An In-Vivo Investigation Of Acute Toxicity Assessment Of Sodium Fluoride To Channa Punctatus (Bloch)

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Abstract

In-vivo median lethal and safe concentrations of sodium fluoride in an Indian snake-headed murrel, Channa punctatus (Bloch), weighing 50-60g, has been assessed. Lorke's method estimated the 96-hour LC50 to be 1385.64 mg/l. As an approximate estimate, the Up-and-Down approach produced a 96-hour-LC50 range of 1500.0 to 1550.0 mg/l. Furthermore, the Behren-Karber, regression, and Finney techniques had 96-hour LC50 values of 1360.0, 1319.78, and 1258.9mg/l, respectively. The cumulative median lethal average of the 96-hour-LC50 dose in Channa punctatus was 1385.64+1525.0+1360.0+1319.78+1258.9 = 1369.86 or 1370 mg/l of sodium fluoride. The dose of sodium fluoride has a significant connection with fish mortality. Sodium fluoride accumulates in aquatic bodies, disrupting the physiological activities of this fish, according to the findings. This fish is more resistant to fluoride toxicity than other fishes. The calculated safety level value indicated that determining an acceptable dose of sodium fluoride for this fish is feasible. Sodium fluoride may even be classified as a slightly/moderately toxic substance to Channa punctatus *based on its toxicity range.*

Keywords: *Channa punctatus, acute toxicity, probit analysis, sodium fluoride.*

INTRODUCTION

Aquaculture involves the culture of freshwater and saltwater fishes under regulated conditions. In India, most fish farming depends on groundwater due to the unequal frequency of rain. Pollution of aquatic resources is a concern, posing a hazard to public health cause damage to underwater life. Every year, multiple ponds and lakes in several Indian states are the sites of mass fish mortality. Water pollution due to human activity is the primary source of this issue. Fluoride, a potentially harmful substance abundantly distributed in the earth's crust, regularly contaminates groundwater due to weathering and volcanic eruption [1]. Fluoride is an ionized form of fluorine. Fluorides are binary compounds or fluorine salts. Fluoride-rich minerals in rocks and soils that eventually make their way into water bodies are the primary cause of elevated fluoride levels. Several workers have observed high fluoride concentrations in groundwater in various Indian regions[2, 3]. Although groundwater appears appropriate for fish farming, it holds greater concentrations of fluoride than the suggested tolerable limit (>1.5)[4]. The ingestion of excess fluoride via food and drinking water causes several adverse effects on human beings [5], domestic animals[6], and experimental fish species inhabiting fluoride-contaminated water[7 – 10].

The literature indicates that sodium fluoride may adversely affect non-target organisms and can bioaccumulate in fish. Sub chronic and chronic studies have reported the toxicity of sodium fluoride in aquatic flora and fauna. However, acute toxicity data in fish are very scarce.

Fish are sensitive to many toxicants and are the best bioindicators for assessing the environmental risk caused by pollution[6]. Channa punctatus, an omnivorous freshwater fish, has been selected as the animal model for the toxicological evaluation of sodium fluoride. Several characteristics of Channa punctatus, such as its wide distribution in the freshwater environment, availability throughout the year, easy acclimatization to the laboratory conditions, and commercial importance, make this species an excellent test animal for toxicity evaluation.

The study may help with environmental risk assessment, regulatory compliance and guidelines, comparative toxicity evaluation, predictive indicators for long-term effects, species sensitivity studies, and industrial and agricultural safety of sodium fluoride.

MATERIALS AND METHODS

The freshwater fish Channa punctatus was collected from the neighboring villages of Arrah, India. Analytical-grade Sodium fluoride of 99% purity (CAS 7681-49-4, Sigma-Aldrich) was used. The sodium fluoride stock solution was prepared following range finding (Table 2) and standard procedure[11].

The experiments were performed according to the standard methods to determine the LC50 of Channa punctatus. The fish of 50-60g and 15-16cm in length were transported to the laboratory in large plastic tanks filled with water. The fish were washed in 1% potassium permanganate to free them from microbial infections.

The fish were acclimatized in 50-liter plastic tubs filled with dechlorinated water before experimentation. The fish were fed ad libitum with commercial feed rice bran and oil cake twice daily. The fish were maintained in tanks under a 12:12 hour light: dark period. The dead fish were removed immediately to keep the water afresh. During the acclimatization and test period, water was renewed every 12 hours following the desired concentration of the test compound. The fish were starved one day before experimentation.

The following methods were used to calculate the LC50 dose of sodium fluoride, from estimation to confirmation:

1. Median lethal dose method[12]
2. Up-and-Down (Staircase) method[13]
3. Behren-Karber method[14 - 15]
4. Regression analysis method
5. Finney[16]probit analysis method

Sodium fluoride toxicity was classified using a standard table. Various methods were used to calculate the safe levels of sodium fluoride [17 - 22].

This study follows the proposed recommendations of the Departmental Ethical Committee for Experiments of the Veer Kunwar Singh University of Arrah—the ethical guidelines followed for fish samplingand other purposes. The calculation was done by applying probit analysis using Microsoft Excel Windows 10. The percentage of mortality, along with their logarithmic and probit values, was compiled into Excel sheets. Acute toxicity is typically assessed by exposing fish to a chemical for a standard duration of 96 hours.

OBSERVATIONS AND RESULTS

Physico-chemical characteristics of water: The physicochemical characteristics of test water having a temperature of 25.0 ± 1.0^{0}C, pH: 7.67 ± 0.1, dissolved oxygen: 6.61 ± 0.21 mg/l, free carbon dioxide: 1.43 ± 0.58 mg/l, total alkalinity: 73.0 ± 4.2 mg/l, hardness: 230.67 ± 55.26 mg/l and chloride: 16.7 ± 0.2 mg/l was recorded during the experimental period (Table 1). The observed parameters are within the range of favorable growth performances[23]. Physiological parameters such as antibiotic concentration formulation and exposure impact such studies[24].

Ethological observations: Different doses of sodium fluoride resulted in uncoordinated behavior in the fish. In response to sudden changes in the surrounding environment, fish were alert, stopped swimming, and remained static in position during preliminary exposure. After a while, they tried to avoid the toxic water by swimming and jumping quickly. Because of the stretching of their body muscles, their fins became tough and stretched. They secreted massive amounts of mucus from their entire body, and a thick layer of mucus deposited inside the buccal cavity and gills. The pigmentation of the body increased. Finally, the fish exhibited vertical posture for a few minutes, with its mouth close to the water surface, attempting to gulp air, and its tail pointing downward. They soon settled at the bottom of the tank, and after a while, their bellies grew upward, and the fish died while the opercula remained wide open, exposing the gills.

Stress-induced behavioral changes are the most sensitive indicator of potential toxic effects. The numerous behavioral changes observed in this fish, such as restlessness, abnormal swimming behavior, vigorous jerks, loss of balance, myotonia, and anorexia, are similar to previous observations in fish exposed to various toxicants[25 - 26].

96hr-LC50 dose determination: The LC50 value recorded in this study attributed to the size of fish with potentially immune systems for biotransformation of Sodium fluoride from the body.

(1) Lorke's[12]method of median lethal dose: The procedure includes two stages.

(A) Three groups of three fish formed from the nine. Each group of fish received a different dose of Sodium fluoride (800, 1000, and 1200 mg/l). The fish are kept under observation for 96 hours to see how they behave and if they will die.

(B) Three fish in this stage were divided into three groups of one fish each. The fish are given higher doses of Sodium fluoride (1600, 2000, and 2400 mg/l) and then observed for 96 hours for behavior and mortality.

Therefore, 96hr-LC50 $= \sqrt{C_0 \times C_{100}} = \sqrt{1200 \times 1600}$ = 1385.64mg/l Sodium fluoride (Table 3). Where, C_0 = the maximum concentration with no mortality of fish and C_{100} = the minimum concentration with total mortality of fish.

(2) Bruce[13]Up-and-Down (Staircase) Method: The Sodium fluoride is dosed sequentially ona single fish in this method. Following the administration of the first dose, the next dose is determined by the outcome of the subsequent dose. If the fish survives the next dose, the dose is adjusted upward; if mortality is recorded at the next dose, the dose is adjusted downward. A constant factor is used to fix the dose either upward or downward. Testing is stopped when the upper limit (2000-5000 mg/l) is reached without mortality or when the LD50 is determined from the test. It gives an approximation of the MLC50 dose.

If a fish survives, a constant factor is added to the dose for the next fish and vice versa. It estimates the median lethal dose (MLC50) in 6 fish over 96 hours using arithmetic, geometric, and harmonic means. Using this method, MLC50 was calculated to be 2450.0mg/lSodium fluoride (Table 2). When no mortality is observed, even at concentrations of 2000-5000 mg/l, the method is abandoned [13]. The 96hr-LC50 dose of Sodium fluoride was calculated to be 1525.0 mg/l (Table 4).

(3) Behren[14]Karber[15] method: It is a non-parametric method requiring at least partial mortality, but the data does not fit the probit model. This method used equal spacing between dose intervals and an equal number of fish at each dose level for observations ranging from 0% to 100%. Many fish were sacrificed because the calculated dose did not kill a single fish. For the first four hours, 24 hours, and daily for 14 days, the fish were doused with the test substance and monitored for signs of toxicity. The 96hr-LC50 dose of Sodium fluoride was calculated to be 1360.0 mg/l (Table 5).

(4) Regression analysis method: It is used when there is no partial mortality. The Sodium fluoride doses were converted into a mortality rate. This method resulted in the death of many fish. Sodium fluoride 24, 48, 72, and 96hr-LC50 doses calculated using this method were2794.78, 1842.00, 1544.65, and 1319.78mg/l, respectively (Table 6). Sodium fluoride dosage was gradually reduced during treatment (Plate I; Figures 1-4).

(5) Finney's[16]probit analysis method: After calculating percent mortality, it is a parametric maximum likelihood method that calculates net/corrected percent mortality from 10% to 100%. Then, based on the straight line obtained in the graph, values of empirical probit ranging from 3.72 to 6.64 (Table-7) were noted from the Fischer and Yates table. Using empirical probit values ranging from 3.72 to 8.72, the expected/provisional probit was calculated. The working probit (from 3.42 to 6.68) and weighing coefficient (from 0.238 to 0.614) values were calculated to determine the mean and deviation of the Sodium fluoride doses and mortality. Finally, Sodium fluoride's median lethal concentration was calculated to be 96hr-LC50 = Antilog 3.10 = 1258.9mg/l. Because the calculated value of χ^2 is less than the tabulated value, the data is homogeneous, indicating that the 'eye fit' line is in good agreement.

The cumulative median lethal average of the 96-hour-LC50 dose in Channa punctatus was 1385.64+1525.0+1360.0+1319.78+1258.9 = 1369.86 or 1370 mg/l of sodium fluoride was calculated for Channa punctatus.

DISCUSSION

The 96hr-LC50 calculated using the Behren [14]-Karber [15]method, the regression analysis method, and the Finney probit analysis method are nearly in a range. These are the most common methods for determining 96hr-LC50 used by workers. The Behren-Karber method, on the other hand, can be used to calculate the 24, 48, 72, and 96hr-LC50 doses from the same set of experiments.

Higher amounts of fluoride have been found in fish, mainly due to the presence of fluoride-containing minerals in various components of the lithospheres and their dumping into water bodies [27]. A large number ofpeople in many states of India are living at risk of higher fluoride levels than the permissible limit of 1.5mg/l in their drinking water [28].

Earlier worker reported 96hr-LC50 dose of sodium fluoride 986.28mg/l in Cyprinus carpio haematopterus[29], 734.51mg/l in Clarias gariepinus[30], 616.50mg/l in Anabas testudineus[31], 460mg/l in Gasteroesteus aculeatus[32], 315mg/l in Pimephalkes promelas[33], 200mg/l in Oncorhynchus mykiss[32], 164.5mg/l in Salmo truta[30]and 126.12mg/l in Puntius sophore[34]. The acute toxicity test indicates the slightly/moderately toxic nature of sodium fluoride for fish (Table 8).

From this account, it may be inferred that the differences in LC50 values in the sensitivity of different fish species to the type of toxicant exposures. Several factors, such as species and strain differences, genetic variability, size and life stage, health and feeding status, time dependency, chemical properties of toxicant, synergistic and antagonistic effects, environmental and behavioral factors, etc.,affect LC50 dose.Channa punctatus seems more sensitive than Anabas testudineus and Clarias batrachus. It may be one of the reasons for the difference in LC50 doses.

It is reported that safe levels are added to account for uncertainties in data and evaluation processes. It is also used in case of lacking data on acute toxicity. The mean safety level of sodium fluoride was calculated at 61.58 mg/l from 13.19×10^3 to 131.98mg/l in Channa punctatus (Table 9). The range indicates that it decide the acceptable safe concentration of sodium fluoride in Channa punctatus. Moreover, 6.158 mg/l (1.319×10^3 to 13.198mg/l) and 0.6158 mg/l (0.1319×10^3 to 1.3198mg/l) of sodium fluoride may allow a safe level for rats and men respectively (https://en.wikipedia.org/wiki/Toxicity). The United States Health and Human Services Department recommends a maximum of 0.7 mg/l of water updating and replacing the recommended range of 0.7 to 1.2 milligrams issued in 1962.

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Table-1. Certain physico-chemical features of experimental water.

S. No.	Physico-Chemical features	Value
1.	Temperature	$25.0 \pm 1.0\,^{0}C$
2.	pH	7.67 ± 0.19
3.	Dissolved oxygen	6.61 ± 0.21 mg/L
4.	Free Carbon dioxide	1.43 ± 0.58 mg/L
5.	Hardness	230.67 ± 55.26 mg/L
6.	Total alkalinity	73.0 ± 4.2 mg/L
6.	Chloride	16.7 ± 0.2 mg/L

Table-2. Range finding of toxicity of sodium fluoride to Channa punctatus (body weight: 50-60g).

S. No.	Concentration of sodium fluoride (mg/L)	Percentage of mortality
1.	2.0	0
2.	20.0	0
3.	200.0	0
4.	2000.0	30
5.	20000.0	100

PLATE I

Figure 1: 24-hr LC_{50} of NaF to *Channa punctatus*

$y = 0.0232x - 14.286$
$R^2 = 0.6859$
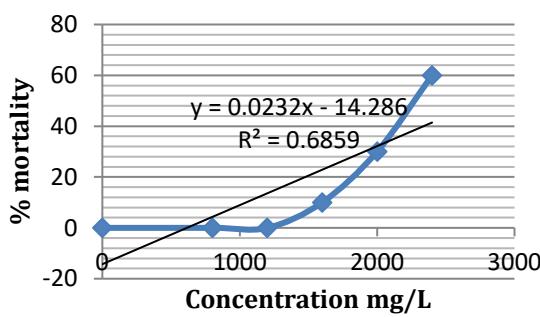
Figure 2: 48-hr LC_{50} of NaF to *Channa punctatus*

$y = 0.0361x - 16.429$
$R^2 = 0.8547$
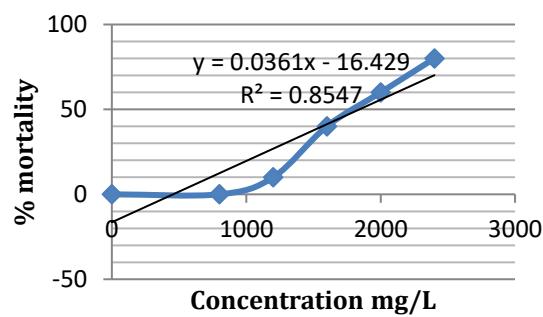
Figure 3: 72-hr LC_{50} of NaF to *Channa punctatus*

$y = 0.0436x - 16.429$
$R^2 = 0.8768$
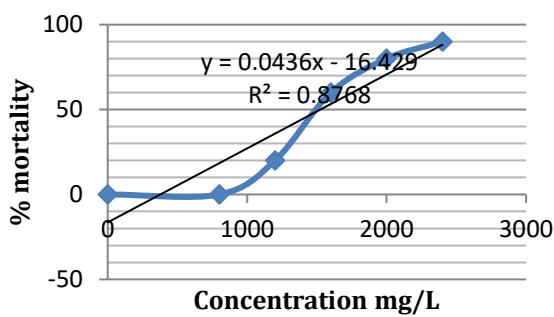
Figure 4: 96-hr LC_{50} of NaF to *Channa punctatus*

$y = 0.0468x - 10.714$
$R^2 = 0.9411$
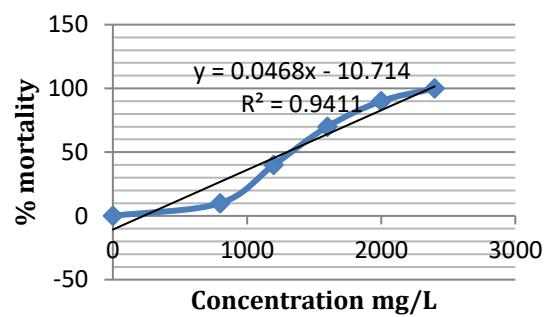
Table 3. Lorke's (1983) method for LC_{50} dose of Sodium fluoride for Channa punctatus (body weight: 55.0g).

S. No.	Dose of Sodium fluoride(mg/L)	Log dose of Sodium fluoride(mg/L)	96hr % mortality (n = 3)	S. No.	Dose of Sodium fluoride(mg/L)	Log dose of Sodium fluoride (mg/L)	96hr % mortality (n = 1)
1.	800	2.903	0	1.	2400	3.380	1
2.	1000	3.000	0	2.	2000	3.301	1
3.	1200	3.079	0	3.	1600	3.204	1

Lorke's (1983) method: $96hr\text{-}LC_{50} = = \sqrt{C_0 \times C_{100}} = \sqrt{1200 \times 1600} = 1385.64 mg/L$ Sodium fluoride

Table 4: Up-and-Down method (Bruce, 1985) for determination of LC_{50} dose of Sodium fluoride for Channa punctatus (body weight: 55.0g).

Number	Dose (mg/L)	Survival/Mortality	Number	Dose (mg/L)	Survival/Mortality
1st	1200	Survival	1st	1800	Mortality
2nd	1400	,,.	2nd	1700	,,
3rd	1600	Mortality	3rd	1600	..
4th	1400	Survival	4th	1500	Survival
5th	1600	Mortality	5th	1400	,,
6th	1800	,,	6th	1300	,,
Arithmetic Mean	1500.0	LC_{50}	Arithmetic Mean	1550.0	LC_{50}
Standard deviation	209.76	2.322 (Default dose)	Standard deviation	93.541	1.971 (Default dose)
Standard error of Mean	85.634	17.34 (Confidence interval)	Standard error of Mean	76.36	20.58 (Confidence interval)
Geometric Mean	1487.62	LC_{50}	Geometric Mean	1540.52	LC_{50}
Harmonic Mean	1475.12	LC_{50}	Harmonic Mean	1531.01	LC_{50}

Mean $LC_{50} = \dfrac{1500.0 + 1550.0}{2} = 1525.0 mg/L$ Sodium fluoride.

Table – 5: Behren-Karber method for 96hr-LC_{50} determination of Sodium fluoride for Channa punctatus (body weight: 55.0g).

Group	Dose of Sodium fluoride (mg/l)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96hr	Mean mortality between two consecutive dose (B)	A x B
				24hr	48hr	72hr	96hr			
1	0	0	10	0	0	0	0	0	0	0
2	800	400	10	0	0	0	1	1	0.5	200.0
3	1200	400	10	0	1	2	4	4	2.5	1000.0
4	1600	400	10	1	4	6	7	7	5.5	2200.0
5	2000	400	10	3	6	8	9	9	8.0	3200.0
6	2400	400	10	6	8	9	10	10	9.5	3800.0
										10400.0

$96hrLC50 = LC100 - \dfrac{\Sigma AB}{N}$

$= 2400 - \dfrac{10400}{10}$

$= 2400 - 1040$

$= 1360 \ mg/L$

Table – 6: Statistical relationship between dose of Sodium fluoride (mg/L) and percent mortality of Channa punctatus (body weight: 55.0g).

Sl. No.	Exposure period (hours)	Regression equation y =bx +a	Lethal Concentration (mg/L)	Toxicity Factor	t value (df=5)	F value (u = 1; v = 4)	95% Confidence limit	
							Lower	Higher

		(% mortality)								
1	24	y= 0.023x – 14.28	LC_{10}	1055.65	1.000	2.554 (p>0.05)	6.523 (p<0.05)	254.48	2365.79	
			LC_{50}	2794.78				673.74	6263.30	
			LC_{90}	4366.43				1052.62	9785.48	
2	48	y = 0.036x – 16.42	LC_{10}	733.89	1.517	4.20 (p<0.05)	17.64 (p<0.01)	176.92	1644.70	
			LC_{50}	1842.00				444.00	4180.05	
			LC_{90}	3233.89				779.60	7247.38	
3	72	y = 0.043x – 16.42	LC_{10}	614.42	1.809	4.68 (p<0.01)	21.90 (p<0.01)	148.12	1376.96	
			LC_{50}	1544.65				372.37	3461.67	
			LC_{90}	2707.44				652.69	6067.57	
4	96	y = 0.046x – 10.71	LC_{10}	450.22	2.112	6.93 (p<0.001)	48.02 (p<0.01)	108.53	1008.97	
			LC_{50}	1319.78				318.16	2957.72	
			LC_{90}	2392.84				576.84	5362.53	

Table 7: Probit analysis for toxicity of Sodium fluoride in *Channa punctatus*.

Dose of Oxytetracycline (mg/L)	Log dose of Oxytetracycline (mg/L)	Number of fish exposed	Mortality of fish	% of mortality of fish	Net/corrected mortality of fish	Empirical probit	Expected/Provisional probit	Working probit	Weighing Coefficient	nw	nwx	nwy	nwx^2	nwy^2	nwxy
	x	n		p			Y	y	w						
0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
800	2.903	10	1	10	10	3.72	3.42	3.82	0.238	2.38	6.91	9.10	20.06	34.73	26.39
1200	3.079	10	4	40	40	4.75	4.75	4.72	0.614	6.14	18.91	28.98	58.21	136.79	89.23
1600	3.024	10	7	70	70	5.52	5.54	5.48	0.564	5.64	17.05	30.91	51.27	169.37	93.46
2000	3.301	10	9	90	90	6.28	6.32	6.34	0.334	3.34	11.03	21.18	36.39	134.25	69.90
2400	3.380	10	10	100	100	8.72	6.68	6.64	0.204	2.04	6.89	13.55	23.31	89.94	45.78
-	-	-	-	-	-	-	-	-	-	19.54	60.79	103.72	189.54	565.08	324.76

96hr-LC$_{50}$ = Antilog 3.10= 1258.9mg/L of Sodium fluoride.

Table 8: Classification of substances/toxicants on the basis of toxicity range (Loomis and Hayes, 1996; Zucker, 1985**;** EPA, 1970; FIFRA, 1947 and GHS, 1992).

Sl. No.	Toxicity range (mg/L)	Toxicant Classification (Loomis and Hayes, 1996)	Sl. No.	Toxicity range (mg/L)	Toxicant Classification Environmental Protection Agency (Zucker, 1985; EPA,1970), The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 1947) and Globally Harmonized System of Classification and Labeling of Chemicals (GHS, 1992)
1.	<5	Extremely Toxic	1.	<50	Most Toxic
2.	5-50	Highly Toxic	2.	>50 and <500	Highly Toxic
3.	50-500	Moderately Toxic	3.	<5000	Moderately Toxic

4.	500-5000	Slightly Toxic	4.	>5000	Least Toxic
5.	5000-15000	Practically Non-toxic			
6.	>15000	Relatively Harmless			

Table 9: Estimation of safe levels of Sodium fluoride at 48/96hr exposure of Channa punctatus (body weight: 55.0g)

Sl. No.	Method	Dose of Sodium fluoride (mg/L)	Accumulation Factor Sodium fluoride (mg/L)	Safe level Sodium fluoride (mg/L)
1.	Canadian Council of Resource and Environment Minister (=CCREM) (1991) and International Joint Commission (=IJJ) (1977)	96hr-LC50 = 1319.78	0.05	1319.78 x 0.05 = 65.99
2.	Committee on Water Quality Criteria (=CWQC) (1972)	48hr-LC50 = 1842.00	0.01	1842.00 x 0.01 = 18.42
3.	Hart et al, (1948)	96hr-LC50 = 1319.78	$0.03 \times \left(\frac{24hr-LC50}{48hr-LC50}\right)^2$	1319.78 x 0.03 x $\left(\frac{2794.78}{1842.0}\right)^2$ = 91.48
4.	National Academy of Sciences/ National Academy of Engineering (=NAS/NAE) (1973)	96hr-LC50 = 1319.78	0.00001	1319.78 x 0.00001 = 13.19×10^{-3}
5.	Sprague (1971)	96hr-LC50 = 1319.78	0.1	1319.78 x 0.1 = 131.98
			Average	61.58