Benchmark Doses for Tumors in Sprague Dawley Rats fed N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE)

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Introduction

The carcinogen risk assessment guidelines proposed by the U.S. Environmental Protection Agency (1999) recommend the use of a benchmark dose (BMD) approach for low dose cancer risk assessment. Unless stipulated otherwise, the BMD is the dose at which the excess lifetime tumor incidence is 10%, denoted by BMD_{10}. A value of 10% was selected as this is about the lowest incidence that can be estimated with adequate precision from typical chronic bioassays in rodents. Further, a lower 95% confidence limit is calculated for the benchmark dose ($BMDL_{10}$) to account for the experimental variation of the bioassay. The $BMDL_{10}$ is then used as a point-of-departure for low dose cancer risk assessment. When a nonlinear dose response curve is expected in the low dose range, a margin of exposure between the $BMDL_{10}$ and anticipated human exposure levels is considered. Otherwise, linear extrapolation from the $BMDL_{10}$ to zero is used for low dose cancer risk estimation. In either case, the $BMDL_{10}$ serves as the point-of-departure.

Bioassay Data

The data used for calculation of the $BMDL_{10}$ were collected in the 104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats. The BMDL is calculated for thyroid follicar cell adenomas and carcinomas combined in males and for hepatocellular adenomas and carcinomas combined for females. All tumors were adenomas except for one carcinoma in the 30 mg/kg group in males and one carcinoma in the 100 mg/kg females.

In order to calculate lifetime incidence rates for each dose group, it is necessary to calculate the number of animals at risk. Clearly, animals that were removed from the study for interim sacrifices or that died before the terminal sacrifice were not at risk for a lifetime. The Poly-3 approach developed by the National Toxicology Program (Bailer and Portier, 1988) is used here to calculate the effective number of animals at risk. Obviously, an animal that survives for the lifetime of the study until the terminal sacrifice counts as a whole lifetime exposure. Also, any animal that is removed from the study with a tumor of interest (thyroid follicular cell in males or hepatocellular in females) prior to the terminal sacrifice lived long enough to develop the tumor is counted as a lifetime exposure. All other animals are given a weight of $(t/T)^3$, where t is the week that an animal was removed from the study without the tumor of interest and terminal sacrifices began at

week T=105. Relatively little weight is given to an animal removed early in a study. For example, the animals removed at an interim sacrifice halfway through the study at 53 weeks receive a weight of $(53/105)^3 = 0.13$ of a lifetime, whereas an animal that died on week 96 receives a weight of $(96^3/105) = 0.76$ of a lifetime. The weights are summed for each dose group to obtain the effective number of animals at risk for each group.

The number of male rats with thyroid follicular cell adenomas or carcinomas combined, effective number of animals at risk, and average serum levels of PFOS at 14 weeks for each dose group are displayed in Table 1.

Table 1. Results from the 104-week carcinogenicity study in male SD rats fed N-EtFOSE.

Group	Dose (ppm)	Serum PFOS at 14 weeks (ug/ml)	Number of rats with thyroid tumors[a]	Effective number of rats at risk
1	0	0.078	0	34
2	3	6.14	3	35
3	30	59.1	2	36
4	100	192	6	37
8	0	0.039	1	34
9	1	2.19	2	37

[a] Thyroid follicular cell adenomas except one carcinoma in Group 3.

The number of female rats with hepatocellular adenomas or carcinomas combined, effective number of animals at risk, and the average serum levels of PFOS at 14 weeks are shown in Table 2.

Table 2. Results from the 104-week carcinogenicity study in female SD rats fed N-EtFOSE.

Group	Dose (ppm)	Serum PFOS at 14 weeks (ug/ml)	Number of rats with liver tumors[a]	Effective number of rats at risk
1	0	0.196	0	32
2	3	12.2	1	32
3	30	104	3	33
4	100	268	7	38
8	0	0.126	2	33
9	1	3.26	1	36

[a] Hepatocellular adenomas except one carcinoma in Group 4.

Benchmark Dose Calculations

The numbers of animals with hepatocellular adenoma/carcinoma and the effective number of animals at risk were entered into the U.S. Environmental Protection Agency benchmark dose software program (BMDS). Estimates of the benchmark dose were obtained using the multistage model

$$P = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + q_3 d^3 + q_4 d^4)]$$

where P represents the proportion of animals with tumors, d is the dietary dose or serum level, and the q's are estimated from the experimental dose response data. Goodness-of-fit p-values for the multistage model are above 0.1 indicating an adequate fit of the model. Small p-values would indicate a statistically significant deviance from the multistage model. The goodness-of-fit p-values, BMD_{10}, and $BMDL_{10}$ in terms of dietary concentration of N-EtFOSE and 14-week serum levels of PFOS for males and females are displayed in Table 3.

Table 3. Goodness-of-fit p-values for the multistage model, BMD_{10}, and $BMDL_{10}$ values obtained using the US EPA benchmark dose software program (BMDS).

Sex	p-value	BMD_{10}	$BMDL_{10}$
	Dietary Concentration		
Male	0.23	89 ppm	40 ppm
Female	0.99	58 ppm	32 ppm
	14-Week Serum Level		
Male	0.23	171 ug/ml	77 ug/ml
Female	0.98	166 ug/ml	91 ug/ml

References

Bailer, A.J. and Portier, C.J. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* 44:417-431 (1988).

U.S. Environmental Protection Agency. *Guidelines for Carcinogen Risk Assessment.* NCEA-F-0644, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. July, 1999.

Benchmark Doses for Liver Tumors in Sprague Dawley Rats fed Perfluorooctane Sulfonic Acid Potassium Salt (PFOS)

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Introduction

The carcinogen risk assessment guidelines proposed by the U.S. Environmental Protection Agency (1999) recommend the use of a benchmark dose (BMD) approach for low dose cancer risk assessment. Unless stipulated otherwise, the BMD is the dose at which the excess lifetime tumor incidence is 10%, denoted by BMD_{10}. A value of 10% was selected as this is about the lowest incidence that can be estimated with adequate precision from typical chronic bioassays in rodents. Further, a lower 95% confidence limit is calculated for the benchmark dose ($BMDL_{10}$) to account for the experimental variation of the bioassay. The $BMDL_{10}$ is then used as a point-of-departure for low dose cancer risk assessment. When a nonlinear dose response curve is expected in the low dose range, a margin of exposure between the $BMDL_{10}$ and anticipated human exposure levels is considered. Otherwise, linear extrapolation from the $BMDL_{10}$ to zero is used for low dose cancer risk estimation. In either case, the $BMDL_{10}$ serves as the point-of-departure.

Bioassay Data

The data used for calculation of the $BMDL_{10}$ were collected in the 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats. The BMDL is calculated for hepatocellular adenomas and carcinomas combined for males and females. All tumors were adenomas except for one carcinoma in the high dose females.

In order to calculate lifetime incidence rates for each dose group, it is necessary to calculate the number of animals at risk. Clearly, animals that were removed from the study for interim sacrifices or that died before the terminal sacrifice were not at risk for a lifetime. The Poly-3 approach developed by the National Toxicology Program (Bailer and Portier, 1988) is used here to calculate the effective number of animals at risk. Obviously, an animal that survives for the lifetime of the study until the terminal sacrifice counts as a whole lifetime exposure. Also, any animal that is removed from the study with a hepatocellular adenoma/carcinoma prior to the terminal sacrifice lived long enough to develop the tumor is counted as a lifetime exposure. All other animals are given a weight of $(t/T)^3$, where t is the week that an animal was removed from the study without a hepatocellular adenoma/carcinoma and terminal sacrifices began at week T=105.. Relatively little weight is given to an animal removed early in a study. For example, the animals removed at an interim sacrifice halfway through the study

at 53 weeks receive a weight of $(53/105)^3 = 0.13$ of a lifetime, whereas an animal that died on week 96 receives a weight of $(96^3/105) = 0.76$ of a lifetime. The weights are summed for each dose group to obtain the effective number of animals at risk for each group.

The number of animals with hepatocellular adenoma/carcinoma, effective number of animals at risk, and average serum levels of PFOS at 14 weeks for each dose group are displayed in Table 1.

Table 1. Results from the 104-week carcinogenicity study in SD rats fed PFOS.

Dose (ppm)	14-wk Serum (ug/ml)	Number of animals with liver tumors[a]	Effective number of animals
		Males	
0	0.05	0	33
0.5	4.04	3	32
2.0	17.1	3	37
5.0	43.9	1	38
20.0	148	7	38
		Females	
0	2.67	0	39
0.5	6.96	1	35
2.0	27.3	1	29
5.0	64.4	1	37
20.0	223	6	41

[a] Hepatocellular adenomas except one hepatocellular carcinoma in the high dose females.

As noted before, the effective numbers of animals at risk reflect the lower survival in the controls and low dose males and the females fed 2 ppm and the higher survival in the high dose females.

Benchmark Dose Calculations

The numbers of animals with hepatocellular adenoma/carcinoma and the effective number of animals at risk were entered into the U.S. Environmental Protection Agency benchmark dose software program (BMDS). Estimates of the benchmark dose were obtained using the multistage model

$$P = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + q_3 d^3 + q_4 d^4)]$$

where P represents the proportion of animals with tumors, d is the dietary dose or serum level, and the q's are estimated from the experimental dose response data. Goodness-of-fit p-values for the multistage model are above 0.1 indicating an adequate fit of the model. Small p-values would indicate a statistically significant deviance from the multistage model. The goodness-of-fit p-values, BMD_{10}, and $BMDL_{10}$ in terms of dietary concentration and 14-week serum levels of PFOS for males and females are displayed in Table 2.

Table 2. Goodness-of-fit p-values for the multistage model, BMD_{10}, and $BMDL_{10}$ values obtained using the US EPA benchmark dose software program (BMDS).

Sex	p-value	BMD_{10}	$BMDL_{10}$
	Dietary Concentration		
Male	0.24	18.2 ppm	7.9 ppm
Female	0.54	16.7 ppm	8.0 ppm
	14-Week Serum Level		
Male	0.23	135 ug/ml	62 ug/ml
Female	0.54	193 ug/ml	92 ug/ml

References

Bailer, A.J. and Portier, C.J. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**:417-431 (1988).

U.S. Environmental Protection Agency. *Guidelines for Carcinogen Risk Assessment.* NCEA-F-0644, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. July, 1999.