

US EPA ARCHIVE DOCUMENT

UNIT STATES ENVIRONMENTAL PROTECTION AGENCY

Chitlik
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DATE: February 7, 1980

2/11/80

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SUBJECT: Proposed tolerances for residues of the herbicide metolachlor; PP#8F2081, Registration#100-583; PP#8F2098, Reg#100-597; PP#9F2213, Reg.#100-583 and 100-597; PP#9F2203, Reg.#100-583 and 100-597, held in abeyance, (Conditional registration proposed). CASWELL#188DD

File petition?

FROM: Laurence D. Chitlik, Toxicologist
Toxicology Branch (TS-769)

Chitlik

TO: Willa Garner, PM#23
Registration Division (TS-767)

THRU: M. Adrian Gross, Chief
Toxicology Branch (TS-769)

M. Gross

Common Name: Metolachlor (formerly CGA-24705)

Product Names: Dual 6E
Dual 8E

Chemical Name: 2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methylethyl)acetamide

Metabolites: 2-[(2-ethyl-6-methylphenyl)amino]-1-propanol and (4-[2-ethyl-6-methylphenyl]-2-hydroxy-5-methyl-3-morpholinone)

Related Petitions: 5G1553, 5F1606, 6G1708, 7F1913

Note: Memo of 1/21/80, L. Chitlik, additional reference for metolachlor 6 month dog study.

Requested Actions:

8F2081 - (Amended registration 100-583)

Deleted 12/11/79, 0.1 ppm Fresh Corn including Sweet Corn (Kernels
in revised Section F Plus Cobs, Husks removed).

Deleted 12/11/79, 0.1 ppm Popcorn (grain)
in revised Section F

proposed 1.0 ppm Corn forage and fodder

proposed 2.0 ppm Soybean forage and fodder

#8F2098 (Amended registration 100-597)

0.3 ppm sorghum grain
2.0 ppm sorghum forage and fodder

#9F2213 (Amended Registrations Dual 6E 100-583 and Dual 8E 100-597)

0.1 ppm Peanuts
1.0 ppm Peanut hulls
3.0 ppm Peanut forage and hay

#9F2203 (Amended Registrations, Dual 6E 100-583 and Dual 8E 100-597)

Held in Abeyance, Ciba-Geigy letter, 12/11/79, 0.1 ppm potatoes.

Existing Tolerances: 0.1 ppm corn grain
Existing, but established 0.1 ppm soybeans
with conditions (PP#7F1913,
see discussion section) 0.02 ppm meat, milk, poultry, eggs

Background/Discussion:

The soybeans and milk and meat tolerances (PP#7F1913) were established conditionally based upon the following requirements:

1. Re-evaluation of histopathology from the 90-day dog study would be submitted by 3/15/79. (Ciba-Geigy letter of 10/25/78)
2. The two-year rat study would be submitted by 3/15/79. (Ciba-Geigy letter of 10/25/78)
3. No additional tolerances would be considered until favorable review of the above studies. (Agreed upon in meeting held with Ciba-Geigy 10/20/78).
4. Ciba-Geigy suggested repeating their mouse oncogenic study, which although acceptable to TOX reviewers, was far removed from the ideal because of GLP problems. The pathology data clearly demonstrated no oncogenic potential for metolachlor, but husbandry was poor. TOX accepted the proposal for a new study.
5. Ciba-Geigy would initiate a 6 month dog study (Ciba-Geigy letter of 10/25/78.)

Histopathology from the 90-day dog study was re-read by a qualified pathologist. This data was submitted in a timely fashion and evaluation of this data has elevated this study from core-supplementary to core-minimum data.

The two-year rat study was also submitted, but while undergoing IBT validation review, it was classified as invalid as a chronic feeding study and only supplementary for oncogenic, memo of L. Anderson, 12/17/79.

Hence, the soybeans and milk, meat, and egg tolerances of PP#7F1913 were not fully supported by the 2-year rat feeding study. Ciba-Geigy immediately agreed to repeat this study, Dec. 11, 1979.

According to the IBT Policy Statement of October 3, 1979, memo of Marcia Williams, all major IBT studies had to go through an IBT validation process. This effected re-evaluation of the 18-month mouse oncogenic study and the 3-generation rat reproduction study. Data supporting the 18-month mouse study were found to support study findings, memo of H. Spencer, 1/31/80. On the other hand, the 3-generation rat reproduction study was found to provide useful but not definitive information and classified as supplementary. It was also concluded that the study must be repeated (Memos of L. Anderson 1/31/80 and W.L. Burnam, 1/31/80), leaving another significant data gap.

Previously, during the 12/6/79 meeting, Ciba-Geigy was informed that favorable validations for both the 18-month mouse study and the 3-generation reproduction studies were critical to any further consideration of proposed tolerances, as well as an acceptable 6-month dog study (in lieu of the unacceptable rat chronic study). After the determination that the 3-generation study had been classified as supplementary and needed to be repeated, Ciba-Geigy immediately responded with their letter of 1/30/80. They agreed to initiate a new multi-generation study with the hope that this would suffice for the proposed tolerances. (Note: Other information included in that memo is erroneous. The memo states that TOX agreed at that time that the study could still be used for tolerance setting purposes.)

On Jan. 31, 1980, a TOX meeting was held to discuss the status of this 3-generation study. The original reviewer, W.L. Burnam, the study validator L. Anderson, and the petition reviewer, L. Chitlik met with Adrian Gross to discuss the study. It was concluded at this meeting that (1) there were no reproductive effects evident at levels up to 1000 ppm determined under the poor conditions in this study, (2) the study must be repeated, (3) the data is classified as supplementary, (4) the data is useful in that it does not reflect any serious reproductive effects, but this is not definitive information.

Chemistry reviews of PP#8F2081, memo of 4/2/79, PP#8F2098, memo of 4/5/79, and PP#9F2213, memo of 11/16/79, indicate that secondary residues in milk, meat, eggs and poultry are expected due to proposed tolerances in corn, soybean, sorghum and peanut forage and fodder. Chemistry Branch indicated that established permanent tolerances of 0.02 ppm in eggs, milk, meat, fat, and meat by-products of livestock are adequate.

Recommendations/Conclusions:

Evaluation of the data supporting the proposed tolerances has revealed that there are a number of significant data gaps as listed below:

1. Rat, oncogenic evaluation;
2. Rat, chronic feeding study;
3. Teratology study in a second species;
4. Rat, multi-generation reproduction study.

Also of note is the fact that the only acceptable oncogenicity study, an 18-month mouse, was conducted at Industrial Bio-Test and is far from the ideal; plagued with GLP and animal husbandry problems. It has, however, gone through the IBT validation process and raw data has been found to support negative study results. The 2-year rat chronic feeding study, also IBT, was determined to be useful only as supplementary oncogenic data by the IBT validation team. This additional rat study is useful in that no oncogenic potential was demonstrated for metolachlor, and although these findings are not definitive, and a data gap exists for this study, increased confidence for the lack of oncogenic potential of metolachlor is gained.

When the problems were uncovered in the IBT rat 2-year feeding study after the IBT validation had been completed, a meeting was held with Ciba-Geigy representatives, 12/6/79. TOX Branch indicated that if the 18-month mouse oncogenicity study and rat 3-generation reproduction were validated favorably and the six month dog study was satisfactory, TOX may have sufficient data to support some of the conditional registrations proposed by Ciba-Geigy. The IBT validation of the 3-generation reproduction study was not favorable, the study was classified as supplementary and must be repeated. Information gained from this study does not indicate metolachlor to induce reproductive effects, but this is not definitive. Hence, the only reliable and acceptable data which supports the safety of metolachlor in the realm of reproductive toxicology is a single rat teratology study.

In response to the above findings, Ciba-Geigy has agreed to initiate a new rat multi-generation study, a teratology study in a second species, a 2-year chronic feeding study with oncogenic evaluation, and has already initiated a new mouse oncogenic study. If RD considers the proposed conditional registrations, careful time tables should be established for receipt of acceptable studies.

Ciba-Geigy has also revised Section F of PP#8F2081 to exclude fresh corn and popcorn. They have also asked that PP#9F2203 (potatoes) be held in abeyance (mainly due to CB deficiencies) and the resultant increase in TMRC from the proposed tolerances would therefore be significantly reduced to 4.27% (Sorghum contributes a 1.22% increase in TMRC while peanuts contribute 3.05%). The ADI must now be based upon the 100 ppm NOEL in the 6-month dog study and 2000 fold safety factors, instead of the preferred 2 year rat feeding study. The last problem associated with the IRDC 6-month dog study was finally resolved with the additional statistical evaluations received 2/6/80. (See data summary and review).

In consideration of the proposed conditional registrations, RD should carefully consider the data deficiencies for milk, meat, poultry and soybean tolerances already established under PP#7F1913. (See discussion section) Available data have revealed no serious toxicological concerns related to metolachlor use, but 3 major data gaps exist.

Proposed amended registrations (100-583 and 100-597) have previously undergone a favorable label safety review by TOX Branch. Labeling is acceptable as proposed.

<u>Study</u>	<u>Received</u>	<u>Test Laboratory</u>	<u>Data Summary</u>	<u>Review Status</u>	<u>Findings</u>
6-Month Dog Study	12/11/79	IRDC		Significant GLP problems discovered & reported in 1/21/80 memo.	Unable to determine NEL because of erroneous final body weight, organ/body weight ratios, and APTT values.
Addendum I to 6-Month Dog Study	1/17/80	IRDC		Review of addendum completed 1/17/80; Ciba-Geigy notified that this addendum did not answer reviewer's questions and Ciba-Geigy agreed that it was inadequate.	Addendum included original report pages not submitted 12/11/79, in original submission.
Addendum 2 (2 volumes of data) to 6-Month Dog Study	1/21/80	IRDC		Initial Review of data to resolve source of problems completed 1/24/80. Ciba-Geigy notified that body weight problem resolved but APTT questions remained.	Problems were found to be associated with poor quality assurance at IRDC. A faulty balance in the necropsy room was determined to be the source of invalid data; APTT questions still remained unanswered.
Addendum 3	1/29/80	IRDC		Addendum review completed 1/29/80, and APTT issue resolved.	APTT values in this study (initially indicating effects at 2 doses levels) were verified to be valueless as clinical findings in this study due to poor methodology. Missing ophthalmologic data also submitted.

(Continued from last page)

Addendum 4
to 6-Month
Dog Study

2/6/80

Ciba-Geigy

Review of 6-month dog
study completed 2/6/80.
submission included
additional statistical
evaluations not complete
in 1/21/80, addendum.

NOEL is 100 ppm

(7)

<u>Study</u>	<u>Received</u>	<u>Testing Laboratory</u>	<u>IBT Validation Status</u>	<u>Review Status</u>	<u>Findings/Comments</u>
2-Year rat feeding study with oncogenic evaluation.	2/15/80	IBT	(A) INVALID as a chronic feeding study. (B) <u>Supplementary study for oncogenic evaluation</u> (memo of L. Anderson 12/17/79)	N/A	No evidence that metholachor is oncogenic, but dose levels not verified by analysis of diet. This study does not fulfill the regulatory requirement for a chronic feeding study, nor for oncogenicity and it therefore must be repeated.
Mouse, 18 month oncogenicity study.	1/18/78	IBT	Determined to be VALID. (1/13/80, memo of H. Spencer)	Completed 9/18/78	Not oncogenic when fed at levels of 30,1000 and 3000 ppm.
Rat, 3-generation reproduction study.	1/18/79	IBT	Data supports findings in original report but classified as a supplementary study. (memo of W.L. Burnam, 1/31/80 Because of poor animal husbandry at IBT, study must be repeated.	Completed initial review 2/27/78; Addendum to previous review based upon IBT validation findings, 1/31/80.	No adverse reproductive effects suggested up to 1000 ppm, however study classified as supplementary and must be repeated.

<u>Study</u>	<u>Received</u>	<u>Testing Laboratory</u>	<u>Review Status</u>	<u>Findings/Comments</u>
90-day, rat	3/1/74	Oncins Research and Breeding Center	Completed 1/25/78	Rat 90-day NOEL 300 ppm feeding histopathology is incomplete and the study is classified as supplementary.
90-day, dog	9/24/74 histopathology received 1/25/79	Oncins Research and Breeding Center	Original review, 9/12/78 Revised, 2/6/80.	Classified as supplementary in review of L. Chitlik, 9/12/78 because tissues were not read by pathologist.
				The submission of 1/25/79, which included a re-examination by a qualified pathologist, elevates the study to core-minimum. The NOEL is 5 ppm.

Additional Data Considered:

1. Rat LD₅₀ = 2780 mg/kg
2. Mouse Mutagenic (Dominant Lethal) - Negative
3. Salmonella/Mammalian - Microsome Mutagenicity test - Negative
4. Rat, Teratogenic - Not fetotoxic or teratogenic at 360 mg/kg

DATA REVIEW:

Dog, 6-Month Oral Toxicity Study, CGA-24705 Technical, International Research and Development Corporation, Report#382-054, November 2, 1979

Twenty-eight male and 28 female beagle dogs 4 to 6 months of age were used in this study. Dogs were housed individually in wire-mesh cages and conditioned in the laboratory for 9 weeks prior to initiation of the study. They were also vaccinated for hepatitis, distemper, leptospirosis, treated for intestinal worms, checked for heart worms and given an ophthalmologic examination. Blood and urine samples were also taken during this period and unhealthy dogs were eliminated from the study. Dogs were then randomized and assigned to the following groups:

<u>Test Level (ppm)</u>	<u>Number of Dogs</u>	
	<u>Male</u>	<u>Female</u>
0 (control)	8	8
100	6	6
300	6	6
1000	8	8

CGA-24705 Technical was dissolved in ethanol to prepare a 50% (w/v) solution used to make a premix and mixed with the remaining Purina Dog Chow.

Observations

Dogs were observed daily for appearance, behavior, and mortality. Tissue mass incidence, body weights and food consumption were determined weekly. Compound intake was calculated weekly.

During the pretest period and at 6 months an ophthalmoscopic examination was performed on each dog.

Clinical Tests

Hematology and blood chemistry tests were performed initially and at monthly intervals including the recovery period. Urinalysis was performed initially and at 2, 4, 6 months and during the recovery period. The following determinations were made:

Hematology

1. hemoglobin
2. hematocrit
3. erythrocyte count
4. total and differential
leuckocyte count
5. platelet count
6. reticulocyte count
(beginning at 4 months)
7. prothrombin time
8. activated partial-throm-
boplastin time (APTT)
9. methemoglobin
10. Heinz bodies

Blood Chemistry

1. BUN
2. fasted blood glucose
3. total cholesterol
4. total protein
5. serum calcium
6. serum potassium
7. serum sodium
8. serum chloride
9. direct and total
bilirubin
10. SGOT
11. SAP
12. SGPT
13. LDH

Urinalysis

1. specific gravity
2. microscopic sediment
3. protein
4. glucose
5. ketones
6. bilirubin urobilinogen
7. pH
8. occult blood (only months 4, 6, 7)

Gross Pathology

Dogs were sacrificed by exsanguination after an over-dose of sodium pentobarbital and then necropsied. "Selected tissues" and liver, kidneys, heart, brain, spleen, gonads, adrenals, thyroids (with parathyroid) and the pituitary were weighed. Recovery animals (2/sex, group I and IV) were sacrificed in the same manner, 1 month after dosing was completed.

Histopathology

The following tissues were stained with hematoxylin and eosin and examined microscopically.

adrenal gland	liver	prostate
aorta	lung with bronchi	salivary gland
bone marrow	lymph node	(submaxillary)
brain (3 levels)	(cervical and	skeletal muscle
cecum	mesenteric)	skin
colon	mammary gland	small intestine
esophagus	muscle	(3 levels)
eye	thymus	spinal cord (2
gall bladder	optic nerve	levels)
gonads	pancreas	spleen
heart	parathyroid	stomach (3 levels)
kidney	peripheral nerve	thyroid with
	(sciatic)	parathyroid
	pituitary	trachea
		urinary bladder
		uterus
		any gross lesion

Statistical Evaluation

Statistical analysis included one-way analysis of variance, Bartlett's test for homogeneity of variances and the appropriate t-test for equal or unequal variances using Dunnett's multiple comparison tables to determine significance. The Wilcoxin, Mann, Whitney, Rank Sum test was also used in the 1/21/80 and 2/6/80 addendums.

Diet Analysis

Prior to the start of the study and at one week intervals 100g diet samples were sent to Ciba-Geigy for analysis. Also, 1 gram of the technical material was sent for analysis at 3 and 6 months.

RESULTS

Diet analysis revealed that the average low dose was 92 ppm, the intermediate group received 273 ppm and the high dose received 952 ppm. By time weighting these, the low dose received 88.6 ppm, the intermediate group 270 ppm and the high dose 964.8 ppm. The percentage error is - 4-11% and not significant.

Observations

Emesis, soft stool and ocular discharge, interdigital cysts, relaxed nictitating membrane and slight dermatitis was observed in all groups including controls at some point during the study.

A "thickened area" along the mammary cord was noted in two female controls, three of the 300 ppm females and five 1000 ppm females. Individual animal observations were not included in the report.

No deaths occurred during the study.

Mean body weight data demonstrated that 1000 ppm males and females gained less body weight than controls and other test groups.

Although food consumption was slightly reduced in 1000 ppm males (2% reduction) and females of the 100 ppm level (6% reduction), 300 ppm level (5% reduction) and 1000 ppm level (9% reduction), the only significant decrease in food consumption is demonstrated by the 1000 ppm females. The average compound consumption for male dogs was determined to be 2.92 (100 ppm level), 9.71 (300 ppm level), and 29.61 mg/kg/day and for female dogs it was determined to be 2.97 (100 ppm level), 8.77 (300 ppm level) and 29.42 mg/kg/day (1000 ppm level). After examinations of 4 randomly selected dogs for ophthalmologic examinations at 6 months, it was concluded that the increase in punctate corneal opacities (epithelial or subepithelial lesions) in all groups was suggestive of trauma and not compound related.

Hematology

Male dogs at the 300 and 1000 ppm levels demonstrated significantly reduced activated partial thromboplastin time (APTT) at 5 and 6 months ($p < 0.01$) as follows:

	<u>Control (seconds)</u>	<u>100 PPM</u>	<u>300 PPM</u>	<u>1000 PPM</u>
5 months	11.6	11.0	10.0*	10.0*
6 months	11.5	12.4	9.6*	9.7*

*designated in the report as significant

In female dogs APTT values were also statistically significantly less than controls at month 4 (100 ppm level), at month 6 at 300 ppm level, at month 5 for the 1000 ppm level and at the end of the recovery period in the 1000 ppm females. As noted below:

<u>Month</u>	<u>Control (seconds)</u>	<u>100 PPM</u>	<u>300 PPM</u>	<u>1000 PPM</u>
4	11.0	10.4*	11.8	10.9
5	10.9	11.0	11.0	9.8**
6	11.3	11.6	9.9*	10.3
7 (recovery)	11.0	-	-	9.3**

* $p < 0.05$

** $p < 0.01$

Considering that the APTT is reduced (rather than lengthened) in both males and females and to statistically significant levels, potential methodology error is considered. Sample preparation is the most likely source of error and the test must also be run under carefully controlled conditions.

These points were discussed with Dr. Darrel Summer of Ciba-Geigy on 1/9/80, and the precise procedure as well as explanations for these irregular values were requested.

Dr. Summer telephoned on 1/10/80, after consulting with IRDC personnel. He explained that IRDC agreed that they also had not observed shortened APTT values except in this study and that they were at a loss to explain it. The question of sample preparation was discussed as well as which test group was sampled first. Dr. Summer agreed to further research the methodology for the source of the problem and submit this in writing. He also agreed to submit copies of unrevised pages in the report (Note: A number of report pages are labeled as "revised" and he explained that most revisions were due to typing errors and that some additional explanation had been added to the report on the SAP findings).

Considering that Prothrombin times did not demonstrate significant effects, added credence was given to the theory that the APTT findings (since shortened) could be considered as erroneous due to faulty methodology. Dr. Summer agreed to submit the APTT methodology used, and make an effort to determine the source of the problem.

Since a number of APTT findings were significant of $p < 0.01$, and the effect appeared dose related, it was decided to pursue this issue further.

On 1/17/80, Ciba-Geigy submitted an addendum to their 6 month dog study in an attempt to answer questions related to their very irregular APTT values. The document included the following:

1. Dade instructions for the use of Actin (Activated Cephaloplastin Reagent) for APTT determinations.
2. An instruction manual for the MLA Electra Coagulation timers E620 and E650.
3. APTT values from 4 other study control groups.
4. Dade I and Dade II calibration control data.
5. A short letter from Dr. A. Clark Kahn III, Director of Clinical Pathology.
6. Copies of original report pages not submitted along with the report.

Unfortunately, the information provided (especially the letter from Dr. Kahn) did not seriously attempt to resolve the APTT methodology problems at IRDC. No information related to sample gathering (ie - redomization) nor sample preparation (the most likely source of error) were included. The only attempt at resolving the questions raised including the following statement by Dr. Kahn:

"There is a possibility of interference from elevated temperature and interference by unknown particulates."

Other points brought out in the letter from Dr. Kahn which are of interest include:

1. Dr. Kahn believes that "this particular test cannot be interpreted clinically based on shortened reaction times."
2. He also indicated that APTT values are within the range based on other control values obtained from other studies.

A comparison of APTT data from this study to the four other control APTT groups, demonstrated that a number of test group values were above and below this range.

Dr. Barnett of Ciba-Geigy was informed by telephone on 1/17/80, that the addendum did not answer the questions posed concerning IRDC methodology, especially sample preparation. Dr. Barnett agreed that the submission was inadequate and indicated that another effort would be made.

On 1/21/80, Ciba-Geigy submitted a second addendum to this study (EPA Accession#099203) which also included discussion of the APTT problems. The following additional information relative to this issue was included in this submission:

1. Summary/discussion of the IRDC APTT method and associated problems (prepared by Ciba-Geigy).

A new attempt was made to use data from 4 previous IRDC control groups to demonstrate that test group values were within the "normal range".

Considering (1) the times were not monitored between sample collection and processing on the MLA-600 coagulation instrument, (2) that controls were sampled first and (3) no SOP was in existence before or during this study (Verified by discussion with Dr. John Barnett of Ciba-Geigy) (4) it must therefore be assumed that the "normal range" based upon 4 previous control groups (where APTT values were determined under the same conditions) is also of no value for comparison purposes. Furthermore, as demonstrated by one of these control groups, the standard deviation varied by more than 4x from one study to another.

Dr. Kahn's letter of 1/10/80, also indicated that a sex related difference may exist, yet the Ciba-Geigy manipulation of the data combined male and female data. This is rather conflicting and certainly not appropriate.

The use of this control data, compiled from four previous IRDC studies, to establish the normal range for APTT values is therefore not acceptable. Once correct and uniform procedures are established at IRDC, a normal range should be established excluding the submitted control data values. Furthermore, comparing the normal range proposed by Dr. Kahn to another that he referenced in his 1/21/80 letter, of another facility (Laboratory of Dr. Hugh Lewis, normal range of 9.3 - 11.6 seconds (based upon 3 SDV rule) with a mean of 10.3 seconds) many more of his test and control values would be outside this range since it is approximately half the time of Kahn's range.

2. Ciba-Geigy toxicologists determined after visting IRDC and going through the APTT procedure with Dr. Kahn that possible sources of error included:
 - A. Optical interference induced by a technician pouring off supernatant and including some blood cells. (Not a likely cause)

- B. If insufficient sodium citrate were added, the clotting process could have been initiated prematurely. (Not considered likely problem).
 - C. The procedures used at IRDC did not limit the time between sample collection and analysis.
 - D. Ignoring the significance of point 3 above, IRDC claimed that reported values were within the lower limit of the normal range and that the statistical significance is a Type I error (false positive).
3. Also in reference to the APTT question, Ciba-Geigy submitted a second letter from Dr. Kahn dated 1/21/80. Dr. Kahn indicated neither the time of blood sampling of the dogs nor the time when they were processed was recorded. Dr. Kahn also responded to a telephone request for the APTT SOP that, "all SOP's are the sole property of IRDC and cannot be released." This statement is inconsistent with the Ciba-Geigy letter of Jack Norton, 1/28/80. In this letter Norton states that, "Ciba-Geigy was informed by IRDC that an SOP had not been instituted for APTT."

The Kahn letter also referenced a telephone conversation of 1/20/80, with this reviewer where we discussed the fact that APTT times for dogs at IRDC are several times shorter than human values and perhaps that IRDC was not able to properly control processing time delays in the animal studies and obtain valid APTT normal range tolerances (ie - the IRDC range is 5.4 seconds while another laboratory referenced by Dr. Kahn, that of Dr. Hugh Lewis, had a range of 2.3 seconds).

The question was raised by this reviewer whether IRDC had considered not activating the thromboplastin times and whether the values would then be more reliable. Dr. Kahn didn't answer this question at the time, but his memo indicates that he contacted the "supervisor of coagulation testing" at the laboratory of Dr. Hugh Lewis. She indicated the method is routinely used by them, but as mentioned earlier, the APTT range at this facility is much tighter than at IRDC!

Dr. Kahn then inappropriately tried to compare the lower APTT values in the metolachlor study to the normal range at the Lewis laboratory. He also did not realize that in such a comparison, many values in this study would have then been outside the upper range limit. The wide disparity in ranges definitely indicates APTT methodology problems at IRDC.

Dr. Kahn indicated that some findings were unusually low in this study and time intervals between obtaining the sample and the completion of the APTT were "not rigidly controlled". He also issued a directive that all coagulation tests must henceforth be completed within four hours after obtaining the blood sample.

The letter of Jack Norton, 1/28/80, Ciba-Geigy, also addressed the wider APTT normal range at IRDC and concluded that some variable is not controlled at IRDC and that it is likely due to delays in analysis of samples. He also stated that the position of Ciba-Geigy is "the reduced APTT values reported in this study are not meaningful in regard to the toxicity of metolachlor."

This reviewer agrees with the registrant that APTT values in this study are not related to a compound effect and are due to incorrect methodology.

The platelet count was significantly increased in 300 ppm females during the pretest period ($364 \times 10^3/\text{cmm}$) while decreased in the 1000 ppm females at the first month. An increase was also noted during the 4th month in 1000 ppm females ($p < 0.05$). It was also increased as compared to controls (the control value is relatively low at $161 \times 10^3/\text{cmm}$) in the 100 ppm females at 3 months ($244 \times 10^3/\text{cmm}$) with $p < 0.01$.

Males at 300 ppm showed a significant decrease in platelets at 3 months ($323 \times 10^3/\text{cmm}$ as compared to controls $368 \times 10^3/\text{cmm}$). These fluctuating and inconsistent values are not considered compound related.

In male dogs of the 300 ppm level at 3, 4, and 5 months both the erythrocyte count and the hemoglobin concentration were significantly less than in control dogs. At the 1000 ppm level, during month 3, the erythrocyte count was also reduced in males. The hematocrit was also reduced in males during months 3 & 4. These values were within the normal range, with the slight exception of the low erythrocyte counts at 3 and 4 months in the 300 ppm males (5.71 and $5.54 \times 10^6/\text{cmm}$).

Considering the sporadic nature of these findings, that they did not persist to termination, and the variation in normal range, these findings are not considered compound related. There were no effects demonstrated in the females.

Serum alkaline phosphatase levels generally decreased more slowly in the test groups as compared to controls. At 4 months, the mean SAP level in 1000 ppm males was statistically significantly higher ($p < 0.05$) at 97 int'l u/L than the control level of 66 int'l u/L . At 6 months both the 300 ppm level males at 78 int'l u/L ($p < 0.01$) and the 1000 ppm males at 87 int'l u/L ($p < 0.05$) were significantly higher than the control SAP of 56 int'l u/L . At 100 ppm, males also had a higher SAP level of 77 but this did not show statistical significance. In females at 6 months the 1000 ppm level of 100 int'l u/L was statistically higher than controls at 69 int'l u/L . Both the 100 ppm level of 86 and the 300 ppm level of 83 int'l u/L were also higher than the mean control level at 6 months. After a one month recovery period the SAP level in two 1000 ppm females dropped to 53 int'l u/L indicating that this effect was compound related.

The lower rate of decrease of SAP as noted above may be associated with decreased bone maturation (possibly related to a retarded growth rate), or hepatic disease/dysfunction. Microscopic examination of liver and bone marrow revealed no unusual findings. On the other hand body weight gain, although not statistically significant was slightly reduced in both males and females of the 300 and 1000 ppm groups and males of the 100 ppm group after 6 months on test.

	<u>Control</u>	<u>100 PPM</u>	<u>300 PPM</u>	<u>1000 PPM</u>
Males	2.6 kg	1.6 kg	2.1 kg	1.3 kg
Females	2.1 kg	2.1 kg	1.9 kg	1.4 kg

Food consumption was also slightly reduced in 1000 ppm females which may have been related to effects in this group but it does not explain other findings.

It is therefore concluded that the slower rate of SAP decrease in 1000 ppm males and females and 300 ppm males in a toxic response to the test compound. This reviewer also notes that repeat determinations were carried out for SAP levels (only during month 6) in two males at 100, 300, and 1000 ppm levels and without explanation. Dr. John Barnett of Ciba-Geigy was telephoned on 1/15/80, and an explanation for the repeats was requested.

Dr. Barnett checked with IRDC and determined that the repeats were due to a technical difficulty with the auto-analyzer which ran out of reagents and gave initial values of zero.

Urinalysis revealed no unusual findings.

Pathology

I. Gross Necropsy

At necropsy, no compound related lesions were observed. A number of spontaneous lesions were noted in all groups. These findings included thickening of mammary areas, interdigital cysts, discolored lungs, mottled liver, kidney capsular adhesion, and corneal opacity.

Pathology

II. Organ Weight Data

Evaluations terminal body weights and organ-to-body weight ratios in the original metolachlor dog study received Dec. 11, 1979, was found to be unreliable. A comparison of the tables on pages 150-154 labeled as "Absolute and Relative Organ Weights, Terminal Sacrifice" with the tables pages 22-25, labeled as "Individual Body Weights" revealed many significant differences in terminal body weight versus week 26 body weights. The range of weights varied as much as 3.52 kg with many weights varying 1 or more kilograms. Few weights differed by less than 0.5 kg and all but one weight was reduced at sacrifice. Dr. J.W. Barnett of Ciba-Geigy was informed of these findings on 1/16/80 and individual sacrifice dates and explanations for such questionable differences were requested.

On 1/21/80, Ciba-Geigy submitted an addendum to their 6 month dog study to answer questions related to the body weights and resultant questionable body weight ratios.

The addendum included the findings of two Ciba-Geigy toxicologists (J.M. Charles and J.T. Stevens) who went to IRDC to determine the cause of the unremarkable terminal weights. Laboratory records for weekly weighings, terminal body weights, necropsy records, food consumption data, diet preparation records, compound diet calculations, scale calibration records for scales used in both the necropsy room and for weekly weighings, clinical observation data, and a list of personnel involved in this study were also included in the submission.

The source of the problem was determined to be a faulty balance in the necropsy room used for the final weighings. This balance (manufactured by National Control, Inc. of Scope Inc., Serial#D785660) was different from the balances used for all the previous weekly weighings (Toledo balance Serial#9692). Calibration checks of the balance during each weekly weighing were included in the submission substantiating that weekly weights were valid.

The National Control balance in the necropsy room was designed to provide equal readings across the total surface of a large stainless steel pan, regardless of where a mass was applied. Two screws which secured the position of a central column beneath the pan had loosened during use. Since the balance was calibrated by placing the reference weight in the center, the calibration indicated normal functions, but when the dogs were weighed (off-centered) irregular terminal dog weights resulted and this problem therefore went undetected.

This balance was checked by Ciba-Geigy representatives when they visited IRDC and it was also found to work properly. They were told it had been repaired. In addition, they checked the digital balance used to weigh diets for food consumption estimates by using 0.5 kg and 2 kg reference weights and it was found to be accurate.

Food consumption and diet preparation data indicates animals were fed up to the day before sacrifice and then fasted overnight. This can therefore be ruled out as a contributing factor to the irregular terminal body weights. The addendum clearly indicated that the irregular values are due to the faulty necropsy room balance, which was discovered by IRDC personnel several months later (September 20, 1979).

Re-evaluation of the metolachlor 6-month dog feeding study using data supplied in the addendum of 1/21/80

- A. The following new data were submitted in this addendum:
1. Relative organ weights based upon week 26 weights taken on June 4, 1979, instead of the erroneous terminal weights taken 1-3 days later.
 2. Organs-to-brain weight ratios.

3. Values for some animals were inadvertently omitted from the IRDC submission (Attachments 5 and 6), so Ciba-Geigy submitted these under attachments 5A and 6A. They also included relative spleen, liver, and adrenal relative weights from the recovery animals that were not included by IRDC.
- B. Review of addendum organ-to-body weight ratios, organ-to-brain weight ratios, and week 26 body weights.

Mean week 26 body weights were reduced in 300 and 1000 ppm males, but not at statistically significant levels, as follows:

	<u>Control</u>	<u>100 PPM</u>	<u>300 PPM</u>	<u>1000 PPM</u>
Males (kg)	14.48	13.55	12.83	12.5
Females (kg)	10.53	10.77	10.52	10.40

Males demonstrated a statistically significant increase in pituitary to body weight ratios at 100 ppm (0.54) with $p < 0.05$ and at 100 ppm (0.54) with $p < 0.01$, as compared to control males at 0.44. At 300 ppm, males actually showed a decrease in pituitary weight as compared to controls suggesting the effect at 100 ppm is questionable. Furthermore, there was no statistical significance in pituitary-to-brain weight ratios among any male test groups. One 300 ppm female was determined to have a cranio-pharyngeal cyst which greatly affected mean weights from that group, but this cyst is congenital and not related to treatment.

The thyroid-to-brain weight ratio was statistically significantly increased in 300 ppm females at 1.61 (with $p < 0.05$) as compared to controls at 1.22. Although not indicated as statistically significant, the 1,000 ppm level also showed an elevated value of 1.37. The thyroid to body weight ratio and mean weights (although not statistically significant) were also elevated in the 300 and 1000 ppm females. When the 2 control recovery males were added to the 6 control males, for purposes of statistical comparison, mean thyroid weights of the 300 ppm group males were also significant ($p < 0.05$).

<u>FEMALES</u>	<u>Control</u>	<u>100 PPM</u>	<u>300 PPM</u>	<u>1000 PPM</u>
Thyroid (g)	0.94	0.84	1.26**	1.10
% body wt.	0.92	0.79	1.20	1.08
% brain wt.	1.22	1.07	1.61*	1.37

* significantly increased $p < 0.05$

** statistically significant ($p < 0.05$) when 2 recovery controls included in evaluation.

No thyroid effects were noted in males.

Heart weights were statistically reduced at $p < 0.05$ in females of the 1000 ppm group (Wilcoxin, Mann, Whitney Rank Sum Test).

In the males, a statistically significant decrease was determined in heart weights ($p < 0.05$) by both the t-test and the Wilcoxin, Mann, Whitney Rank Sum Test. This was not indicated as statistically significant in the IRDC revision nor in that submitted by Ciba-Geigy/21/80, but was included in the 2/6/80 submission. Heart and heart-to-brain weight ratios were reduced in all other test levels as well, but not at significant levels.

<u>MALES</u>	<u>Control</u>	<u>100 PPM</u>	<u>300 PPM</u>	<u>1000 PPM</u>
heart (g)	110.89	101.37	94.13	94.98
% brain wt.	138.96	118.02	109.67*	121.77

* Statistically significant, $p < 0.05$

Spleen were reduced in males at 1000 ppm, although not at significant levels. In females, a reduction in spleen-to-brain weight ratio was significant at the 300 ppm level ($p < 0.05$, Wilcoxin, Mann, Whitney Rank Sum Test) and at the 1,000 ppm level, but not at a significant level. This statistically significant decrease in female spleen weight was not noted in the addendum data. Spleen is a unreliable indicator, and such findings likely relates to completeness of exsanguination at terminal sacrifice.

Although not indicated in the addendum submission, female liver-to-brain weight ratios were reduced at the 100 ppm dose level ($p < 0.05$, Wilcoxin, Mann, Whitney Rank Sum Test). No effects were evident in higher dose groups or in males and this finding is therefore not considered compound related.

Recovery Group Organ Weights

Since 1000 ppm recovery groups consisted of only 2 dogs/sex, statistical comparison to recovery controls is of little value.

After the 30 day recovery period elapsed, differences for a number of organs still existed in the 1000 ppm recovery animals. The only statistical difference noted was the reduction in spleen-to-brain weight ratio of 1000 ppm females ($p < 0.05$). Male liver weights and liver-to-brain weight ratios were reduced 15% and 13.7% respectively.

Male and female kidney weights and kidney-to-brain weight ratios decreased. Male gonad and brain weight ratios decreased 23.7% and 22.8% respectively. Male and female heart weights (17% and 30% respectively) and heart-to-brain weight ratios decreased.

Male thyroid weights and brain weight ratios decreased (39% and 37% respectively) as compared to controls.

The significance of these recovery weight differences is of extremely limited value due to the very few animals involved.

III. Histopathology

No lesions considered compound related were noted. Findings unrelated to compound administration included a craniopharyngeal cyst in the pituitary, focal parafollicular cell hyperplasia of the thyroid, mucroliths in medullary tubules of the kidneys, interstitial pneumonia and hypospermatogenesis in maturing dogs. Endometrial hyperplasia, which appeared to be dose related is likely due to a retarded maturation of the female dogs at the 300 ppm and 1000 ppm levels. No control or 100 ppm females demonstrated this finding, while 4 out of 6 300 ppm females and 5 out of 8 1000 ppm females demonstrated minimal to moderate diffuse endometrial hyperplasia.

Mammary hyperplasia in the acini and ductal structures was also observed in some control and test females which is indicative of proestrus or estrus.

Study Conclusions

All problems associated with this 6 month metolachlor dog study (originally submitted Dec. 11, 1979) have been resolved in 4 addendums submitted by the registrant. These problems include:

1. A faulty balance in the IRDC necropsy room negated any validity of terminal body weights and all organ-to-body weight ratios based upon them. New organ-to-body weight ratios have been submitted based upon the final weekly weighing measured several days earlier. Organ brain weight ratios have also been submitted.
2. Significant reductions in APTT values have been determined to be due to faulty methodology at IRDC.
3. The registrant has also submitted revised statistical analyses of organ weight data since the original analysis was determined to be incomplete. (Received 2/6/80)
4. Original report pages, not included in the submission of 12/11/79 have been received.
5. Missing individual animal data from the IRDC report have been supplied.

The NOEL in this study has been determined to be 100 ppm.

A revised IRDC final report reflecting all study revisions and addendums including corrected statistical evaluations should be submitted by the registrant.

This study is classified as Core-Minimum.

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File last updated 1/31/80

ACCEPTABLE DAILY INTAKE DATA

Dog	NOEL	S.F.	PADI	MPI
mg/kg	ppm		mg/kg/day	mg/day/60kg
2.500	100.00	2000	0.0013	0.0750

Published Tolerances

CROP	Tolerance	Food Factor	mg/day/1.5kg
Corn, grain (68)	0.100	1.00	0.00150
Soybeans (143)	0.100	0.92	0.00138
Meat, inc poultry (89)	0.020	13.85	0.00415
Milk & Dairy Products (93)	0.020	28.62	0.00858
Eggs (54)	0.020	2.77	0.00083

MPI	TMRC	% ADI
0.0750 mg/day/60kg	0.0164 mg/day/1.5kg	21.93

Current Action

8F2098

CROP	Tolerance	Food Factor	mg/day/1.5kg
Sorghum (147)	0.300	0.03	0.00014

MPI	TMRC	% ADI
0.0750 mg/day/60kg	0.0166 mg/day/1.5kg	22.11

Current Action 9F2213

CROP	Tolerance	Food Factor	mg/day/1.5kg
Peanuts (115)	0.100	0.36	0.00054

MPI	TMRC	% ADI
0.0750 mg/day/60kg	0.0171 mg/day/1.5kg	22.82
