# The *in Vivo* Effects of Dietary Mirex on Hepatic Lactic Dehydrogenase and Glutamic Oxaloacetic Transaminase Levels of the Rat

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Adult male and female Sprague-Dawley rats were fed standard laboratory chow, *ad libitum*, containing mirex (dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene) in concentrations of 10, 50, 100, and 200 ppm, for 4 weeks. Liver homogenates were analyzed for lactic dehydrogenase and glutamic-oxaloacetic transaminase activity. Male liver LDH levels were reduced approximately 50% in the 100 and 200 ppm of mirex exposed animals, as compared to control animals, by 2 weeks of dietary mirex and persisted through the test period. Liver GOT levels showed a steady decrease in activity, exhibiting a 60–65% decrease at the end of the 4-week treatment period. With 10 ppm of dietary mirex, male liver LDH levels showed no significant change during the 4-week period, while liver GOT levels were reduced about 26% at the end of 3 weeks. Female rat liver homogenates exhibited an approximate 24% decrease in LDH levels after 3 weeks of dietary feeding of 100 ppm of mirex, while liver GOT levels were reduced about 35% when compared to control animals at 3 weeks. Female rats fed 50 ppm of mirex showed an approximate 25% reduction of LDH levels and about a 28% decrease in GPT liver levels after 4 weeks of exposure.

Mirex (dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene), an extremely stable organochlorine compound, is an inducer of microsomal enzymes, as are many of the organochlorine compounds (Abernathy *et al.*, 1971; Baker *et al.*, 1972; Kashyap and Gupta, 1971; Wagstaff and Street, 1971). Little information is available on the *in vivo* organochlorine compound effects on soluble tissue enzymes. The studies which have been reported deal mainly with serum changes in acute dosages of organochlorine compounds (Krampl, 1970; Luckens and Phelps, 1969), although Komer (1972) reported reduction of certain enzymes in liver tissue levels of yearling lamb after 125 days of dieldrin treatment.

This study reports the *in vivo* effects of dietary mirex exposure on lactic dehydrogenase (LDH) and glutamic-oxaloacetic transaminase (GOT) in rat liver. LDH and GOT were selected because of their ubiquitous nature and their use as diagnostic indicators of tissue dysfunction.

## MATERIALS AND METHODS

Mirex diets were prepared by grinding Purina Laboratory Rat Chow to a fine grit. Chromatographically pure mirex (supplied by Allied Chemical Co.) was dissolved in warm soybean oil (4 mg of mirex/ml). Appropriate volumes of stock soybean oil solution were diluted to 1 l./10 kg of chow and thoroughly mixed to a uniform consistency. Dietary concentrations of 10, 50, 100, and 200 ppm of mirex were prepared from the stock solution. The control diet consisted of 1 l. of untreated soybean oil mixed into 10 kg of ground laboratory rat chow.

Adult male and female Sprague-Dawley rats ranging from 290 to 380 g were randomly assigned to test diets. Animals within each test group were within 1 week of age and were fed ground laboratory chow for 3 days before beginning test diets. All diets were fed *ad libitum*. For enzyme determinations, livers were rapidly removed from rats sacrificed by spinal dislocation and immediately cooled to 4°. The tissue samples were gently blotted and a 10% (w/v) homogenate was prepared in cold (4°) 0.2 *M* sodium phosphate buffer, pH 7.4, with 0.32 *M* sucrose added. The homogenate was centrifuged at 600 × *g* for 15 min, and the supernate stored at 4° until enzyme assay. The time from tissue removal to enzymatic analysis never exceeded 4 hr. Preliminary assays run on fractions stored at 4° for 1 week showed little loss of activity. A 0.1-ml sample of the homogenate was diluted with 1.9 ml of the same sodium phosphate buffer (final dilution = 0.5%) for enzymatic analysis. Although the data listed in Table I and Figure 1 are presented by weeks of exposure, individual samples were prepared during a 4-day period and were placed in weekly groupings for ease and simplification of data presentation. All assays were performed with Boehringer-Mannheim Clinical Reagent Kits utilizing the methods of Worblewski and LaDue (1955) and Karman *et al.* (1955) for LDH and GOT determinations, respectively. Total protein was determined by the method of Lowry *et al.* (1951). All enzymatic assays were determined spectrophotometrically on a Beckman DBG recording spectrophotometer. Tissues were examined histologically from paraffin embedded sections, stained with hematoxylin and eosin.

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## RESULTS

Although there was no apparent change in male LDH liver levels at a dietary level of 10 ppm of mirex at the end of the 30-day exposure, in both 100 and 200 ppm of mirex diets, there was an approximate 50% reduction in LDH levels, as compared to control (Table I). Samples were not assayed at 2 weeks of 10 ppm of mirex diet in order to conserve experimental animals for assay at 8 weeks of 10 ppm of mirex diet. At the end of the 8-week exposure period, there was a 17% reduction of LDH and 31% reduction in GOT levels as compared to 8-week controls.

The drop in the tissue enzyme level at 100 and 200 ppm appears between the first and second week of exposure and the enzyme levels remain depressed at about 50% of control until the end of the treatment period, and remained depressed up to 2 weeks after termination of the mirex diet.

There is no dosage effect as indicated by comparing the male rat liver responses at 100 and 200 ppm of mirex treatments. The lack of a dosage effect related to the tissue enzyme response is interesting. This probably indicates that the high treatment levels chosen (100 and 200 ppm) are beyond the linear portion of a typical dosage response curve.

The female response is quite different; the female rat liver LDH levels appear much less sensitive to mirex ex-

**Table I. Liver LDH and GOT Levels of Adult Rats Fed Dietary Mirex[c]**

| Lactic dehydrogenase, ppm | Weeks | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Males | | | | |
| Control | 3.52 ± 0.13 (4)[a] | 3.74 ± 0.47 (5) | 4.30 ± 0.27 (3) | 3.14 ± 0.11 (5) |
| 10 | 3.39 ± 0.08 (4) | | 4.00 ± 0.18 (4) | 3.21 ± 0.18 (5) |
| 100 | 3.05 ± 0.19 (4) | 1.76 ± 0.13[b] (3) | 1.76 ± 0.39[b] (4) | 1.59 ± 0.07[b] (5) |
| 200 | 2.89 ± 0.16 (4) | 1.83 ± 0.12 (4) | 2.01 ± 0.16[b] (4) | 1.56 ± 0.16[b] (5) |
| Females | | | | |
| Control | 2.43 ± 0.23 (4) | 2.35 ± 0.28 (2) | 2.54 ± 0.18 (4) | 2.52 ± 0.06 (2) |
| 50 | 2.51 ± 0.11 (4) | 2.21 ± 0.11 (2) | 2.35 ± 0.22 (4) | 1.82 ± 0.20 (2) |
| 100 | 2.35 ± 0.30 (4) | 2.09 ± 0.09 (2) | 1.89 ± 0.11 (3) | |
| Glutamic oxaloacetic transaminase, ppm | | | | |
| Males | | | | |
| Control | 0.390 ± 0.026 (4) | 0.420 ± 0.039 (5) | 0.429 ± 0.024 (3) | |
| 10 | 0.417 ± 0.043 (4) | | 0.317 ± 0.048 (4) | |
| 100 | 0.301 ± 0.031 (4) | 0.216 ± 0.025[b] (4) | 0.161 ± 0.019[b] (3) | |
| 200 | 0.294 ± 0.047 (4) | 0.226 ± 0.013[b] (4) | 0.163 ± 0.006[b] (4) | |
| Females | | | | |
| Control | 0.293 ± 0.026 (4) | 0.263 ± 0.00 (2) | 0.274 ± 0.013 (4) | 0.424 ± 0.027 (2) |
| 50 | 0.270 ± 0.014 (4) | 0.217 ± 0.022 (2) | 0.175 ± 0.008[b] (4) | 0.297 ± 0.050 (2) |
| 100 | 0.265 ± 0.010 (4) | 0.194 ± 0.045 (2) | 0.168 ± 0.007[b] (4) | |

[a] Number of individuals. [b] Significantly different from control value ($p < 0.005$) according to $t$ test of Cochran and Cox. [c] Values are mean values, expressed as units/mg of protein + standard error. (Unit = 1 μmol of substrate/min).

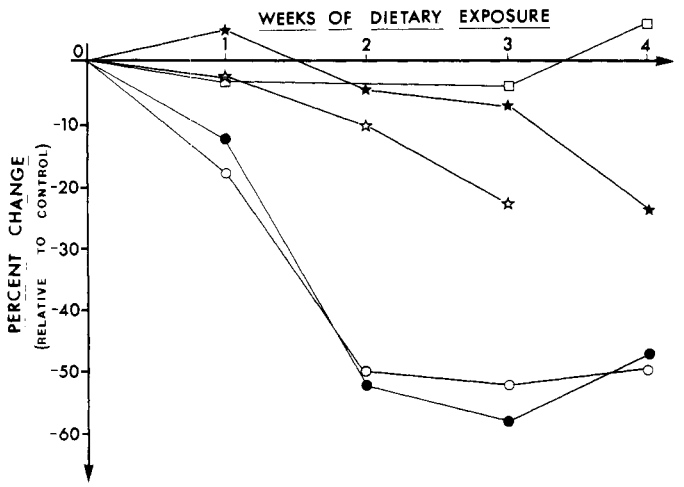


**Figure 1.** The *in vivo* effects of dietary mirex on hepatic lactic dehydrogenase levels of the rat, expressed as percent change relative to control. Males: 10 ppm (□), 100 ppm (●), 200 ppm (○). Females: 50 ppm (★), 100 ppm (☆).

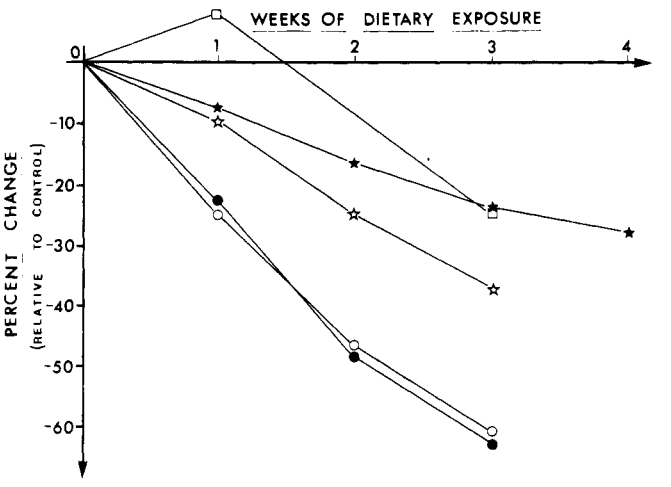


**Figure 2.** The *in vivo* effects of dietary mirex on hepatic glutamic oxaloacetic transaminase levels of the rat, expressed as percent change relative to control. Males: 10 ppm (□), 100 ppm (●), 200 ppm (○). Females: 50 ppm (★), 100 ppm (☆).

posure than male levels (Table I). With the 100 ppm of mirex diet, there was an approximate 25% decrease, relative to control, after 3 weeks of exposure while on the 50 ppm of mirex diet, there was about 25% LDH reduction at the end of 4 weeks. The drop in tissue enzyme levels appears between the second and third week for the 100 ppm treatment, while the response to 50 ppm of mirex appears between the third and fourth week. Statistical analysis ($t$ test) shows that a ±10–20% change, relative to control, is not significant for either male or female LDH or GOT liver levels. The drop in male liver GOT levels at 100 and 200 ppm of mirex appears during the first week of exposure reaching a 60–65% reduction at the end of 3 weeks of exposure. This is similar to the LDH response (Figure 1). At 10 ppm of dietary mirex, a 25% reduction in male rat liver GOT levels appears at the end of 3 weeks.

As was the case with LDH levels, female GOT liver levels appear much less affected than do the male levels. However, whereas the two highest levels used (100 and 200 ppm of mirex) in males were similar in effect, the two highest levels used (50 and 100 of mirex) in females exhibited a difference in response (Figure 2).

## DISCUSSION

It is a well established and accepted fact that "soluble" enzymes are a very important adjunct to clinical diagnosis of tissue damage and disease. Both LDH and GOT in conjunction with other enzymes are used as indicators of liver and heart damage. Physiologically they are involved in cellular metabolism and the energy processes of the cell. LDH is a widely distributed enzyme present in most animal tissues (Worblewski, 1958). It is specifically involved in the interconversion of pyruvic acid and lactic acid. Tissue damage, such as necrosis, leads to a release of cellular LDH and a subsequent increase in serum LDH levels.

GOT is involved in the interconversion of glutamate to α-ketoglutarate and is found both in the soluble cytoplasm and mitochondria of the cell. In conjunction with

other enzymes (GPT), changes in GOT levels are significant in liver damage, skeletal muscle diseases (peripheral myopathies), and acute pancreatitis.

This study clearly indicates that mirex affects tissue-soluble enzyme levels and, in terms of physiological response, the decrease in LDH and GOT levels is indicative of membrane damage (Bergmeyer, 1965). This damage, however, is not apparent on examination of histologically prepared tissue. There is only a slight indication of fatty infiltration in livers of mirex-treated rats and this is not considered significant. Further, reduction in LDH levels is not due to mirex inhibition of LDH activity; in *in vitro* exposure of liver preparations to $10^{-4}$, $10^{-5}$, and $10^{-6}$ *M* mirex, there was no inhibition of LDH activity.

Komer (1972) reported reduction of tissue glutamic dehydrogenase, glutamic-pyruvate transaminase (GPT), and glutamic synthetase in yearling lamb liver with a 27 ppm of dieldrin diet for 125 days. The decrease in levels ranged from 30 to 50% for the enzymes studied. Our data are similar, although the higher treatment levels used produced an approximate 50% reduction of both LDH and GOT in 30 days.

Mirex is about twice as toxic to females as it is to males based on dietary 30-day $LC_{50}$ values of 275 and 607 ppm, respectively (unpublished data). This is not consistent with what has been reported for acute $LD_{50}$ mirex values. These values are 600 mg/kg for females and 306 mg/kg for males (Martin, 1971; Spencer, 1968). There is an apparent sex reversal between acute and short term chronic toxicity. The enzyme reduction pattern apparently correlates with the $LD_{50}$ values, but is the reverse of the $LC_{50}$ toxicity. The reason for this reversal is not apparent, although the difference in males and female control levels of LDH and GOT may possibly be involved in this reversal. Based on toxicity (30 day $LC_{50}$ value), the 100 ppm of mirex diet in males and the 50 ppm of mirex diet in females are equivalent, with both being approximately one-sixth of the respective $LC_{50}$ value. The 100 ppm of mirex diet in females is also comparable to the 200 ppm of mirex dietary level in males, with both being about one-third of the $LC_{50}$ value. There was a twofold reduction of male LDH levels as compared to female levels at both comparison treatment levels. This same pattern is seen with GOT liver levels.

Both LDH and GOT are diagnostic markers used in determining liver necrosis and chronic liver disease. Although no histopathological changes were observed in our study, the enzyme response is similar to what has been reported for carbon tetrachloride toxicity. Schwetz and Plaa (1969) report that histopathological changes seen in $CCl_4$ hepatotoxicity coincide with increases in serum GOT activities. Peak activity of serum GOT occurs 24 hr after $CCl_4$ treatment of rats (Koeferl and Larson, 1970). Although these data report increases in serum levels, the response in tissue is compatible since a decrease in tissue enzyme levels would appear as increased serum levels. In the present study, both serum LDH and GOT levels increased as the tissue enzyme levels decreased. Luckens and Phelps (1969) reported significant increases in serum GOT, GPT, and LDH levels after single, acute doses of DDT, aldrin, dieldrin, and endrin. Heptachlor has also been shown to increase serum GPT and serum aldolase levels in rats (Krampl, 1970).

The reduction of LDH and GOT levels in the mirex-treated animals is symptomatic of liver damage, which is a more subtle response than histological examination indicates. As a sensitive indicator of the cellular response to mirex, monitoring tissue enzyme levels may serve as an index of chronic insecticide exposure and provide a diagnostic tool by which cellular damage could be assayed. With further study, this could be used to measure the total cellular effects of other organochlorine compound exposures.

The primary effect of organochlorine compound exposure is disruption of peripheral nervous function with subsequent respiratory paralysis and death. However, if exposure does not cause immediate death, it may reduce an animal's ability to meet and survive natural environmental stresses, thereby producing a secondary effect which may be more damaging to a population than the initial toxicity would indicate.

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