP1A1 mRNA was 3.5 ng/kg using EPA Benchmark Dose Software Version 1.3.

Sewall et al. 1995: Thyroid Histology

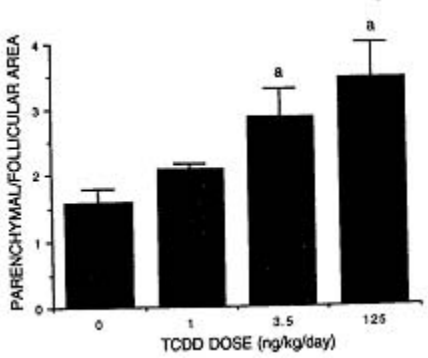
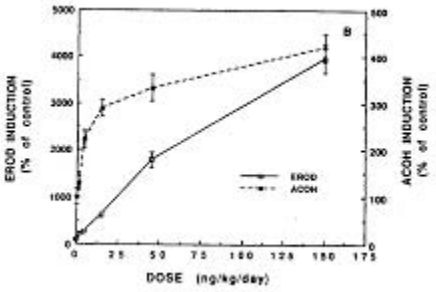


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

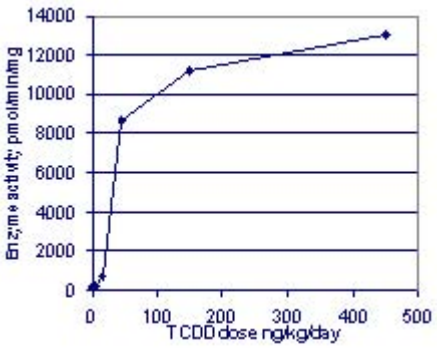
17. **DeVito et al. (1994)**. LOAEL (no NOAEL) of 1.5 ng/kg/day for induction of CYP1A1 and CYP1A2 (publication Fig. 2 attached) and increased phosphorylation of phosphotyrosyl proteins in female B6C3F1 mice gavage fed 1.5–150 ng/kg/day, 5 days per week, for 13 weeks. Approximate body burden after 13 weeks at 1.5 ng/kg/day of 24 ng/kg, based on Diliberto et al. (2001). ED_{01} value calculated at 22 ng/kg for CYP1A1 induction in the liver using EPA Benchmark Dose Software Version 1.3.

DeVito et al. 1994: Enzyme Induction



18. **Diliberto et al. (2001)**. Dose response relationship for CYP1A1 induction in female B6C3F1 mice (60 days old) at all oral gavage doses from 0.15 ng/kg/day (5/7 days, 13 weeks) and higher, corresponding to a radiolabel measured body burden of 2.75 ng/kg. Hepatic CYP1A1 activity (publication Table 5, graph of liver EROD data attached) modeled using EPA BMDS Software Version 1.3 results in an ED_{01} of 9.7 ng/kg/day. Body burden interpolated using linear regression of data from Diliberto et al. (2001; Table 4) with formula: body burden = 6.8782 * daily dose (M-F) (R^2 = 0.9994; Microsoft Excel), resulting in estimated ED_{01} body burden of 67 ng/kg.

Diliberto et al. 2001: EROD Induction



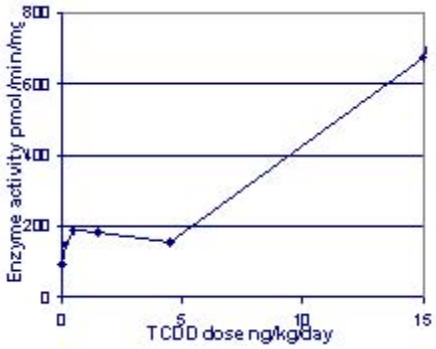
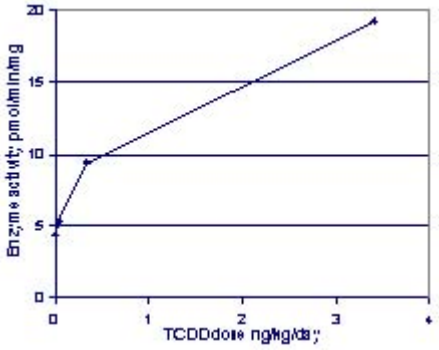


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

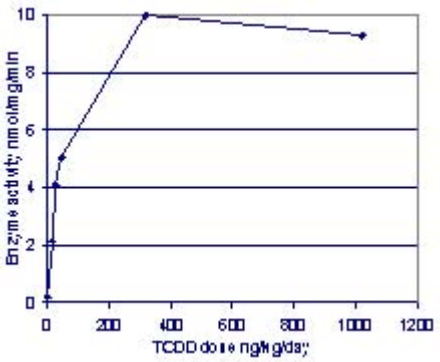
19. **Vogel et al. (1997)**. LOAEL(NOAEL) for CYP1A1 EROD induction (graph of publication Table 3 data attached) at 0.34 (0.034) ng/kg/day to C57 female mice administered 1, 10, 100 ng/kg loading doses followed by weekly injections of 0.2, 2, and 20 ng/kg for 135 days, calculating to 4.9 (0.49) ng/kg body burden (assuming 100% absorption, 10 day halflife). ED_{01} value calculated for CYP1A1 EROD induction of 0.003 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

Vogel et al. 1997: EROD Induction



20. **van Birgelen et al. (1995)**. Significant increases in CYP1A1 (graph of liver EROD data from publication table 3 attached) and CYP1A2, plus decreased relative thymus weights and loss of hepatic retinoids, at all doses tested in 8 week old female Sprague-Dawley rats exposed to dietary matrix intakes of 0, 0.2, 0.4, 0.7, 5, 20 µgTCDD/kg diet, corresponding to 0, 13.5, 26.4, 46.9, 320 and 1024 ng/kg/day oral intake. LOAEL (no NOAEL) calculated from 13.5 ng/kg/day dose to be 243 ng/kg body burden (50% absorption from dietary matrix, 25 day halflife). Calculated no effect levels (CNEL) by the authors of 0.7 to 4 ng TCDD/kg/day (Hill and Weibull models, based on the measured control value plus twice the standard deviation: mathematical calculated corresponding body burdens are 13 and 72 ng/kg at 50% absorption, halflife 25 days). Measured levels by authors in liver and fat of 1400 and 620 ppt, respectively. ED_{01} value calculated for CYP1A1 of 19 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

van Birgelen et al. 1995: EROD Induction



21. **Schrenk et al. (1994)**. CYP1A1 induction in female Wistar rats exposed via biweekly subcutaneous injection to average daily doses of 2, 20, and 200 ng/kg/day for 13 weeks. LOAEL (no NOAEL) for CYP1A1 EROD induction of 2 ng/kg/day (graph of publication table 1 data attached), approximating to 72 ng/kg body burden (25 day halflife, 100% absorption). ED_{01} for CYP1A1 induction of 26 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

Schrenk et al. 1994: EROD Induction

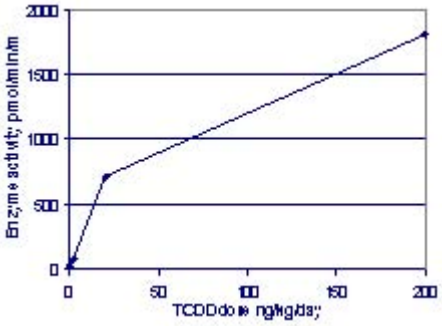


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

22. **Walker et al. (1999)**. Dose-dependent expression of CYP1A1 (graph of publication table 3 data attached) and CYP1A2 RNA (CYP1B1 less sensitive) in female Sprague-Dawley rats gavage fed biweekly for 30 weeks to average daily doses of 3.5 - 125 ng/kg/day. LOAEL value of 3.5 ng/kg/day (no NOAEL), calculates to 76 ng/kg body burden (assuming halflife of 25 days, 60% absorption following gavage). Measured liver level of 447 ng/kg. ED_{01} values calculated for CYP1A1 mRNA and CYP1A2 mRNA at 59 and 270 ng/kg body burdens, respectively, using EPA Benchmark Dose Software Version 1.3. High maximal induction potential of enzymes contributes to high 1% effective dose (ED_{01}).

Walker et al. 1999: CYP1A1 mRNA