Acute Oral Toxicities of Three Pesticides Used in Egyptian Rice Farms to Albino Rats

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ABSTRACT

The present study was carried out to determine the acute rat toxicity of three commercial pesticide formulations recommended against rice pests (i.e., Sumithion 50% EC as insecticide, Sayno 50% EC as herbicide and Fuji-one 40% EC as fungicide). All the tested formulations were administered orally to adult male and female albino rats and the median lethal doses (LD<sub>50</sub>) values were determined after 24, 48, 72 and 96 hours post-treatment. The obtained results revealed that the oral LD<sub>50</sub> values of aforementioned pesticide formulations on adult male and female rats were (1166.983, 1253.818), (895.698, 966.445) and (966.445, 895.698) mg/Kg body weight (b.w.) after 24 hrs, respectively. Moreover, the 48hrs oral LD<sub>50</sub> values of the tested pesticide formulations mentioned above were (1012.383, 1081.599), (721.09, 838.617) and (721.09, 781.164) mg/Kg b.w., respectively. No rat mortality was occurred after 48hrs. Therefore, the LD<sub>50</sub> values after 72 and 96 hrs were the same as determined at 48hrs. The obtained results indicated that the male rats were more sensitive to these toxicants than females. Also, the acute toxicities of these commercial pesticide formulations seemed to be more toxic than their reported toxicities which based on active ingredients. It was surprising that, the candidate fungicide and herbicide exhibited higher acute toxicity to rats than the Sumithion insecticide.

Key words: Pesticide Formulation - LD<sub>50</sub> - Albino Rats - Rice.

Introduction

The toxicity of any chemical can be measured in several ways, but generally human toxicity is estimated based on test results on rats and other animal models. Any substance that is poisonous to rats is not necessarily equally poisonous to humans or other animals. Some pesticides are fatal after one lethal dose (acute toxicity); others can be dangerous after sublethal, repeated doses (chronic toxicity). One simple measure of toxicity use bioassays to measure death rates in order to quantify the effect of the toxin. This measure is commonly known as a lethal dose 50 (LD<sub>50</sub>). The LD<sub>50</sub> is usually an initial screening step in the assessment and evaluation of the toxic characteristic of a substance. Therefore, a pesticide LD<sub>50</sub> normally provides information on health hazards likely to arise from short-term exposure and serve as a basis for labeling and classification and also helpful in establishing a dosage regimen in sub-chronic and chronic studies (Chandra et al., 2014).

Pesticides, in general, perform their activity by acute toxicity to the target organisms (Matsunaka, 1992) and the simplest acute toxicity study employs LD<sub>50</sub> value determination. The toxicity of chemicals has been classified according to their oral LD<sub>50</sub> values, giving simple approximate expression of the degree of toxicity (Matsumura, 1995).

Fenitrothion belongs to the organophosphorus insecticides, it is a contact insecticide and selective acaricide of low ovicidal properties (Spencer, 1981) and it is considered a cholinesterase inhibitor (Kidd and James, 1991). The commercial formulation of fenitrothion insecticide (Sumithion 50% EC) is used for controlling chewing, sucking, and boring insects in cereals, soft fruit, tropical fruit, vines, rice, sugar cane, vegetables, turf, and forestry. Also used as a public health insecticide for control of household insects (flies, cockroaches, and other insects) by application to breeding sites; for control of flies in animal houses; for control of stored product insect pests; for control of mosquito larvae (as a vector control agent for malaria); and for control of locusts (Anonymous, 2003).

Herbicides can cause deleterious effects on organisms and human health, both by their direct and indirect actions (Kortekamp, 2011 and Zeliger, 2011). With respect to the toxicity, some herbicides pose major concerns when applied in regions close to water resources due to their highly toxic potential to many aquatic organisms (Polard et al., 2011). The contamination of aquatic environments by herbicides has been characterized as a major world concern. This aquatic contamination is due to the use of these products in the control of aquatic plants, leachate and runoff of agricultural areas (Ying and Williams, 2000).

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The commercial formulation of thiobencarb herbicide (Sayno 50% EC) is used for Pre-emergence to early post-emergence control of Echinochloa, Leptochloa, and Cyperus spp. and other monocotyledonous and annual broad-leaved weeds in direct-seeded and transplanted rice, at 3-6 kg ha\(^{-1}\) (Anonymous, 2003).

Fungicides are extensively used in industry, agriculture, the home and garden. Fungicides vary enormously in their potential for causing adverse effects in humans. According to the EPA manual, Recognition and Management of Pesticide Poisoning, most fungicides currently in use are unlikely to cause frequent or severe systemic poisonings. Apart from poisonings that affect the body generally, fungicides have probably caused disproportionate numbers of irritant injuries to skin and mucous membranes, as well as some dermal sensitization (Larry et al., 2001 and Morgan, 1999). The acute toxicity of fungicides to humans is generally considered to be low, but fungicides can be irritating to the skin and eyes. Inhalation of spray mist or dust from these pesticides may cause throat irritation, sneezing and coughing (Pesticide Safety Fact Sheet, 2009).

The commercial formulation of isoprothiolane fungicide, Fuji-one 40% EC is used to control the following pathogens and insect pests, at 400-600 g ha\(^{-1}\) (foliar spray), 3.0-6.0 g/nursery box (spreading treatment), 3.6-6.0 kg ha\(^{-1}\) (submerged treatment), 360-600 g tree\(^{-1}\) (soil incorporation): Pyricularia oryzae, Helminthosporium sigmoideum, Fusarium nivale on rice; Rosellinia necatrix on pome fruit, stone fruit and grapes; Delphacidae (planthoppers) on rice. Also used in rice to accelerate rooting, promote root elongation and control non-parasitic damping-off (Anonymous, 2003).

In agriculture, the use of pesticide has been the dominant form of pest management since the 1950s to kill pest organisms including insects, weeds, fungi and nematodes. In recent times, use of pesticides in rice farming has increased rapidly and this scenario contributes significantly towards adverse effects on human health and environment (Ahmad et al., 2014 and Snelder et al., 2008). In rice farming, pesticide application has been promoted among farmers in developing countries to increase their productivity and enhance economic potential for farm households (Ecobichon, 2001). A proportion of the pesticide that is not absorbed by the plants will be moved and transferred to the environment through wind, water and soil. Depending on the physicochemical properties, it can be transported thousands of miles away and can infiltrate into meat, milk, human blood, animal and plants, which may result in serious health implications to humans and the environment. In addition, continuous pesticide application may put farmers’ health at risk, with pesticides being dispersed, spilled or leaked and entering the human body either directly or indirectly. In the literature, there is vast evidence that pesticides do pose a potential risk to humans (Igbedioh, 1991), and other life forms as well as unwanted side effects to the environment (Zhang et al., 2010). In relation to that, various reports of ill health associated with those applying pesticides has been reported by Food and Agricultural Organisation (FAO, 2014).

The LD\(_{50}\) values are generally expressed on the basis of active ingredient. If a commercial product is formulated to contain 50 percent active ingredient, it would take two parts of the material to make one part of the active ingredient. In some cases, other chemicals mixed with the active ingredient for formulating the pesticide product may cause the toxicity to differ from that of the active ingredient alone (Norman et al., 2014). Moreover, because of determining the toxicity of a pesticide to humans is not easy, since humans cannot be used as the test subject, other animals, such as rats, are used (Clyde et al., 2012). The results of these toxicity tests are used to predict the safety of the new chemical to humans and human toxicity from exposure to small doses for long periods of time (Norman et al., 2014).

Thus, the present work was undertaken to evaluate the acute oral toxicity of three rice farming pesticide formulations used by rice farmers in Egypt i.e. Sumithion 50% EC (fenitrothion insecticide), Sayno 50% EC (thiobencarb herbicide) and Fuji-one 40% EC (isoprothiolane fungicide) through the determination of their LD\(_{50}\) values on adult male and female albino rats.

Materials and Methods:

A- Pesticides:

1. Sumithion 50% EC (fenitrothion, organophosphorus insecticide):
IUPAC Chemical name:
\(O,O\)-dimethyl \(O\)-4-nitro-\(m\)-tolyl phosphorothioate.

The commercial formulation of fenitrothion insecticide, Sumithion 50% EC was obtained from Sumitomo Corporation Cairo Company.

2. Sayno 50% EC (thiobencarb, thiocarbamate herbicide):
IUPAC Chemical name:
S-4-chlorobenzyl diethylthiocarbamate.

The commercial formulation of thiobencarb herbicide, Sayno 50% EC was obtained from Egypt for Fertilizers and Chemicals Company (EFC Co.).
3. Fuji-one 40% EC (isoprothiolane, dithiolane fungicide):  
IUPAC Chemical name: di-isopropyl 1,3-dithiolan-2-ylidenemalonate.  
The commercial formulation of isoprothiolane, fungicide, Fuji-one 40% EC was obtained from Shoura Chemicals Company, Cairo. All commercial formulations of pesticides used were obtained from the Research Institute of Plants Protection, Dokki, Giza.

B-Experimental animals:  
Albino rats (Wister strain) were obtained from Helwan Farm of Egyptian Organization for Vaccine and Biological Preparations. Male and female rats ranging in weight from 180-220 gm were used for experimentation. Rats were fed on standard chow diet obtained from El-Salam Factory for Dry Ration-El-Marg - Egypt; and water was freely provided ad libitum. Male and female rats were separated and placed in stainless steel cages, with different capacities i.e. 35 x 25 x 20 cm and 30 x 20 x 20 cm, (5 animals group-caged by dose and by sex). All animals were allowed for acclimatization to the laboratory conditions for 10 days prior to dosing. Temperature 22°C ± 3, relative humidity 30 - 70%and 12 hours light/dark cycle. At the beginning of experiments, unhealthy rats were excluded; and animal body weight was measured to the nearest gram. The total numbers of rats used in the present studies were 105 adult male and 105 adult female rats.

C-Experimentation:  
The acute oral LD$_{50}$ values of Sumithion 50% EC, fenitrothion insecticide, Sayno 50% EC, thiobencarb herbicide, and Fuji-one 40% EC, isoprothiolane fungicide, (i.e. three compounds) on adult male and female rats were determined. The total number of rats for each sex was divided as: 3 compounds × 6 doses for each compound plus the control (i.e.7 treatments) × 5 rats for each treatment = 105 rats. Prior to dosing, animals were deprived of food for 12-15 hrs overnight but given free access to water. Each pesticide was dissolved in corn oil (1 ml/kg b.w. rat), while untreated animals were given 1 ml corn oil/kg b.w..

Oral dosing was carried out using a glass syringe attached to a curved stainless steel animal intubation needle with spherical ball tip. Doses were prepared shortly prior to administration.

The tested doses of Sumithion were 0, 600, 900, 1200, 1500 1800 and 2100 mg/kg body weight (b.w.). In the case of Sayno and Fuji-one, the doses were 0, 400, 600, 800, 1000, 1200 and 1400 mg/kg b.w. for both male and female rats. After toxicant administration, the animals were provided, with free access to food and water, and are observed at least once during the first 30 minutes, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter and the mortality rate was recorded for 4 days. The LD$_{50}$ values were determined according to the “Probit analysis” technique described by Finney (1971).

All experiments of the present study were conducted at the laboratories of pesticides research, Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Results and Discussion  
The types of toxicity tests that are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD$_{50}$ values. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds (Shetty et al., 2007). Pesticides may be classified by target organism, chemical structure and physical state, etc. Chemical structures differ within categories as well as between categories. Thus, toxicity to target and non-targeted species can vary widely within each group. Rampant use of pesticides in agricultural products continues to risk the life of the common man. Thus, it is very important to know the LD$_{50}$ value of the pesticide before using it in the fields (Raj et al., 2013).

It was observed that all rats had signs of intoxication, which varied in severity according to the dose administered. The animals showed toxicity signs of hyperexcitability, profuse salivation, tremors, burrowing behavior, frequent maturation, diarrhea, hunched back, muscular incoordination, weakness, altered gait, startle response, writhing movements of neck, violent twisting movements sometimes lifted from the body from floor, labored breathing, gasping, convulsions and death. There was full recovery of surviving rats (Lyaniwura and Okonkwo, 2004).

The determined LD$_{50}$ values are shown in tables (1 and 2) and Figs. (1 and 2). Generally, it was found that all rat mortalities occurred within 48 hrs from pesticides treatment. The LD$_{50}$ values of an organophosphorus insecticide fenitrothion (Sumithion 50% EC) for male rats were 1166.983 mg/kg b.w. after 24 hrs and 1012.383 mg/kg after 48 hrs post treatment. While the values of LD$_{50}$ for female rats were 1253.818 mg/kg after 24hrs and 1081.599 mg/kg after 48 hrs post treatment, which indicated clearly that fenitrothion insecticide was more toxic to male rats than to female rats. These findings are coincided with.
those obtained previously by other investigators, the acute oral LD<sub>50</sub> values for male and female rats were 1700 and 1720 mg/kg, respectively (Anonymous, 2003). It was reported that the acute oral LD<sub>50</sub> values of fenitrothion to rats ranges between 250-800 mg/kg (OHS, 1993). Worthing and Walker (1987) recorded an oral LD<sub>50</sub> value of fenitrothion of 800 mg/kg for female rats. In addition, Mikami et al. (1977) estimated the acute oral of fenitrothion in corn oil on male and female rats. The LD<sub>50</sub> value was 660 mg/kg b.w. for males and 1050 mg/kg b.w. for females. Consequently, the obtained LD<sub>50</sub> values of fenitrothion are in agreement with that of Rosival et al. (1976) and Kadota et al. (1972). They found that the acute oral LD<sub>50</sub> values of fenitrothion insecticide were 700 and 800 mg/kg for male and female rats, respectively. Whereas Benes and Cerna (1970) and Gaines (1969) reported that the LD<sub>50</sub> values of fenitrothion to adult male rats were 940 and 740 mg/kg, respectively, when administered orally.

It has been indicated that the acute toxicity of fenitrothion to mammals is considered to be low due to its high metabolic rate (Gallo and Lawryk, 1991). Fenitrothion is more readily broken down and does not persist in areas where they are used and it was developed in place of parathion for its highly selective toxicity to insects over humans and animals (Hayes, 1982 and Spencer, 1981).

On the basis of the obtained LD<sub>50</sub> values shown in Tables (1 and 2) and Figs. (1 and 2) the LD<sub>50</sub> values for thio carbamate herbicide thiobencarb (Sayno 50% EC) were 895.698 and 721.09 mg/kg at 24 and 48 hrs after dosing and 721.09 mg/kg at 24 and 48 hrs of dosing for female rats, respectively, which indicated that thiobencarb herbicide was more toxic to male than female rats. These findings are in agreement with those obtained previously by EFSA (2013) who found that the acute oral LD<sub>50</sub> values of technical thiobencarb 96% and 99.2 % were 1033 and 920 mg/kg for male rats and 1130 and 960 mg/kg b.w. for females, respectively. In addition, thiobencarb acute oral LD<sub>50</sub> values in rats ranged from 920 to 980 mg/kg b.w. (Louisiana Suggested Weed Management Guide, 2012).

According to the Anonymous (2003), WSSA (1983) and Nishimura (1985) the acute oral LD<sub>50</sub> values of thiobencarb to male and female rats were 1033 and 1130 mg/kg with lower and upper ranges of 924-1155 mg/kg and 1033-1247 mg/kg, respectively.

However, the present results are in contradiction with those obtained by MSDS (2014) and Bayer Crop Science (2010) who demonstrated that the acute oral LD<sub>50</sub> values of thiobencarb were 1414 and 1231 mg/kg for male and female rats, respectively.

On the other hand, in accordance with the obtained LD<sub>50</sub> values shown in Tables (1 and 2) and Figs. (1 and 2) the LD<sub>50</sub> values for dithiolane fungicide isoprothiolane (Fuji one 40% EC) were 966.445 and 838.617 mg/kg after 24 and 48 hrs after dosing for female rats, respectively; while they were 895.698 mg/kg at 24 hrs and 781.164 mg/kg at 48 hrs after treatment for male rats which indicated that males generally were more sensitive than females. These findings are in agreement with those obtained previously by (Agro-care Chemical Industry Group Limited, 2002 and CAPL, 2009) who found that the acute oral LD<sub>50</sub> values of isoprothiolane were 1190 and 1340 mg/kg for male and female rats, respectively. SANS (2010); Sigma-Aldrich (2010) and Farm Chemicals Handbook (1991) found that the acute oral LD<sub>50</sub> value of isoprothiolane for rats was 1190 mg/kg.

It was reported that isoprothiolane, trade name – Fuji one, a related dithiolane fungicide, belong to organosulphur group of compound is also used as an insecticide (Selvi Arul et al., 2013). These are mainly used to control rice blast disease and to reduce the population of brown plant hopper and leaf hopper in rice plants (Uesugi, 2001). This fungicide has adverse effects on humans resulting in eye irritation, serious eye damage and severe acute toxicity. Isoprothiolane residues in water and soil are of concern as their uptake can lead to the accumulation of primary products (Fushiwaki et al., 1993), and results in the toxic effect to the non target species such as humans through food chain (Uesugi, 2001).

It was surprising that, the candidate fungicide and herbicide exhibited higher acute toxicity to rats than the Sumithion insecticide. It has been reported that several factors influence acute LD<sub>50</sub> values including sex of the animals used and formulation of pesticide (Coombs et al., 1979); route of exposure is critical in assessing the potential of a toxicant. Some of these factors probably contributed to the variation of the oral LD<sub>50</sub> values between male and female rats.

As shown in Tables (1 and 2) and Figs. (1 and 2) the sex differences in susceptibility to pesticides in rats have been well demonstrated. In general, male rats were more sensitive than females. The published literature records cases when male rodents are more sensitive to xenobiotics than females. While these data suggest that the sexes are not equally sensitive to all of the pesticides tested, no clear cut generalizations about sex sensitivity could be made; although females were often more sensitive, this was not always true (Rispin et al., 2000). In a comparison of male and female rat oral and dermal LD<sub>50</sub> values, pesticides showed significant differences in sensitivity in male and female rats (EPA, 1991). Moser et al. (1998) and Timbrell (1991) notes that chlorpyrifos is more acutely toxic to male rats than to females. This may be due to differences in the way that vital organs react to toxins can also have a significant impact on overall toxicity. The insecticides such as aldrin and heptachlor are metabolized more rapidly to the toxic epoxide forms in male rats. These pesticides demonstrate a lower toxicity in the female rat.
In addition, metabolic differences due to gender can also have an effect on sensitivity for acute effects; Sipes and Gandolfo (1991) suggested that because male rats metabolize most foreign compounds faster than females, one would expect the biological half-life of most xenobiotics to be longer in the female than the male rat. However, if a metabolite or intermediate is responsible for the toxic response, male rats would be expected to show the greater susceptibility.

Certain organophosphate pesticides are detoxified by glutathione S-transferases. However, cytochrome P450 (CYP) mediated metabolism can also cause formation of reactive metabolites. Female rats are known to have 10-30% less total CYP as compared with male rats (Kedderis and Mugford, 1998). Conjugative enzymes, i.e. sulfotransferases, glutathione S-transferases and glucuronyltransferases, also play a role in detoxification. Sex-dependent differences have also been found in expression of these enzymes. When such sex-dependent differences are seen, it is generally the male rats which have higher enzyme activities. For example, glutathione protects tissues against electrophilic attack by xenobiotics. DeBethizy and Hayes (1994) noted that glutathione conjugating activity toward dichloronitrobenzene is two- to three-fold higher in male than female rats. Conjugation of xenobiotics may not always lead to more rapid excretion of the conjugated metabolite. In fact, some compounds are toxic only after conjugation with glutathione. Glutathionyl conjugates which are implicated in nephrotoxicity would be likely to show greater toxicity in males than females.

In accordance with the obtained LD50 values, it was reported that the formulations of pesticides affect their toxicities (El-Sebae et al., 1978) and the pesticide formulations seem to be more toxic than their active ingredients alone (El-Sebae et al., 1982). Since the technical grade is usually formulated (mixed with carriers, solvents, etc.) for use in commercial pest control, the toxicity of these other ingredients must be taken into consideration when assessing the toxicity of a formulated product (Williamson, 1989). The WHO (1991) emphasized that the final toxic classification of any pesticide is related to its formulation form which appears to enhance pesticide toxicity. This may be due to the effects of additives such as organic solvents and surfactants in the formulation form of pesticides which increase its penetration through its target tissue of organisms causing more toxicity than the pure active ingredient materials of the pesticides (El-Sebae et al., 1978, 1982; WHO, 1991 and Abou-Zeid et al., 1993).

Consequently, these findings may be due to the fact that many formulations contain adjuvants (stabilizers, penetrants, surfactants) that may have significant irritating and toxic effects in addition to the primary herbicide. A number of premixed products may be combination formulations with additional active ingredients that are more toxic than the principal herbicide. Therefore, it is important to read the label to identify each active ingredient and its associated toxicities. Good hygienic practice should not be disregarded because only the primary pesticide is reported to have a high LD50 in laboratory animals (Roberts et al. 2009 and Eddleston et al. 2002).

Finally, it will be needed to carry out research aims at more effective methods (e.g. Subacute, subchronic and chronic toxicity studies) to prevent and/or reduce the deleterious effects of such compounds on the environment, the biota potentially exposed, and especially to human health (Marin-Morales et al., 2013). Additionally, with further expansion and intensification in rice production, extended research on pesticide use among farmers is then essential. This in turn may help in adapting safe pesticide management practices in rice farming (Ahmad et al., 2014).

### Table 1: The acute oral LD50 values of tested pesticide formulations for male albino rats after 24 and 48 hrs of treatment.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>24 hrs LD50 values (mg/kg)</th>
<th>Confidence limits (mg/kg)</th>
<th>Slope</th>
<th>48 hrs LD50 values (mg/kg)</th>
<th>Confidence limits (mg/kg)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion</td>
<td>1166.983</td>
<td>950.292 - 1389.28</td>
<td>3.185</td>
<td>1012.383</td>
<td>955.511 - 1087.573</td>
<td>3.823</td>
</tr>
<tr>
<td>Thiofanecarb</td>
<td>895.698</td>
<td>722.505 - 1139.334</td>
<td>3.166</td>
<td>721.09</td>
<td>660.841 - 783.373</td>
<td>3.240</td>
</tr>
<tr>
<td>Isothiocyanate</td>
<td>966.445</td>
<td>796.276 - 1256.812</td>
<td>2.958</td>
<td>721.09</td>
<td>660.841 - 783.373</td>
<td>3.240</td>
</tr>
</tbody>
</table>

### Table 2: The acute oral LD50 values of tested pesticide formulations for female albino rats after 24 and 48 hrs of treatment.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>24 hrs LD50 values (mg/kg)</th>
<th>Confidence limits (mg/kg)</th>
<th>Slope</th>
<th>48 hrs LD50 values (mg/kg)</th>
<th>Confidence limits (mg/kg)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion</td>
<td>1253.818</td>
<td>994.236 - 1558.004</td>
<td>3.660</td>
<td>1081.399</td>
<td>991.223 - 1175.028</td>
<td>3.259</td>
</tr>
<tr>
<td>Thiofanecarb</td>
<td>906.445</td>
<td>796.276 - 1256.812</td>
<td>2.958</td>
<td>838.617</td>
<td>672.097 - 1124.164</td>
<td>3.620</td>
</tr>
<tr>
<td>Isothiocyanate</td>
<td>895.698</td>
<td>722.505 - 1139.334</td>
<td>3.166</td>
<td>781.164</td>
<td>637.84 - 971.064</td>
<td>3.134</td>
</tr>
</tbody>
</table>
**Fig. 1:** Toxicity lines of median lethal doses (LD₅₀ values) of the tested pesticides on adult male albino rats dosed orally at different times.

1: Ld-p (Log dose – probit) line after 48, 72 and 96 hrs.
2: Ld-p line after 24 hrs.
Fig. 2: Toxicity lines of median lethal doses (LD$_{50}$ values) of the tested pesticides on adult female albino rats dosed orally at different times.

1: Ld-p (Log dose – probit ) line after 48, 72 and 96 hrs.
2: Ld-p line after 24 hrs.
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