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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review of the Influence of Atrazine Ingestion on Steroid-Receptor Recognition.

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TO: Venus Eagle
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Reregistration Branch
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THRU: Karen Hamernik, Ph.D. 12/15/93
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KB 12/20/93

ACTION: Review the report of a Chronic Toxicity Study in Rats: Influence of Atrazine Ingestion On Steroid-Receptor Recognition. Study No. 852214, By Ciba Geigy and the James Brown Cancer Center, Louisville, Kentucky. Dated: October 15, 1990.

CONCLUSIONS: This reviewer finds that the study report data suggest that hormone receptors may be affected in the 2-year study. However, the data are so varied and from such small numbers of samples that any firm conclusions would be difficult to make concerning the receptor binding of various tissues with the several steroid hormones tested. In addition, the results reported may have been confounded by earlier <u>in utero</u> treatment with atrazine.

See " Discussion " section for additional comments.

This study report is classified as supplementary data and is not covered by a Guideline designation. The data are not upgradable.

REVIEW: A two year study was started with dietary exposure of atrazine at 0, 10, 50, or 500 ppm dose levels. Each dose level consisted of 50 animals per sex. The experimental design is extracted from the report and added below.

Group	Daily Dose-Concentration (ppm)	Number of Rats Male	Number of Rats Female	Sacrifice Interval (Study Weeks)
1	0	10	10	8[a]
		10	10	35[b]
		40		52[c]
			30[e]	104[d]
2	10	10	10	8[a]
		10		35[b]
		40		52[c]
			30	104[d]
3	50	10	10	8[a]
		10		35[b]
		40		52[c]
			30	104[d]
4	500	10	10	8[a]
		10		35[b]
		40		52[c]
			30[e]	104[d]

a - Ten animals/sex/group were sacrificed after at least 7 weeks on study and evaluated endocrinologically.

b - Ten female animals/group were sacrificed after at least 34 weeks on study and evaluated endocrinologically.

c - The remaining male animals (approximately 40/group) were sacrificed after at least 52 weeks on study and evaluated endocrinologically.

d - The remaining females were sacrificed after at least 103 weeks on study and evaluated endocrinologically. Portions of the mammary gland and associated tumors, if any, as well as the uterus were shipped to the University of Louisville for receptor recognition analysis.

e - Ten control animals were placed on 500 ppm diet, and 10 Group 4 (high-dose concentration) animals were placed on the control diet from week 65 until study termination. The first ten animals from Group 1 and the last ten

Young Sprague-Dawley rats were used which were obtained from parents derived from a reproduction study using atrazine as the test substance. Acclimation of the young animals was for a period of 9 days before being placed on study. Selection of the test animals for each group was by a randomization table to insure a proper spread of the litters across all treatment groups.

Animals were provided Purina rodent chow # 5002 ad libitum. Temperature was maintained at 73 degrees +\- 5 degrees F, with humidity at 50% +\- 20%. Light was provided as 50% on/off in 24 hrs.

The test diet was found to be stable for at least 40 days at room temperature. Samples of the diet were collected at approximately 4 week intervals for the 1st year and at 8 week intervals during the 2nd year.

Details for collection of various materials and data which included: clinical observations, body weight, gross pathology, organ weights, sacrifice, and tissue for hormone analyses have been presented in this Study Report No. 852214 reviewed here.

Tissue collection:

Tissues taken at study termination for histopathology included: the uterus, mammary gland and the pituitary from female rats and the remainder of these organs were stored at - 86 C. for hormone receptor studies. The hormone studies were carried out at the James Graham Brown Cancer Center Hormone Receptor Laboratory, Louisville, Kentucky.

Studies performed:

Receptor Binding Studies for estrogen, progestin, and prolactin receptors were performed on the cytosol of the various tissues collected.

Extensive methodologies have been reported in other studies and are only extracted and inserted below for completeness.

TB Review dated 12/22/93

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RESULTS: Generally, animals derived from test material studies are not used unless they are derived from untreated controls. The results reported in this study may have been confounded by <u>in utero</u> exposure of all animals assigned to the treatment groups.
With regard to the experimental design: Endocrinology data for the male animals, if collected, were not submitted in the study report. If collected, the endocrinological data for the females at 8 weeks and 35 weeks were not provided. Additionally, the results may have been confounded by <u>in utero</u> exposure of all animals assigned to treatment groups.

Table 1 below shows that at 50 ppm there were 13/50 (43%) tumor bearing animals. Since the table notes "after lifetime dietary administration of atrazine", this should be something other than 50 animals because of the interim sacrifices made (see design).

Table 1: The Incidence of Mammary Tumors Noted in Female Sprague-Dawley Rats After Lifetime Dietary Administration of Atrazine.

Mammary Tumor - Type	Tumor Level (ppm)					
	0	10	50	500	500 --> 0[1]	0 --> 0[2]
Tubular Adenoma	0/20[0][3]	0/30[0]	1/30[0]	0/19[0]	1/10[10]	0/10[0]
Fibroadenoma	4/20[20]	6/30[20]	10/30[33]	7/19[37]	4/10[40]	2/10[20]
Carcinoma	8/20[40]	4/30[13]	5/30[17]	6/19[32]	5/10[50]	2/10[20]
Tumor Bearing Animals	11/20[55]	10/30[33]	13/50[26] 30	11/19[58]	8/10[80]	4/10[40]

[1] Females switched at 65 weeks from diet containing 500 ppm of atrazine to control diet.

[2] Females switched at 65 weeks from control diet to diet containing 500 ppm of atrazine.

[3] Number in parenthesis equals the percent incidence.

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Table 2 below shows the binding capacities and constants for estradiol in normal and tumor tissues of mammary glands, and pituitary glands as well as the normal uterus of treated females.

NOTE: The table reports the number of animal tissues and not the number of animals. It is not clear which entities are counted in the table. In any case, the tumorous mammary and normal pituitary tissue appear to have decreased existing binding of estradiol. However, in order to be able to more properly evaluate the data, the animal with its individual finding will need to be displayed.

Table 2: BINDING CAPACITIES AND CONSTANTS FOR ESTRADIOL ASSOCIATED WITH CYTOSOLIC RECEPTORS IN THE MAMMARY GLAND, MAMMARY GLAND TUMORS, UTERUS AND PITUITARY OF FEMALE SPRAGUE-DAWLEY RATS EXPOSED TO ATRAZINE FOR A LIFETIME.

Feeding Level (ppm)	Mammary Glands: Normal Tissue N[1]	SBC[2] (fmol/Mcp)	Kd[3] ($\times 10^{-10}$M)	Mammary Glands: Tumored Tissue N	SBC (fmol/Mcp)	Kd ($\times 10^{-10}$M)	Uterus N	SBC (fmol/Mcp)	Kd ($\times 10^{-10}$M)	Pituitary Gland: Normal N	SBC (fmol/Mcp)	Pituitary Gland: Tumored N	SBC (fmol/Mcp)
0	7	14.1±8.4[4]	1.3±0.5	5	59.6±76.8	3.7±5.4	6	484±190	1.29±0.53	4	168±269	3	163±133
10	11	12.1±11.6	1.1±0.8	8	63.1±58.1	0.9±1.3*	9	481±188	1.05±0.77	3	714±693	8	92±115
50	12	13.7±8.3	0.9±0.4	13	54.5±65.4	2.4±3.0	11	366±140	0.84±0.37	10	504±1033	2	212±144
500	9	16.8±0.4	1.1±0.4	15	24.0±28.2	1.8±4.4	9	417±82	1.05±0.39	3	23±10	6	119±141

[1]N = Number of animal tissues examined.

[2]Specific binding capacity in fmol (femtomoles)/mcp (mg of cytosolic protein).

[3]Binding constant $\times 10^{-10}$M (molar).

[4]Mean or average ± standard deviation.

*Different from the control at $P \leq 0.05$.

Table 3 shows the binding capacities and constants for progestin associated with the mammary gland, mammary gland tumors, uterus and pituitary gland and pituitary gland tumors.

NOTE: Data on the mammary tissues are quite variable. Tumor data suggests that there is decreased binding over control tissues, however, the number of tumorous controls is very small and quite variable data as indicated by the large S.D.. Pituitary data are also variable but are so few in number as to provide no means to a supportable conclusion.

Table 3. BINDING CAPACITIES AND CONSTANTS FOR PROGESTIN ASSOCIATED WITH CYTOSOLIC RECEPTORS IN THE MAMMARY GLAND, MAMMARY GLAND TUMORS, UTERUS AND PITUITARY OF FEMALE SPRAGUE-DAWLEY RATS EXPOSED TO ATRAZINE FOR A LIFETIME.

Feeding Level (ppm)	Mammary Glands: Normal Tissue N[1]	SBC[2] (fmol/Mcp)	Kd[3] (x10^{-10}M)	Mammary Glands: Tumored Tissue N	SBC (fmol/Mcp)	Kd (x10^{-10}M)	Uterus N	SBC (fmol/Mcp)	Kd (x10^{-10}M)	Pituitary Gland: Normal N	SBC (fmol/Mcp)	Pituitary Gland: Tumored N	SBC (fmol/Mcp)
0	7	49.7±77.1	6.9±9.2	15	370±794	15.6±20.6	6	641±170	9.6±1.9	4	77±96	3	97±147
10	11	14.5±26.7	10.3±22.3	8	148±222	7.4±6.5	9	462±158	6.5±3.9	3	137±131	8	9±20
50	12	15.5±17.1	5.8±7.5	13	176±307	10.1±7.8	11	846±482	9.8±3.6	10	105±49	2	42±43
500	9	34.1±61.8	11.6±15.5	15	136±276	5.9±7.1	9	555±453	8.2±5.6	3	0±0	6	11±18

[1]N = Number of animal tissues examined.
[2]Specific binding capacity in femtomoles/mg of cytosolic protein.
[3]Binding constant x10^{-10}M (molar).
[4]Mean or average ± standard deviation.

Table 4 shows the data on the binding capacities for prolactin associated with receptors in mammary gland, mammary tumors, uterus and pituitary glands of female SD rats exposed to atrazine for a lifetime.
NOTE: This reviewer is unable to establish the exact number of tissues or their origin which were evaluated for prolactin SBC in either mammary or pituitary tissues whether normal or tumorous.
The 500 ppm mean data suggest that binding may occur less in normal but treated mammary tissue than in controls. Mean data in the uterus is so variable as indicated by the S.D. that no conclusion could be made.

Table 4. BINDING CAPACITIES FOR PROLACTIN ASSOCIATED WITH RECEPTORS IN MAMMARY GLAND, MAMMARY TUMORS, UTERUS AND PITUITARY GLAND OF FEMALE SPRAGUE-DAWLEY RATS EXPOSED TO ATRAZINE FOR A LIFETIME.

Feeding Level (ppm)	Mammary Gland				Uterus		Pituitary Gland			
	Normal		Tumored				Normal		Tumored	
	N[1]	SBC[2] (fmol/mcp)	N	SBC (fmol/mcp)	N	SBC (fmol/mcp)	N	SBC (fmol/mcp)	N	SBC (fmol/mcp)
0	7	53.8±128.1[3]	6	10.9±10.2	7	5.2±11.5	4	180±220	3	14±19
10	11	14.7±26.0	7	76.9±169.3	10	24.1±51.0	3	71±54	8	40±41
50	13	14.0±22.2	17	82.4±206.9	12	12.0±21.7	9	10±17	2	0±0
500	9	7.6±12.0	16	32.3±67.8	9	25.3±43.0	3	7±9	16	13±11

[1] Number of tissues evaluated.
[2] Specific binding capacity in femtomoles/mg of cytosolic protein.
[3] Mean or average ± standard deviation.

Table 5 is a summary comparison of SBC and/or Kd values for estrogen, progestin and prolactin receptors in the cytosolic fractions of normal and tumorous tissue from the pituitary and mammary glands of Sprague-Dawley rats. The origin of the values is not clear since the values don't correlate with those in previous tables.

NOTE: The values in the table appear to be based on the number of animal tissues and not the number of animals which were sampled. Additionally, there is no indication as to when the individuals were sampled since the number of animals in table 5 for estrogen does not equal those in table 3. However, the S.D.s are quite large and showed extreme variation in the data.

Table 5. A COMPARISON OF SBC AND/OR KD VALUES FOR ESTROGEN, PROGESTIN AND PROLACTIN RECEPTORS IN THE CYTOSOLIC FRACTIONS OF NORMAL OR TUMORED TISSUE FROM THE MAMMARY AND PITUITARY GLANDS.

Gland	Type	Receptor							
		Estrogen			Progestin			Prolactin	
		N[1]	SBC[2] (fmol/mcp)	Kd[3] ($\times 10^{-10}$M)	N	SBC (fmol/mcp)	Kd ($\times 10^{-10}$M)	N	SBC (fmol/mcp)
Mammary	Nontumor	45	15.5±11.5[4]	1.5±1.6	39	45.6±47.3	8.6±14.7	39	19.7±36.0
	Tumor	48	56.1±69.0*	2.2±3.6	41	179.5±363.4*	8.7±9.5	41	54.7±146.2*
Pituitary	Nontumor							40	40.0±22.4
	Tumor							46	46.5±24.7

[1]Number of animal tissues examined.
[2]Specific binding capacity in femtomoles/mg of cytosolic protein.
[3]Binding constant $\times 10^{-10}$M (Molar).
[4]Mean ± standard deviation.
*Different from the control at P≤0.05.

DISCUSSION:

The incidence of tumors in Table 1 do not indicate an increase in tumors over controls following the 2 years of treatment with atrazine. The histopathology results from interim sacrifices was not included in this report.

The data has been reported only as summary information and as such, the review can not be as extensive as would normally be undertaken. The individual information on such activities as the scintillation work and the complete methodologies should have been submitted for review.

The SBC (specific binding capacity) for estradiol reported in Table 2 for normal mammary gland appears to be insignificant in comparison to controls. However, the data is suggestive of some alteration in hormone binding in atrazine treated tissue, ie. 500 ppm in mammary tumors. This same atrazine effect is also suggested by the data in the normal pituitary (500 ppm vs controls).

The data in table 3 indicating the "N" number of tissues do not appear applicable to the individual data and require further interpretation by the registrant before the data can be further evaluated.

Though variations in the data values are recognized for prolactin, none were considered by the authors as statistically significant. This is most likely because

of the extremes in S.D. calculated.The numbers of tissues examined in the receptor binding studies are so small that with the concomitant extremes in values obtained, any firm conclusions on receptor binding are difficult, if not impossible to be drawn from this data.

The reviewer, therefore, agrees with the conclusion noted by the study authors namely: "that SBC..... and Kd..... constants did not reveal any consistent treatment related response at the receptor level." As well as the possible confounding of the results by in utero exposure prior to starting the study, the reviewer considers the information (study) to be so incompletely reported that the data does not support any definite conclusion. Furthermore, the lack of a treatment-related response in the mammary gland (ie. there was no overall increase in mammary tumor incidence in Atrazine treated animals) further confound data interpretations.