UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 23 1992

SEP 23 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Atrazine Two-Generation Reproduction Study

FROM: Ad hoc Committee for Atrazine Reproductive Issue Mike Bowers for
Health Effects Division (H7509C)

TO: Amal Mahfouz
Senior Toxicologist
Human Risk Assessment Branch
Health and Ecological Criteria Division
Office of Science and Technology (WH-586)

Conclusions:

This memorandum discusses HED's position on the Atrazine Two-Generation Reproduction Study in Rats. The issue was evaluated by reproductive and statistical experts in HED. This additional information is being provided for the meeting scheduled for September 28th. In short, HED does not believe the difference in pup weight between the control group and 50 ppm F_2 males at day 21 is biologically significant and HED also questions the statistical significance of the body weight changes. A number of points support a dose level of 500 ppm as the appropriate LEL for the atrazine two-generation reproduction study.

Biological Significance:

1. There was no significant effect on pup weight gain in either generation (although it is less in all treated groups).

2. There was no adverse effect on pup survival at any dose in either generation (all treated groups in the F_2 generation had better survival than controls).

3. It is rare to find an effect in the F_2 generation that is not seen in the F_1 generation (see M.S. Christian in "Pre-meeting Comments for Workshop on One vs. Two Generation Reproductive Effects Studies," U.S. EPA, 1987).

Recycled/Recyclable
Printed on paper that contains
at least 75% recycled fiber

4. There were more pups/litter in treated groups than in the control groups in both the F_1 and F_2 generations. Day 0 litter sizes were 11.5, 13.3, 13.4 and 12.8 for the 0, 10, 50 and 500 ppm groups in the F_2 generation. However, there was not a dose-relationship for litter size and the larger litter sizes in treated animals appears to be due to chance. It has been reported that smaller litter size confers both a growth and survival advantage which persists even after culling (see Current Issues in Toxicology, Khera, Grice and Clegg, eds., Springer-Verlag, 1989, p. 29 for a discussion of the effects of litter size and culling on pup weight and survival). This is illustrated by the relationship of lactational pup body weight and litter size in the F_1 generation in that the litters of the low dose group were much larger than controls and somewhat larger than other treated groups. However, only the low dose group had a significantly lower mean body weight than controls at day 21.

5. Litter weights (a more biologically relevant measure than pup weights) were similar at day 4 (pre-culling) despite differences in mean pup body weight which existed even at that time period. Respective litter weights were 73.37, 80.07, 83.48 and 79.62 g for 0, 10, 50 and 500 ppm. After culling, mean litter weights do not provide useful information since study sensitivity has been limited by culling. This has been put forward as an argument against culling by A.K. Palmer (undated personal communication, available upon request).

6. The Agency guidelines acknowledge the importance of litter size as a confounding factor in analyzing pup weight gain. "Individuals tend to be smaller in larger litters than individuals in smaller litters. Thus, reduced birth weights that can be attributed to larger litter size should not be considered an adverse effect unless the increased litter size is treatment related and the ability of the offspring to develop is compromised... Postnatal weights are dependent on birth weight, sex and normality of the individual, as well as the lactational ability of the dam. With large litters, small or weak offspring may not compete successfully for milk and show impaired growth. Because this situation is unlikely to occur in humans, **impaired postnatal growth that is attributable solely to large litter size should not be considered an adverse effect** (emphasis added)."

7. A review of the total toxicological data base (see Attachment 1) indicates that the systemic NOEL is somewhere between 70-100 ppm and 300 ppm for long duration feeding studies (subchronic, chronic, 3-gen. repro. studies). It indicates that there does not appear to be any bioaccumulation of atrazine (metabolism studies) after repeated doses which might account for an effect in the second generation toward the end of the weaning period. Finally, the two developmental toxicity studies show that the developmental alterations are occurring at dose levels well above those which the 50 ppm pups are receiving at day 21 (approximately 5 mg/kg/day, excluding the contribution of milk). They are also occurring at maternally toxic doses which suggest that there is not necessarily any unique developmental toxicity component to atrazine, i.e., both the delays in ossification in fetuses and the depressions in body weight in adult animals, represent general chemically-induced reductions in normal growth rate. This is consistent with the findings in the reproduction study which do

not support any indication of pre- or post-natal toxicity. In conclusion, the ancillary data does not support the suggested finding of a biologically significant depression in male F_2 pup weights at 50 ppm.

Statistical Significance:

HED believes that the use of the F test as a prerequisite for paired testing is appropriate; however, a more meaningful parameter, which relates the rate of growth (body weight gains) rather than body weights per se, should be evaluated. Evaluations of body weight changes should be performed post-culling for obvious reasons. The number of post-culled pups/litter remained essentially the same throughout the remainder of the lactation period.

Attached is an analysis of body weight gains from day 4 (post-culling) through days 14 and 21, and days 14-21 for F_1 and F_2 control and 50 ppm male dose groups (see Attachment 2). Included are individual litter mean male pup weights, summary statistics and a two sample t test.

For F_1 pup body weight gains, the means and S.D.s were similar at all time periods although very slightly less for days 14-21 (92%) and days 4-21 (95%). The t test did not show any significance in mean body weight gains. For the F_2 pups, the mean weight gains and S.D.s were similar at days 4-14, but lower for the treatment group at days 14-21 (84%) and days 4-21 (90%). The t test did not show any significant difference at any period of lactation.

OW has stated that the objective of the study was "to determine at which dose level the mean pup body weights were statistically lower than the control group mean weight." HED believes this is a gross oversimplification of the objective of this type study, since pup body weights are only one parameter from a whole range of effects that may be observed in such a study. Furthermore, a definitive statistical analysis of pup weights must take into account:

(a) the repeated measures aspects of the design: i.e., within each generation, the pups are weighed repeatedly,
(b) the dose-response aspect of the experimental design,
(c) multiple comparison considerations,
(d) lack of randomization of litters in the second generation.

OW's analysis looks only at pup weights on day 21 of the second generation, and ignores points (a), (b) and (d).

HED also does not agree with OW's argument that we are dealing with *a priori* as opposed to *a posteriori* tests. The latter are appropriate for making tests suggested by an inspection of the data, and a significant overall test for differences among the means is required (see Kirk, p. 112). However, even if pairwise comparisons without a significant

F-test on day 21 were appropriate, the alleged statistical significance of the difference at 50 ppm should be discounted by taking into account the possibility for testing at multiple time periods.

Weight-of-Evidence:

It is very important to remind ourselves often that all of the toxicology studies which we review are simply biological screening tests of limited sensitivity and that it is possible to over interpret the data. Thus, when we have a situation in which a single parameter appears to be affected, the most reasonable way to view this is to look at all the relevant data--not just one or two data points. In this regard, the biological analysis should hold precedence over any statistical analysis when the observation seen is a minimal effect in the gray area of interpretation. Thus, the possible alteration in male pup weights at day 21 should be examined in light of:

1. The overall systemic/developmental toxicity of this compound.

2. The observation that in both generations the control litter sizes were lower at day 0 of lactation than the treated groups (an effect which could carryover throughout the lactation period).

3. The lack of any other finding of reproductive toxicity.

4. The lack of a clear dose-response even though the dose levels increase over 50X from the low to high dose.

5. The lack of an effect in the F_1 male pups, or either F_1 or F_2 females.

6. The consistent effect of atrazine in the maternal and paternal animals at 500 ppm.

7. The use of the F test as a prerequisite for paired testing is appropriate for pup mean weights.

8. Evaluation of body weight gains, a more meaningful parameter, does not result in statistical significance using the the t test.

On these bases, the effect does not appear to be of biological or statistical significance.

Committee Members:

Mike Beringer, HED/CCB 9/23/92
Lori Brunsman, HED/SAB 9/23/92
Gary Burin, HED/SAB 9/23/92
Marion Copley, HED/TOXI 9/23/92
Karen Hamernik, HED/TOXI 9/23/92
Hugh Pettigrew, HED/SAB 9/23/92 for H.M.
Jim Rowe, HED/TOXII 9/23/92
Hank Spencer, HED/TOXI 9/23/92
Karl Baetcke, HED/TOXI 9/23/92

ATTACHMENT 1

The following is an analysis of ancillary data which should be considered in evaluating the potential effect in F_2 50 ppm males (taken from the Atrazine Tox Oneliners; 9/02/92; Core Minimum/Acceptable except for the 3-generation reproduction study, 1989 developmental toxicity study, Core Supplementary).

Study Type	Results
2 year rat (005940, 006937) 1986	systemic NOEL = 70 ppm, LOEL = 500 ppm (based on statistically significant body weight decreases in both sexes)
90 day rat (001895) HO-atrazine 1989	systemic NOEL = 100 ppm, LOEL = 300 ppm (renal effects; reduced hematopoietic parameters)
developmental toxicity (rat) (006131, 006761, 006937, 009652) 1984	dev. tox. NOEL = 10 mg/kg/day, LOEL = 70 mg/kg (based on delayed ossification) maternal NOEL = 10 mg/kg/day, LOEL = 70 mg/kg (based on reduced body weight)
developmental toxicity (rat) (009497) 1989	dev. tox. NOEL = 25 mg/kg/day, LOEL = 100 mg/kg (based on delayed ossification, skull bones) maternal NOEL = 25 mg/kg/day, LOEL = 100 mg/kg (based on reduced body weight)
3-generation repro. (002917, 000525) Atrazine 80W 1966	systemic & reproductive NOEL > 100 ppm (HDT)
Metabolism (rats) (006718, 006937, 006937) 1987	rats exposed to 100 mg/kg for 10 days did not accumulate atrazine in tissues, except, perhaps, red blood cells; first order kinetics of distribution; elimination in urine and feces (50:50 each in single dose vs 75:25 in repeated doses)

ATTACHMENT 2

VIEW DATA

CASE	MID14	MID21	MID1421	CTRL14	CTRL1421	CTRL21
1	23.100	41.100	17.700	23.100	20.670	43.770
2	20.700	34.950	14.250	24.620	21.340	45.960
3	19.450	37.360	17.910	19.750	15.300	35.050
4	25.370	44.080	18.710	18.470	19.340	37.810
5	22.140	40.190	18.050	22.880	21.420	44.300
6	23.540	41.370	17.830	21.600	18.330	39.930
7	19.930	36.340	16.410	21.820	17.670	39.490
8	23.240	42.530	19.290	21.170	18.290	39.460
9	22.790	36.680	13.890	23.070	18.650	41.720
10	9.8200	23.030	13.210	18.820	16.020	34.840
11	19.290	35.510	16.220	18.920	13.030	31.950
12	23.180	41.400	18.220	26.180	20.880	47.060
13	17.650	32.740	15.090	20.420	15.810	36.230
14	19.250	34.400	15.150	21.900	20.540	42.440
15	23.600	42.850	19.250	15.820	13.880	29.700
16	19.030	35.080	16.050	23.570	23.640	47.210
17	20.350	36.110	15.760	27.260	23.630	50.890
18	22.810	39.060	16.250	22.140	18.770	40.910
19	23.920	44.780	20.860	20.000	18.680	38.680
20	27.080	50.210	23.130	24.000	21.080	45.080
21	19.960	36.720	16.760	22.650	19.010	41.660
22	23.770	41.520	17.750	21.370	14.830	36.200
23	23.010	43.700	20.690	22.550	19.390	41.940
24	18.420	35.840	17.420	20.990	19.160	40.150
25	21.730	38.950	17.220	21.780	23.130	44.910
26	20.740	37.840	17.100	23.140	18.470	41.610

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
MID14	21.30	3.272	26	21.93	9.820	27.08
MID21	38.63	5.113	26	38.39	23.03	50.21
MID1421	17.31	2.235	26	17.32	13.21	23.13
CTRL14	21.85	2.426	26	21.86	15.82	27.26
CTRL1421	18.88	2.825	26	18.89	13.03	23.64
CTRL21	40.73	4.905	26	41.26	29.70	50.89

TWO SAMPLE T TESTS FOR CTRL14 VS MID14

VARIABLE	MEAN	SAMPLE SIZE	S.D.	S.E.
CTRL14	21.85	26	2.426	4.759E-01
MID14	21.30	26	3.272	6.416E-01

	T	DF	P
EQUAL VARIANCES	0.68	50	0.4997
UNEQUAL VARIANCES	0.68	46.1	0.5000

	F	NUM DF	DEN DF	P
TESTS FOR EQUALITY OF VARIANCES	1.82	25	25	0.0710

CASES INCLUDED 52 MISSING CASES 0

TWO SAMPLE T TESTS FOR CTRL21 VS MID21

VARIABLE	MEAN	SAMPLE SIZE	S.D.	S.E.
CTRL21	40.73	26	4.905	0.962
MID21	38.63	26	5.113	1.003

	T	DF	P
EQUAL VARIANCES	1.51	50	0.1369
UNEQUAL VARIANCES	1.51	49.9	0.1369

	F	NUM DF	DEN DF	P
TESTS FOR EQUALITY OF VARIANCES	1.09	25	25	0.4184

CASES INCLUDED 52 MISSING CASES 0

VARIABLE	MEAN	SAMPLE SIZE	S.D.	S.E.
CTRL1421	18.88	26	2.825	5.541E-01
MID1421	17.31	26	2.235	4.382E-01

	T	DF	P
EQUAL VARIANCES	2.22	50	0.0309
UNEQUAL VARIANCES	2.22	47.5	0.0312

	F	NUM DF	DEN DF	P
TESTS FOR EQUALITY OF VARIANCES	1.60	25	25	0.1238

CASES INCLUDED 52 MISSING CASES 0

VIEW DATA

CASE	MID14	MID21	MID1421	CTRL14	CTRL21	CTRL1421
1	19.900	37.850	17.950	18.480	35.480	17.000
2	16.310	30.880	14.570	18.160	37.420	19.260
3	19.950	33.640	13.690	20.580	40.680	20.100
4	15.570	33.160	17.590	22.730	38.460	15.730
5	18.270	34.060	15.790	21.360	41.130	19.770
6	24.330	41.780	17.450	15.530	28.310	12.780
7	21.360	39.300	17.940	19.610	35.030	15.420
8	18.720	29.320	10.600	18.420	34.010	15.590
9	13.360	24.690	11.330	24.490	47.510	23.020
10	16.970	29.040	12.070	15.090	30.390	15.300
11	19.750	35.050	15.300	20.850	40.920	20.070
12	15.900	32.820	16.920	27.470	56.640	29.170
13	22.560	38.800	16.240	16.150	30.810	14.660
14	23.950	44.380	20.430	19.170	36.460	17.290
15	18.960	35.320	16.360	18.230	35.390	17.160
16	24.020	37.080	13.060	23.540	41.990	18.450
17	19.000	36.980	17.980	19.410	40.100	20.690
18	19.070	34.650	15.580	21.310	39.710	18.400
19	20.620	35.200	14.580	16.900	36.100	19.200
20	18.480	37.950	19.470	22.760	42.770	20.010
21	15.100	27.610	12.510	M	M	M
22	13.300	27.030	13.730	M	M	M
23	17.930	32.130	14.200	M	M	M
24	21.760	30.300	8.5400	M	M	M
25	18.180	37.510	19.330	M	M	M
26	25.520	47.310	21.790	M	M	M
27	19.660	33.180	13.520	M	M	M
28	20.540	34.480	13.940	M	M	M

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
MID14	19.25	3.147	28	19.04	13.30	25.52
MID21	34.70	5.097	28	34.56	24.69	47.31
MID1421	15.44	3.083	28	15.44	8.540	21.79
CTRL14	20.01	3.156	20	19.51	15.09	27.47
CTRL21	38.47	6.295	20	37.94	28.31	56.64
CTRL1421	18.45	3.538	20	18.42	12.78	29.17

TWO SAMPLE T TESTS FOR CTRL14 VS MID14

VARIABLE	MEAN	SAMPLE SIZE	S.D.	S.E.
CTRL14	20.01	20	3.156	7.057E-01
MID14	19.25	28	3.147	5.947E-01

	T	DF	P
EQUAL VARIANCES	0.82	46	0.4139
UNEQUAL VARIANCES	0.82	41.0	0.4146

	F	NUM DF	DEN DF	P
TESTS FOR EQUALITY OF VARIANCES	1.01	19	27	0.4848

CASES INCLUDED 48 MISSING CASES 8

TWO SAMPLE T TESTS FOR CTRL21 VS MID21

VARIABLE	MEAN	SAMPLE SIZE	S.D.	S.E.
CTRL21	38.47	20	6.295	1.408
MID21	34.70	28	5.097	0.963

	T	DF	P
EQUAL VARIANCES	2.29	46	0.0267
UNEQUAL VARIANCES	2.21	35.5	0.0337

	F	NUM DF	DEN DF	P
TESTS FOR EQUALITY OF VARIANCES	1.53	19	27	0.1540

CASES INCLUDED 48 MISSING CASES 8

VARIABLE	MEAN	SAMPLE SIZE	S.D.	S.E.
CTRL1421	18.45	20	3.538	7.912E-01
MID1421	15.44	28	3.083	5.826E-01

	T	DF	P
EQUAL VARIANCES	3.13	46	0.0030
UNEQUAL VARIANCES	3.06	37.4	0.0041

	F	NUM DF	DEN DF	P
TESTS FOR EQUALITY OF VARIANCES	1.32	19	27	0.2506

CASES INCLUDED 48 MISSING CASES 8