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Acute Toxicity Determination Of Two New Benzothiazino Phenoxazine Dyestuffs In Mice

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ABSTRACT

Two new heterocyclic benzothiazinophenoxazine dyestuffs have been subjected to acute toxicity test using mice as the test animal. For each dyestuff, a total of thirty albino mice with average weight range of 14 – 25g were used. The first dyestuff-Dibenzo[a,j][1,4]benzothiazino[3,2-c]phenoxazine had LD_{50} value of 291.7mg/kg while the second dyestuff-8,-chloro-11-oxa-10-thia-5,9,18-triazadibenzo[a,r]pentaphene had LD_{50} value of 363.07mg/kg. These values are within the limits of acceptable values for dyestuffs.

Keywords: Toxicity, LD_{50}, Mice, Benzothiazino Phenoxazine

Phenoxazine is the basic structure of some groups of heterocyclic dyestuffs (Pearson 1957, Okafor 1977). The present synthesized dyestuffs have been found satisfactory as disperse dyes for polyester, nylon, candles and soaps (Okafor and Akpuaka 1992). Dyes and pigments have to be screened for toxicity since they find their way into the human body through materials such as clothing, hair dye, and also by injestion. A good dye for use must therefore be non-toxic or should have low toxicity. There are two types of toxicity test available – Acute toxicity by LD_{50} and by the Fixed Dose Procedure requiring fewer animals; the other method is the long-term toxicity test, which deals with the teratogenicity and carcinogenicity of the compounds. LD_{50} determination is a simple preliminary assessment of the toxicity of a compound by determination of the median lethal dose, which is capable of killing 50% of animal under stated conditions (Goldstein 1974). Although Tachon et al (1986) had suggested the replacement of LD_{50} test with a growth inhibition test for isolated cells for 19 hair dyes, but the comparison between this test method and the LD_{50} determination showed good correlation of results.

A survey of acute oral toxicity, as measured by the 50% lethal dose (LD_{50}) demonstrated that of 4461 colourants tested, only 44 had $LD_{50} < 250$mg/kg while 3669 exhibited practically no toxicity (Zollinger, 1991). In order to minimize the possible damage to man and the environment, an international association, Ecological and Toxicological Association of Dyestuff Manufacturing Industry (ETAD) was founded in 1974. ETAD coordinates the ecological and toxicological consequences of organic dyestuffs and pigments (Zollinger, 1991). To keep in line with the objectives of ETAD, the present toxicological tests on the two new dyestuffs were undertaken. The compounds are Dibenzo[a,j] [1,4] benzothiazino [3,2-c]phenoxazine 1 and 8 – chloro-11-oxa-10-thia-5, 9, 18 – triazadibenzo[a, r]pentaphene 2.

MATERIAL AND METHODS

For the test compound 1, a total of thirty albino mice ranging in weight from 14 – 25g were used. The mice were obtained from the animal house of the Pharmacology Department of the University of Nigeria, Nsukka. The mice were weighed and separated into five groups of six mice and put in different cages. They were fed with feeds and water *ad labitum* for fourteen (14) days in order to acclamatize them to the environment. A calculated dose of a suspension of the test compound suspended in Tween 80 (solvent) was injected intraperitoneally ranging in values 200mg/kg, 250mg/kg, 300mg/kg 350mg/kg 400mg/kg and 450mg/kg to the mice in the five cages according to their individual body weights. The number of animals dead was observed between 24 – 72 hours. The percentage of the animal dead was calculated, corrected and converted to 'probit' (probability units) (Akpuaka 1990). A graph of probit against $\log_{10}$ dose was plotted and from there the LD_{50} was calculated. The same procedure was repeated for compound 2.

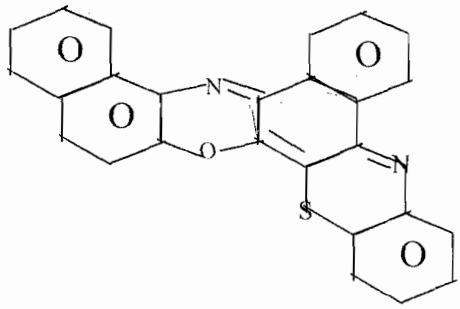
RESULTS AND DISCUSSION

For each test compound, the number of animals dead after 72 hours was recorded. This number

was converted to percentages. Table 1 and Figure 1 show the results for compound 1,- Dibenzo[a,j][1,4]benzothiazino[3,2-c]phenoxazine. The LD_{50} value is 291.7mg/kg. Table 2 and Fig 2 show the results for compound 2, - 8-chloro-11-oxa-10- thia-5, 9, 18 – triazadibenzo[a, r]pentaphene. The LD_{50} value is 363.07mg/kg. These LD_{50} values show low toxicity. These values are within the values quoted for no toxicity for LD_{50} test done on 4461colourants and only 44 had LD_{50} < 250mg/kg while 3669 exhibited practically no toxicity i.e LD_{50} above 250mg/kg.

Of the two compounds tested, it is seen that the chloro aza analogue 2 is relatively less toxic than the unsubstituted compound 1. This is explained by the strong inductive effect (-1) of the chlorine atom, which decreases electron density in the aromatic rings and so less electrons are to be donated to the biological centres in the mice. This leads to decreased binding of compound 2 in the mice and so leads to decreased toxicity. Higher value of LD_{50} show reduced toxicity.

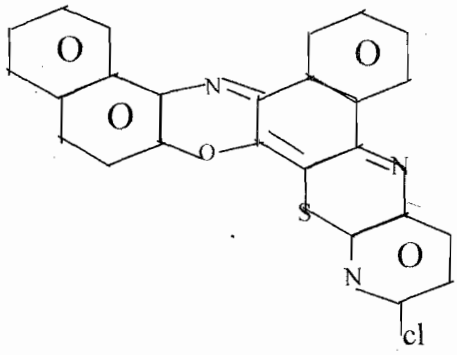
Table I: LD_{50} Determination of Dibenzo[a, j][1, 4]benzothiazino[3, 2-c]phenoxazine.



Group	No in cage	Dose mg/kg	Log_{10} dose	No. of animals dead	% of animal dead	% correction	Probit corresponded to %
1	6	200	2.30	1	16.67	16.67	4.05
2	6	250	2.39	2	33.33	33.33	4.56
3	6	300	2.47	3	50.00	50.00	5.00
4	6	350	2.54	4	66.67	66.67	5.44
5	6	400	2.60	5	83.33	83.33	5.95

Fig.1 Graph of Probit of % Animal dead against log_{10} dose of Dibenzo[a, j][1,4]benzothiazino[3,2-c]phenoxazine

Table 2 LD_{50} Determination of 8-chloro-11-oxa-10-thia-5,9–18-triazadibenzo [a,r] pentaphene.



Group	No in cage	Dose mg/kg	Log_{10} dose	No. of animals dead	% of animal dead	% correction	Profit corresponded to %
1	6	250	2.39	0	0	4.16	3.25
2	6	300	2.47	1	16.67	16.67	4.05
3	6	350	2.54	3	50.00	50.00	5.00
4	6	400	2.60	4	66.67	67.67	5.44
5	6	450	2.65	5	83.33	83.33	5.95

Toxicity determination in Mice

Graph of Probit of % Animal dead against log_{10} dose of

Dibenzo[a, j][1,4]benzothiazino[3,2-c]phenoxazine

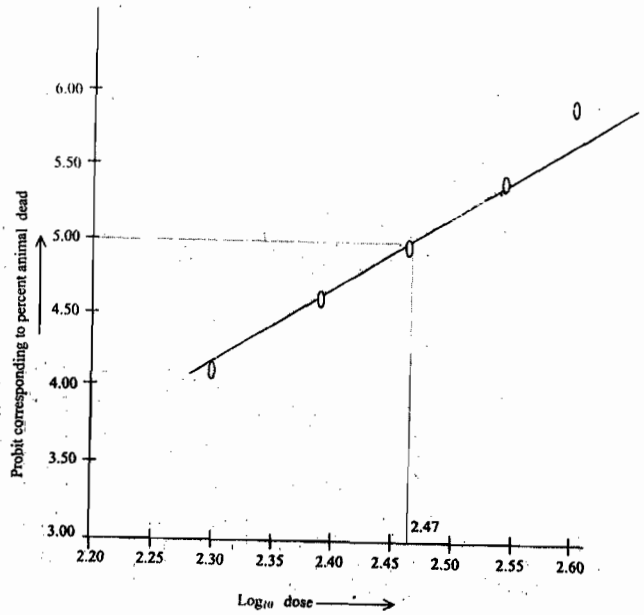
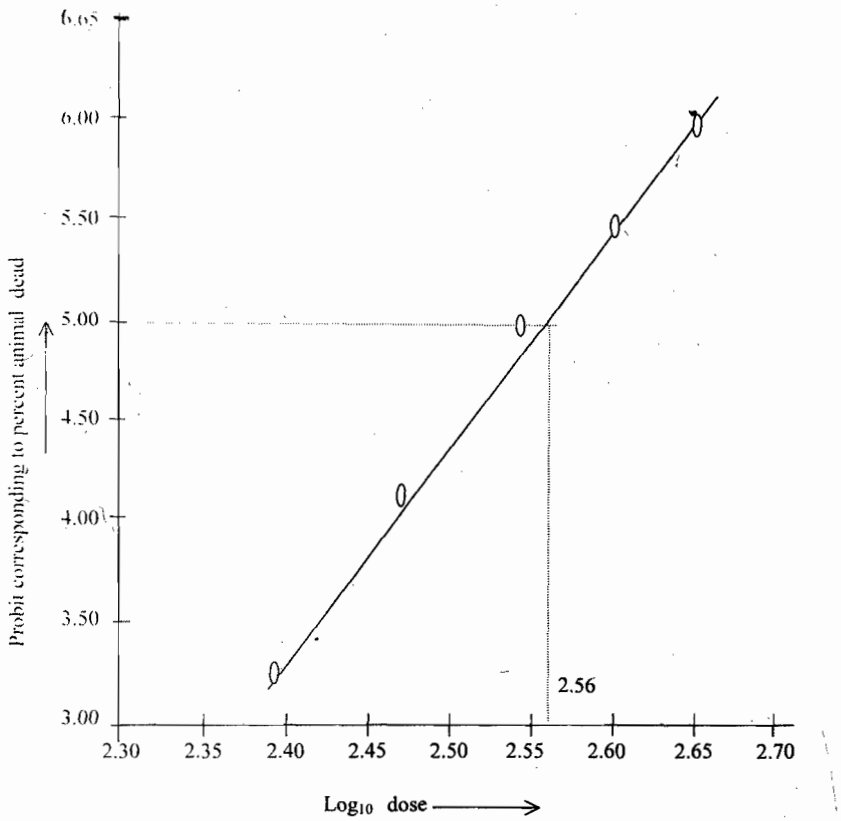


Fig. 1

Log_{10} dose at 50% dead = 2.47

LD_{50} = 291.7mg/kg



Log_{10} dose at 50% dead = 2.56

LD_{50} = 363.07mg/kg

Fig. 2

Graph of Probit of % animal dead against log_{10} dose for 8-chloro-11-oxa-10-thia-5, 9, 18- triazadibenzo [a, r] pentaphene.

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