# The Toxicity of 2,4-D and Picloram Herbicides to Fish[1]

M. Sergeant, D. Blazek, J. H. Elder, C. A. Lembi and D. J. Morré
Department of Botany and Plant Pathology
Purdue University, Lafayette, Indiana 47907

*Abstract*

Two common herbicides, picloram or Tordon (4-amino-3,5,6-trichloropicolinic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and their salts exhibit low toxicity to fish. Certain formulated derivatives (especially esters) tend to be more toxic than acid salts as is an impurity from technical picloram. Even with picloram containing impurities, adaptive and/or detoxification responses by the fish are indicated. These herbicides (picloram and 2,4-D) seem to present a low potential hazard to fish from normal agricultural or industrial practice.

Growth regulating herbicides have been developed over the past 30 years which control broad spectra of plant species. They are used extensively for control of brush and herbaceous species in non-crop areas as well as for weed control in agricultural, recreational and urban areas. In addition to the standard herbicides used for these purposes such as 2,4-D and 2,4,5-T, a newer material called picloram or Tordon herbicide (Table 1) has also received extensive testing and widespread use in non-crop areas. The mode of action of picloram is similar to that of 2,4-D and 2,4,5-T (4, 5, 6, 8, 16, 19, 21) but with a greater herbicidal effectiveness due to increased mobility and resistance to breakdown within the plant (6). As a consequence of its resistance to breakdown, picloram introduced into the biosphere may persist for a year or more (7, 9, 12, 18) in contrast to 2,4-D and 2,4,5-T which are broken down more rapidly (11). The present communication constitutes a preliminary study from our laboratory dealing with the toxicity of 2,4-D and picloram-containing herbicides to green sunfish. This study was part of a more extensive program concerning the mode of action of herbicides of the auxin type, their derivatives and formulations, and their effects on aquatic ecosystems.

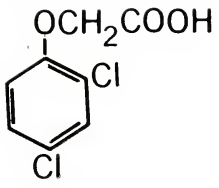
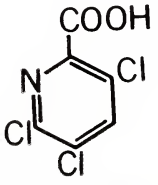
## Materials and Methods

Green sunfish *(Lepomis cyanellus)*, 50-150 g, were maintained in pond water at 24°C with continuous aeration and on artificial diets. Fish mortality was recorded and at the end of the study, fish were examined for tissue abnormalities. The livers were excised, weighed and sampled for histological examination.

For electron microscopy, portions of the livers were fixed at 0-4° C in 2.5% glutaraldehyde (Fisher, Biological Grade) in 0.1 M sodium phosphate at pH 7.2 followed by a buffer rinse and post fixation

[1] Supported in part through a project sponsored by the Joint Highway Research Project, Department of Civil Engineering, Purdue University and The Indiana State Highway Commission. Journal Paper 4252. Purdue University Agricultural Experiment Station.

TABLE 1. *Characteristics of 2,4-D and picloram derivatives.*

| Compound | Abbreviation | Chemical Structure[1] |
|---|---|---|
| 2,4-Dichloro-phenoxyacetic acid | 2,4-D |  |
| 4-Amino-3,5,6-trichloropicolinic acid | Picloram (Tordon[2]) |  |

[1] Acid (R=H); salt (R=metal ion or organic amine); ester (R=long chain alcohol).

[2] Registered trademark of the Dow Chemical Company.

in 1% osmium tetroxide in the same buffer. Specimens were dehydrated in a graded acetone series and embedded in Epon (24). Thin sections were post-stained with lead citrate (22) after being mounted on carbon-coated parlodion-covered grids and were viewed with a Philips EM-300. Magnifications are approximate.

TABLE 2. *Effect of herbicide formulations on the swimming response of green sunfish* (Lepomis cyanellus).

| Herbicide | Concentration | Formulation | Acid Equivalent | Average Response Time |
|---|---|---|---|---|
| 2,4-D | $5X10^{-4}$ M | Acid | 99% | No effect in 41 hr |
| | | Li+Salt | 95% | No effect in 41 hr |
| | | Isopropyl-Diethylanolamine Salt | 28% | No effect in 41 hr |
| | | Butoxyethanol Ester | 43% | 60 min |
| Picloram | $5X10^{-4}$ M | Acid | 99% | No effect in 41 hr |
| | | Technical | 91% | 5 min |
| | | K+Salt | 22% | 5 min |

## Results

With 2,4-D, neither the acid nor commercial salt formulations were toxic to green sunfish at a concentration of $5 \times 10^{-4}$ M (110 ppm acid equivalent) (Table 2). However, at this same concentration, the

butoxyethanol ester of 2,4-D proved toxic after 60 minutes of exposure. Similar results were reported previously by Butler (1) (Table 3) in tests using three salt water species of fish. With picloram, the 99% analytical grade material was also nontoxic at 5 x $10^{-4}$ M (1.2 ppm, acid equivalent). However, both the 91% technical picloram and the 22% commercial formulation were toxic (Table 2) suggesting the presence of an impurity in these preparations. Similar toxicities of picloram to fish were reported by Kenaga for rainbow trout and other species (17) with the isocotyl ester being at least 10 times more toxic than the corresponding amine salts (Table 4).

TABLE 3. *Comparative toxicity of 2,4-D formulations to fish* (Leiostomus xanthurus, Fundulus similus *and* Mugil cephalus)[1]

| Formulation | $LC_{50}$—ppm ae 48 hr Exposure |
|---|---|
| Acid | no effect at 50 ppm |
| Dimethylamine salt | no effect at 15 ppm |
| 2-Ethylhexyl ester | no effect at 10 ppm |
| Propyleneglycolbutylether ester | 4.5 |
| Butoxyethanol ester | 5.0 |

[1] From Butler (1).

TABLE 4. *Comparative toxicity of picloram formulations to rainbow trout* (Salmo gairdnerii).[1]

| Formulation | Equivalent | ($LC_{50}$—ppm ae) Exposure Time 24 hr | 96 hr |
|---|---|---|---|
| Triisopropanolamine salt | 55.8% | 279 | 209 |
| Triethylamine salt | 70.5% | 43 | 29 |
| Isooctyl ester | 68.3% | 10 | 3 |

[1] From E. E. Kenaga (17).

In our studies, fish were quickly immobilized by 5 x $10^{-4}$ M technical picloram but did not die. Fish treated for up to one hour with this concentration of technical picloram recovered motility and swam normally upon return to pond water without herbicide. The recovery time varied as an approximately linear function of the treatment time (Fig. 1). After recovery, the fish were then given a second exposure to technical picloram. After the second exposure and return to pond water without herbicide, the fish again began to swim normally but the recovery times were generally shorter (Fig. 1) than after the first exposure. This trend continued through a third exposure to technical picloram and after a fourth exposure many of the fish failed to even respond, *i.e.* 0 recovery time.

Liver changes were observed even in those fish exposed to $10^{-4}$ M technical picloram (Table 4), a concentration which did not affect

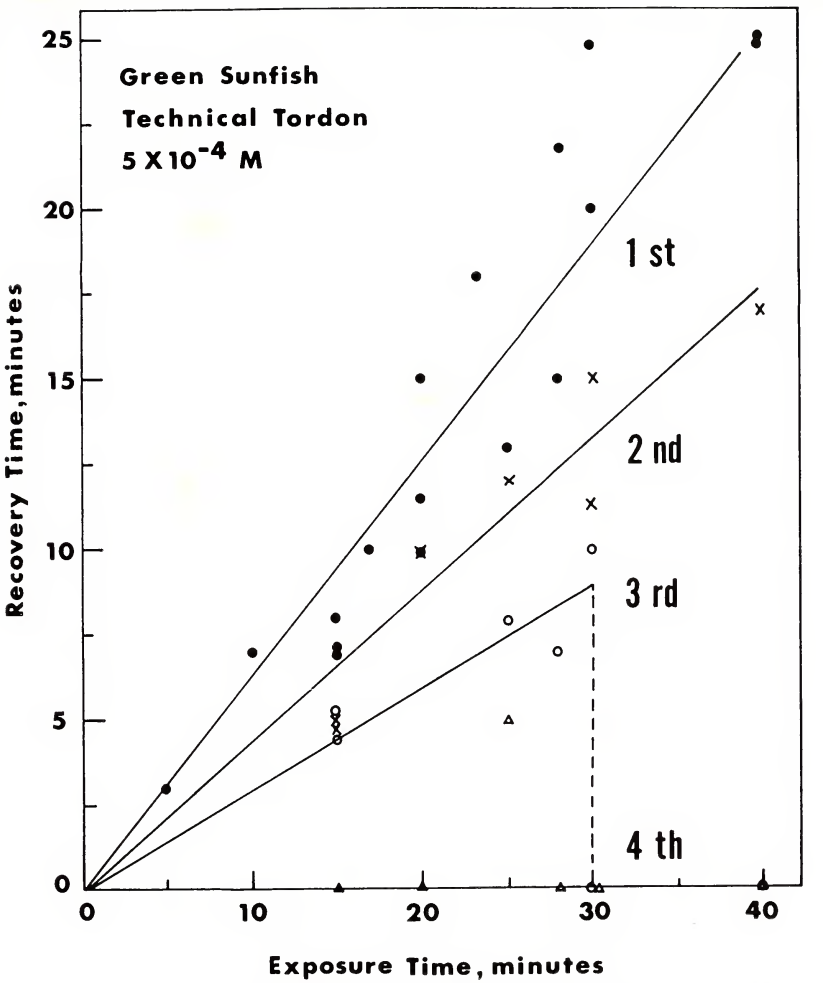



FIGURE 1. *The relationship between exposure time to 5 X 10⁻⁴ M technical picloram (91% acid) and subsequent recovery of normal swimming response of green sunfish* (Lepomis cyanellus).

the swimming response. Ultrastructural changes associated with exposure to technical picloram included the disappearance of rough-surfaced endoplasmic reticulum sheets which were observed in all hepatocytes from untreated fish (Fig. 2) and a conspicuous increase in a tubular or vesicular smooth (lacking ribosomes) form of the endoplasmic reticulum (Fig. 3). Treatment of fish for periods of up to 5 days at this same concentration of technical picloram did not result in significant changes in the ultrastructural pattern (Fig. 4) from that observed after 1 day (Fig. 3).

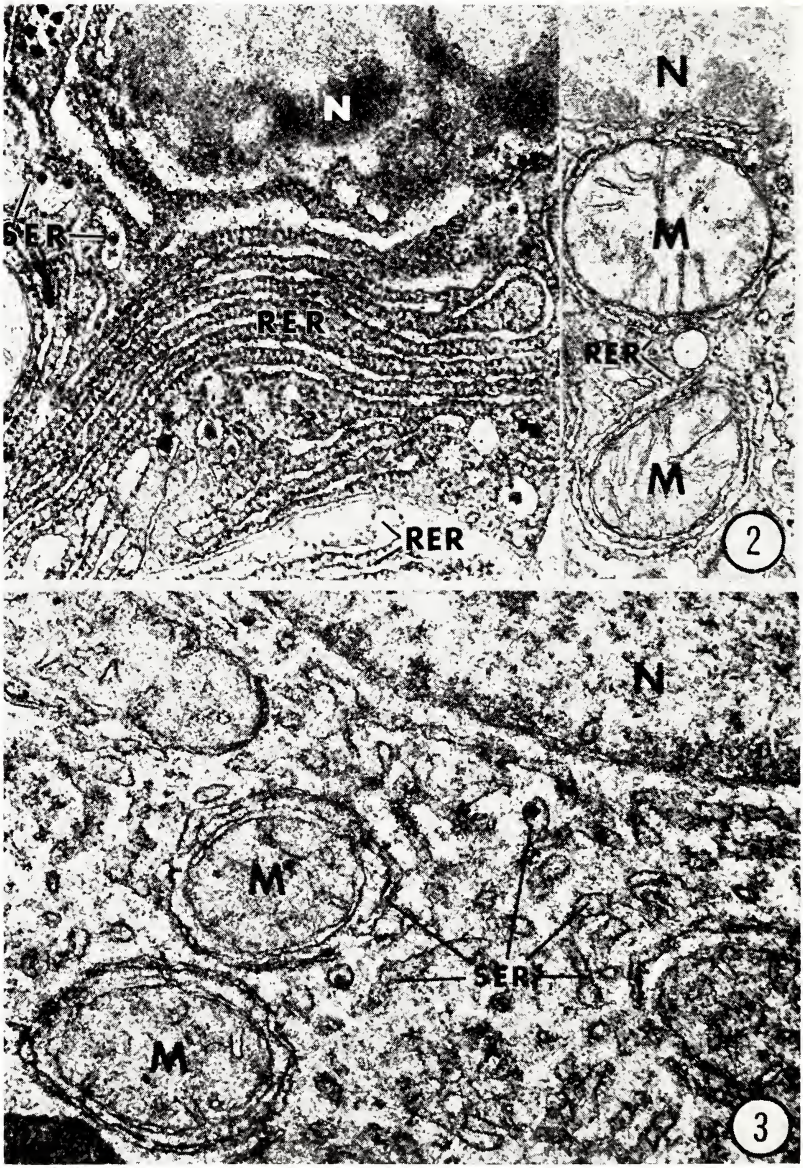



FIGURE 2. *Electron micrographs of fish* (Lepomis cyanellus) *hepatocytes. Of interest are endoplasmic reticulum profiles, most of them rough-surfaced (covered with ribosomes) in stacked or whorled arrays (RER) interspersed with short tubular or vesicular profiles of smooth endoplasmic reticulum (SER) (Left X 36,000). Most, if not all, mitochondria (M) of fish hepatocytes are surrounded by lamellae of rough-surfaced endoplasmic reticulum (Right X 32,000).* N=*nucleus.*

FIGURE 3. *Electron micrograph of a fish hepatocyte after 24 hours exposure to* $10^{-4}$ M *(91% acid) technical picloram. The large sheets of rough endoplasmic reticulum are absent and in their place are expanses of a tubular or vesicular network of smooth endoplasmic reticulum* (SER). *Ribosomes are lost from most of the endoplasmic reticulum around the mitochondria* (M) *as well, especially from the cytoplasmic surface. N=nucleus. X 35,000.*

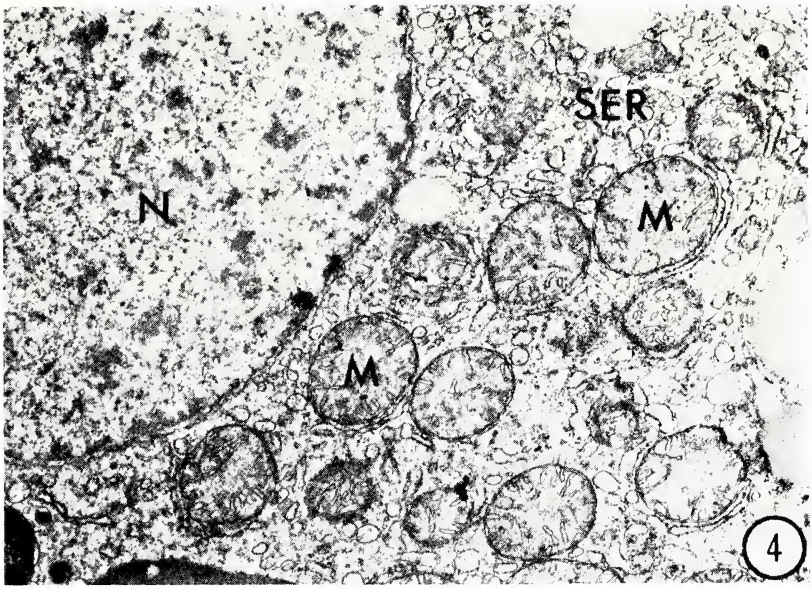
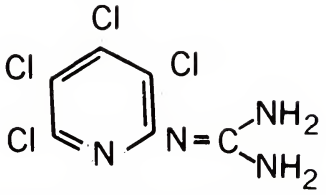



FIGURE 4. *Electron micrograph of a fish hepatocyte after 5 days of exposure to* $10^{-4}$ M *technical (91% acid) picloram. Most of the endoplasmic reticulum* (SER) *is smooth-surfaced and vesiculated. Mitochondria* (M) *and nuclei* (N) *appear unchanged from 1 day of exposure to technical picloram but considerable variation was encountered from cell to cell. The appearance of hepatocytes examined after 2, 3, 4 and 5 days of exposure to this concentration of technical picloram were similar. X 13,700.*

As a first step in determining the nature of the toxic impurity present in technical picloram, the following compounds were obtained through the courtesy of the Dow Chemical Company, Midland, Michigan:

1) 4-amino-3,5,6-trichloropicolinonitrile
2) 2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine
3) 4-amino-2,3,5,6-tetrachloropyridine
4) 6-amino-3,4,5-trichloropicolinic acid

These compounds were known to be present in technical picloram as impurities and Compound 2, illustrated below, was toxic to fish (Fig. 5).




2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine

The other 3 impurities tested were not toxic to fish at a concentration of $10^{-4}$ M (Fig. 5) and the swimming response of the fish in the presence of the compounds was normal.

TABLE 5. *Effect of technical picloram (91%) on liver weight of green sunfish* (Lepomis cyanellus).

| Treatment | Weight of Liver, % of Body Weight[1] | |
|---|---|---|
| | 5 Days | 9 Days |
| Control | 0.65 ± 0.28 | 0.56 ± 0.16 |
| Technical picloram (91% acid) $10^{-4}$M | 1.36 ± 0.61 | 1.08 ± 0.36 |

[1] Averages from 10 fish ± standard deviation.


FIGURE 5. *Green sunfish* (Lepomis cyanellus) *representative of the fish used in this study following treatment with various impurities from technical picloram (see text). Impurity 2 was toxic. In solutions containing the other three compounds (all at $10^{-4}$ M concentrations), the fish maintained a normal swimming response. 1/5 actual size.*

## Discussion

With its relatively low toxicity of 8,200 mg/kg and its rapid excretial rate from the animal body, picloram or Tordon Herbicide seems to present no serious hazard to man or terrestrial animals (17). 2,4-D is even less toxic. The toxicities of these herbicides to birds has been reported to be low (17) and no effect on reproduction of either birds or fish have been noted (17). As summarized in this report, the acute toxicity of picloram or 2,4-D to fish is also low. However, the use of ester formulations tends to increase toxicity emphasizing the need for testing specific commercial formulations, combinations of herbicides and combinations of herbicides with other types of water pollutants.

Pure picloram seems to be virtually without effect on fish. The fact that commercial picloram or Tordon Herbicide has an $LD_{50}$ to fish in the range 10-500 ppm (depending on species, size and conditions of exposure) seems to arise from a toxic impurity present in technical and commercial picloram formulations. At concentrations at or near the $LD_{50}$, the loss of swimming response is reversible and upon repeated

exposure, the fish are able to adapt to the herbicide. The subacute response is accompanied by liver enlargement, loss of large sheets of rough-surfaced endoplasmic reticulum, and the appearance of a vesicular or tubular smooth form of endoplasmic reticulum. Proliferation of smooth endoplasmic reticulum is a phenomenon commonly associated with the response of liver cells to a variety of drugs and pesticides (2, 15, 20) and with the induction of relatively non-specific steroid and drug hydrolyzing enzymes. The action of these enzymes of the smooth reticulum is to make the drugs more water soluble and more readily excreted from the organism.

The response of the endoplasmic reticulum of fish liver to technical picloram reported here is different. It involves disappearance of the rough-surfaced sheets of endoplasmic reticulum. It appears that the smooth endoplasmic reticulum of the livers from fish exposed to technical picloram is derived from rough-surfaced endoplasmic reticulum through loss of ribosomes and vesiculation. This interpretation is supported by the observation that the lamellae of rough-surfaced endoplasmic reticulum which envelops most of the mitochondria also lose ribosomes and appear smooth-surfaced in the treated cells.

Again, the liver changes were studied only with the technical picloram and may very well be a response to one or more of the impurities present rather than to the actual herbicide.

It has been the practice of some investigators and many field biologists to regard all formulations of 2,4-D or picloram as equal

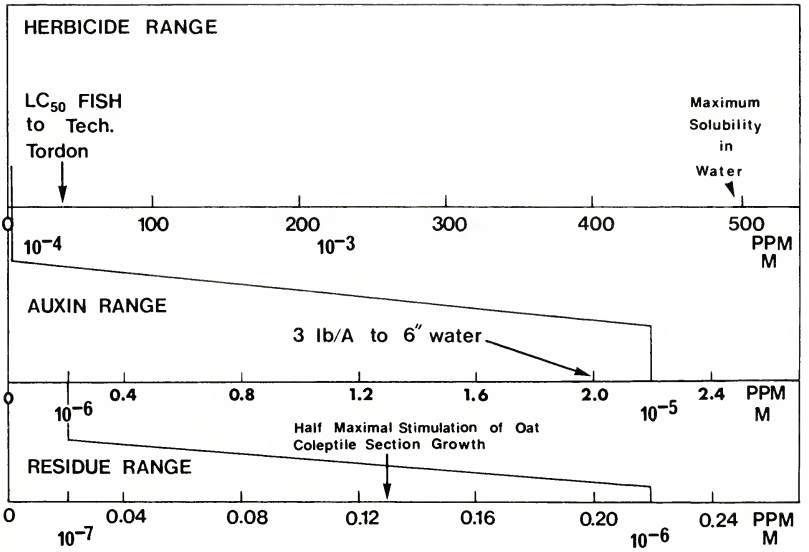



FIGURE 6. *Diagram illustrating some biological responses to picloram and 2,4-D herbicides over the range 0 to 3 X $10^{-3}$* M. *The midportion of the diagram shows an expansion of the range 0 to $10^{-5}$* M *and the bottom scale is an expansion of the range 0 to $10^{-6}$*M.

in phytotoxicity and toxicity to fish. It is apparent from our data and the data of others (1, 3, 13, 14, 17) that the formulation used is important. In shallow water, the amount of an ester formulation required to secure optimum results for weed control (Fig. 6) may result in dramatic kill of fish, not from the basic herbicide, but the manner in which it is prepared for commercial use. However, as illustrated in Figure 6, the concentrations of salt formulations of even technical picloram which are toxic to fish approach the maximum solubility of picloram acid in water and are at least an order of magnitude greater (10-fold) than those which might be expected from accidental or direct contamination of lakes or streams through normal use practices. On the same basis, the toxic concentrations reported in the study are 100-1000 fold higher than what might be expected in terms of water pollution resulting from runoff of treated soil or vegetation. Consequently, these herbicides present a low potential hazard to fish from normal agricultural or industrial practices.

## Literature Cited

1. BUTLER, P. A. 1965. Effects of herbicides on estuarine fauna. Southern Weed Conf. Proc. (18th) 576-580.

2. CONNEY, A. H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. **19**:317-366.

3. COPE, O. B., E. M. WOOD and G. H. ALLEN. 1970. Some chronic effects of 2,4-D on the bluegill (*Lepomis macrochirus*). Proc. Amer. Fish. Soc. **99**:1-12.

4. EISINGER, W. R., S. KRAWIEC, D. J. MORRÉ and R. J. HULL. 1968. Picloram: Its auxinic properties and interactions with 2,4-D. Weed Sci. Soc. Amer. Proc. 28-29.

5. ————, and D. J. MORRÉ 1966. Tordon, a new synthetic growth regulator. Proc. Indiana Acad. Sci. **75**:62.

6. ————, ———— and C. E. HESS. 1966. Promotion of plant growth by Tordon herbicide. Down to Earth **21**:8-10.

7. GORING, C. A. I., C. R. YOUNGSON and J. W. HAMAKER. 1965. Tordon herbicide disappearance from soils. Down to Earth **20**:3-5.

8. HAMAKER, J. W., H. JOHNSTON, R. T. MARTIN and C. T. REDEMANN. 1963. A picolinic acid derivative: A plant growth regulator. Science **141**:363.

9. ————, C. R. YOUNGSTOWN and C. A. GORING. 1967. Prediction of persistance and activity of Tordon herbicide in soils under field conditions. Down to Earth **23**:30-36.

10. HARDY, J. L. 1966. Effect of Tordon herbicide on aquatic chain organisms. Down to Earth **22**:11-13.

11. HEMMETT, R. B., and S. D. FAUST. 1969. Biodegradation kinetics of 2,4-dichlorophenoxyacetic acid by aquatic microorganisms. Residue Rev. **29**:191-207.

12. HERR, D. E., E. W. STROUBE and D. A. RAY. 1966. The movement and persistence of picloram in soils. Weeds **14**:248-250.

13. HUGHES, J. S. 1962. The toxicity to fish of different formulations of 2,4,5-T, 2-(2,4-DP) and Silvex. Southern Weed Conf. Proc. (15th) 265-266.

14. _____, and J. T. DAVIS. 1963. Variations in toxicity to bluegill sunfish of phenoxy herbicides. Weeds **11**:50-53.

15. JONES, A. L., and D. W. FAWCETT. 1966. Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital. J. Histochem. Cytochem. **14**:215-232.

16. KEFFORD, N., and O. H. CASO. 1966. A potent auxin with a unique chemical structure-4-amino-3,5,6-trichloropicolinic acid. Bot. Gaz. **127**:159-163.

17. KENAGA, E. 1969. Tordon herbicide—evaluation of safety to fish and birds. Down to Earth **25**:5-9.

18. KEYS, C. H., and H. A. FRIESEN. 1968. Persistence of picloram activity in soil, Weed Sci. **16**:341-343.

19. KRAWIEC, S., and D. J. MORRÉ. 1968. Interactions of TORDON herbicide applied in combinations. Down to Earth **24**:7-10.

20. KUPFER, D. 1967. Effects of some pesticides and related compounds on steroid function and metabolism. Residue Rev. **19**:11-30.

21. MALHOTRA, S. S., and J. B. HANSON. 1970. Picloram sensitivity and nucleic acids in plants. Weed Sci. **18**:1-4.

22. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Stain Technol. **35**:313-323.

23. SMITH, G. E., and B. G. ISOM. 1967. Investigation of effects of large scale applications of 2,4-D on aquatic fauna and water quality. Pesticides Monitoring J. **1**:16-21.

24. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. **26**:31-43.