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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Endosulfan: Re-assessment of Dog and Rat Chronic Studies in Response to ATSDR Representative's Objections to the NOEL Chosen and Their Use in Setting the RfD

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Comment: Because of concerns expressed by one of the RfD/RfC Work Group (a ATSDR Representative) regarding the apparent increase in alkaline phosphatase and lactate dehydrogenase in dogs in the chronic study, verification of the RfD for Endosulfan was postponed (meeting held November 4, 1992) pending a reconsideration of these data by the HED RfD Committee. In a memo dated 1/29/93, the ATSDR Representative provided the bases for setting the ATSDR Minimal Risk Level (MRL) using the chronic dog study and reasons why the rat study should not be used in setting the RfD.

It is stated that the increased serum levels of alkaline phosphatase and plasma lactate dehydrogenase at dose levels of 0.6 mg/kg and above [in the dog study] indicate hepatotoxicity and therefore, the next lower dose (0.2 mg/kg) was chosen as the NOAEL. With regard to the rat study, the increased incidence of progressive glomerulonephrosis in males, a common spontaneously occurring renal disease, was viewed as obscuring interpretation of the renal effects observed, and the increased incidence of blood vessel aneurysms in males was considered as related to the renal lesions; therefore, the study was considered (by the ATSDR representative) to be inappropriate for use in setting the RfD. (1)

ISSUES

DOG STUDY

1. Alkaline phosphatase levels - males: a "dose-related" increase displayed prior to study and throughout study, with the magnitude of the difference from control value increasing with time only at the high-dose level; females: a similar "dose-related" increase throughout the study, but not prior to study, and the magnitude of the difference from control value varied with time. There was no evidence of any lesion in the liver in either sex, and the liver function test was negative. The increase at the high-dose (30 ppm) level may be treatment-related, but there are no microscopic lesions and liver function was not affected at any dose level. Additionally, the values are considered to be within the normal range of biological variation. Therefore, the apparent increase at the 10 ppm dose level is not considered to be of any toxicological significance. In general, all groups displayed lower alkaline phosphatase values relative to pre-test values with each subsequent interval (as would be expected with age), but the treated groups displayed a lesser decrease than the controls. One would expect these values to increase in an animal with liver damage. No histopathology was observed in the liver to indicate an adverse effect of Endosulfan on the liver.

Interval / Dose (ppm)	ALKALINE PHOSPHATASE U/L				
	0	3	10	30	30♦
MALES					
preliminary	220	224	230	235	193
intermediate 1	137	151	156*	214*	205
intermediate 2	118	139	157*	196*	199
intermediate 3	73	98	117*	136*	
intermediate 4	75	91	92	141*	
final	61	80	99*	146*	266
FEMALES					
preliminary	182	164	224	205	195
intermediate 1	135	128	175*	245*	201
intermediate 2	131	125	152*	201*	212
intermediate 3	99	87	117	168*	
intermediate 4	117	108	135	199*	
final	95	99	129	183*	281

♦ 30/45/60 ppm; * $p < 0.05$

2. Lactate dehydrogenase - both sexes displayed a "dose-related" decrease prior to the start of dosing, which was statistically significant at all dose levels; there was an increase at the intermediate time interval prior to termination and at termination at 10 and 30 mg/kg (both sexes and dose-related), but only the high-dose was statistically-significantly increased at termination. There were no microscopic findings and liver function was not affected. As stated above, one would expect to see an increase in subsequent values relative to the pre-test values had Endosulfan produced a toxic effect on the liver.

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Interval Dose (ppm)	LACTATE DEHYDROGENASE U/L				
	0	3	10	30	30♦
MALES					
preliminary	164	154*	129*	121*	25*
intermediate 1	33	27	39	31	31
intermediate 2	44	36	51	48	30
intermediate 3	40	35	38	20	-
intermediate 4	45	34	55*	50*	-
final	42	40	55	57*	-
FEMALES					
preliminary	155	147*	122*	119*	28*
intermediate 1	31	27	35	27	29
intermediate 2	37	35	46	45	55
intermediate 3	25	25	33	29	-
intermediate 4	35	34	55*	60*	-
final	37	35	47	63*	17

* $p<0.05$

RAT STUDY

1. The effects of concern are the increase in severity (**marked**) of progressive glomerulonephrosis, which was evident in both sexes at the high-dose level, and the increase in blood aneurysm(s), which was observed in the high-dose males (see tables below).

Progressive Glomerulonephrosis (N=70)

Parameter/Dose	0 ppm	3 ppm	7.5 ppm	15 ppm	75 ppm
MALES					
progressive glomerulonephrosis	55	52	64	61	44?58
minimal/moderate	35	34	42	37	28
marked	20(36)♦	18(35)	22(34)	24(39)	30(51)
FEMALES					
progressive glomerulonephrosis	29	37	42	39	37
minimal/moderate	28	31	36	34	29
marked	1(3)	6(16)	6(14)	5(13)	8(22)

♦ (%)

Incidence of Blood Vessel Aneurysm(s)

Parameter/Dose	0 ppm	3 ppm	7.5 ppm	15 ppm	75 ppm
MALES blood vessels aneurysm(s)	10	6	14	10	19
FEMALES blood vessels aneurysm(s)	1	2	5	4	3

2. There was a reduction in body weight gain in both sexes at the high-dose level, which was not mentioned by the ATSDR representative.

3. There were no consistent changes in protein parameters (blood clinical chemistry values) in either sex (see tables below), nor were BUN values affected. Urinary protein values were increased at the two highest dose levels (dose-related) in males only, but only at termination. Since the values observed at termination at these dose levels were (1) within the range of values observed at other time points and in the control, and (2) many of the rats measured at this time point had not been measured during the study, the apparent increase is not considered treatment-related.

4. There were no treatment-related histological lesions in the liver of either sex.

Male Clinical Chemistry (Blood) Parameters

Parameter/Dose	0 ppm	3 ppm	7.5 ppm	15 ppm	75 ppm
Total Protein					
week 13	7.1	6.9	6.6**	6.8**	6.7**
week 26	7.0	7.1	7.2	7.5**	7.3**
week 52	7.2	7.2	7.2	7.3	7.2
week 78	7.5	7.4	7.1	7.4	7.5
week 103	7.2	7.3	7.1	7.0	7.1
Albumin					
week 13	4.3	3.9**	3.9**	4.0**	4.0**
week 26	4.2	4.2	3.9**	4.2	4.2
week 52	3.9	4.1	3.9	4.0	4.0
week 78	3.9	4.0	3.8	3.9	3.9
week 103	3.9	3.9	3.8	3.7	3.6*
Globulin					
week 13	2.9	3.0	2.7	2.8	2.7
week 26	2.8	3.0	3.3**	3.3**	3.1**
week 52	3.2	3.1	3.3	3.3	3.2
week 78	3.6	3.4	3.3	3.5	3.6
week 103	3.2	3.4	3.4	3.4	3.6*

Female Clinical Chemistry (Blood) Parameters

Parameter/Dose	0 ppm	3 ppm	7.5 ppm	15 ppm	75 ppm
Total Protein					
week 13	6.7	6.4	6.5	6.6	6.4*
week 26	7.6	7.7	7.4	7.6	7.3
week 52	8.0	8.0	7.1**	7.4**	7.4**
week 78	8.1	7.9	7.6	7.8	7.9
week 103	8.2	7.8	7.7*	7.5**	7.6**
Albumin					
week 13	4.2	4.1	4.0	4.0*	3.8**
week 26	4.5	4.7	4.5	4.7	4.4
week 52	4.7	4.7	4.3	4.7	4.7
week 78	4.4	4.4	4.2	4.2	4.3
week 103	4.5	4.4	4.4	4.2*	4.2*
Globulin					
week 13	2.5	2.3	2.5	2.7	2.6
week 26	3.1	3.0	2.9	2.9	2.9
week 52	3.3	3.3	2.8*	2.7**	2.7**
week 78	3.7	3.5	3.4	3.6	3.6
week 103	3.6	3.4	3.3	3.3	3.3

Urinalysis Findings

Protein (mg/dL)/ Dose	0 ppm	3 ppm	7.5 ppm	15 ppm	75 ppm
MALES					
week 12	17	23	27	24	17
week 25	83	97	114	96	132
week 51	421	1252	614	512	1041
week 77	345	214	145	213	237
week 102	375	400	533	600*	620**
FEMALES					
week 12	0	0	2	0	0
week 25	18	58	76	21	62
week 51	150	202	173	159	183
week 77	361	521	521	314	586
week 102	197	322	351	207	348

CONCLUSION

TB II does not agree with the ATSDR representative's conclusions regarding either study. The differences observed in the enzyme values (alkaline phosphatase and lactate dehydrogenase) in the dog study were not accompanied by any histopathological lesion or decrement in liver function at any dose level. Increases in the levels of these non-specific enzymes without supporting evidence is not considered adequate confirmation of liver damage. The increase in the severity of progressive glomerulonephrosis in rats of both sexes at the high-dose level is regarded as an adverse effect, in that the spontaneously occurring renal disease was exacerbated by exposure to the test material. The NOEL chosen in both of these studies remains as stated in the RfD/Peer Review Report of Endosulfan, dated October 13, 1992; i.e., 0.6 mg/kg/day.