TOXICITY EFFECT OF EXPIRED PESTICIDES TO FRESHWATER FISH, *Labeo rohita*

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ABSTRACT

We selected 15 widely used pesticide formulations to investigate possible chemical, biological and toxicological effects of expired pesticides that may enter into our ecosystem. They comprised of organophosphate and pyrethroid based insecticides, azole based fungicides, acetamide, propionate, acetic acid based herbicides, and fungicides mixtures containing two actives of azole and dithiocarbamates. The selected compounds were tested for acute toxicity to fish, Labeo rohita using Organisation for Economic Co-operation and Development (OECD) guideline no. 203 under static condition. Five to six concentrations (ranges from 0.005 – 80 mg/L) of both expired and unexpired pesticide formulations were tested along with controls under similar conditions at Department of Ecotoxicology, International Institute of Biotechnology and Toxicology. The results of the LC_{50} (Lethal Concentration)demonstrated significantly lower LC_{50} values for the expired pesticides as compared to the corresponding unexpired counter part. From this data, it is clear that expired pesticide formulations must be disposed carefully and, care should be taken to avoid their discharge to water bodies and thus prevent loss to fish.

Keywords: Fish toxicity, freshwater, *Labeo rohita*, pesticides

INTRODUCTION

The United Nations Food and Agriculture Organization (FAO) define obsolete pesticides as stocked pesticides that can no longer be used for their original purpose or any other purpose and therefore require disposal (FAO, 1995). FAO estimate that half a million tonnes of obsolete pesticides are in storage world-wide (Shah and Devkota, 2009). A broad definition of obsolete pesticides includes all pesticides - technical and formulations - past their expiry date (2 years post manufacture date), all banned pesticides, damaged and degraded products, unusable formulations and packages, unidentified products, associated contaminated empty containers and old application equipment, other contaminated materials and equipment, buried pesticides and containers and heavily contaminated soils (Mwandia and Touni, 2006). In developing economies, expired pesticides may enter into water bodies due to usage of expired pesticides, purchased due to lack of knowledge about expiry and due to malpractices in trading.

Expired pesticides, if present in ecosystem, may pose new issues of toxicological concern, which are not similar with unexpired product. Data and information are scanty on the possible altered toxicological effects and since considerable amount of expired pesticides do exist in our society, there is need to characterize the risk of such products. India has at least 200 tonnes of obsolete pesticides (Jayashree, 2010). According to some reported inventories and surveys, the quantity of expired pesticides is estimated above 5000 tons in North West Frontier Province, Pakistan (Khwaja et.al., 2006). A survey indicated that around 1000 tonnes of obsolete pesticides may be remained in Punjab (OECD-FAO-UNEP, 2000).

In some situations, it was observed that the dealers gave those pesticides to farmers, which were reportedly expired. The Pesticide Dealers trapped most of the farmers who had

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insufficient knowledge about selection of proper pesticides and no money to buy them. They bought pesticides on credit (Khooharo, 2008). Unfortunately, farmers may not be aware of the source of the pesticides sold to them and unwittingly introduce dangerous chemicals into the environment (Kreisler and Heiss, 2008). When croplands are treated, some impacts of pesticides occur on non-target terrestrial and aquatic ecosystems, as well as on adjoining agro ecosystems (Surendra, 2010). Surface water also can be contaminated directly by pesticide spray drift the travel and deposition of fine pesticide spray droplets away from their intended target when the spray is applied too close to water. Drift incidents can result in greater surface water contamination than either runoff or leaching. Obvious, acute effects such as fish kills can occur (Don et al., 1994). As pesticides degrade, they may leach into soil and water, or they may be windswept or volatilized reaching neighboring, or far away, areas (Kreisler and Heiss, 2008). Women who eat lots of fish from contaminated waters have been shown to have babies with elevated rates of mental, developmental, and behavioral disorders (Ram, 2002). The effects on fish occurred largely during the reproductive cycle (i.e., at the time that the yolk sac was absorbed) (Burdick et al., 1967). Some farmers reportedly underestimated the toxicity of the pesticides and thought that throwing of leftover pesticides in running water was their safe disposal. The only option to dispose of the expired pesticides was to bury them in fields (Incineration) (Khooharo, 2008). In the majority of cases, disposal of obsolete pesticides in developing countries entails repackaging, transportation to Europe and incineration in dedicated high temperature toxic waste incinerators. It costs about US$3,000-US$4,000 per tonne of waste. Other destruction options include incineration in local cement kilns, mobile incinerators, burial and low technology chemical treatments. They also need high levels of training, sophisticated equipment and long term maintenance, which are often inappropriate in developing countries (PAN UK, 1998).

Environment Impact Quotient calculated by Researchers at Cornell University by combining information on dermal toxicity, chronic toxicity, systemicity, fish toxicity, leaching potential, surface-loss potential, bird toxicity, soil half-life, bee toxicity, beneficial arthropod toxicity and plant surface half-life for individual pesticides. Calculations revealed a significant relative toxicity of many active ingredients stored in each stockpile for fish (Blankespoor et al., 2009). Hence, we focused on toxicity effects of expired pesticides on fish.

Labeo rohita is a prime cultured and important staple freshwater fish generally found in rivers, ponds, and reservoirs (Dube and Hosetti, 2010) and popular in Thailand, Bangladesh, northern India and Pakistan (Rainer et al., 2005). Hence, we investigated the effect of certain expired pesticides to *Labeo rohita* in acute exposure test.

The objective of this investigation was to evaluate the toxicity effect of expired pesticide formulations to freshwater fish, *Labeo rohita* using a standard test guidelines OECD # 203 and Gaitonde Committee Guidelines (6.4.0.D.ii) to determine 96 hour LC_{50} and NOEC (No observed Effect Concentration).

MATERIALS AND METHODS

The test pesticides and chemicals were selected based on their wide use in India (or elsewhere). Fifteen different pesticide products in combination under five groups of pesticides were assay studied (Table 1).

The above products were purchased from market and they were all in within the mandatory 2 years shelf life. The same 15 products as obtained from IIBAT's repository, but were expired (expiry ranged between 10 months to 24 months after the 2 year shelf life) were tested simultaneously as given in Table-1. Manufacturer's names and brand names are withheld.

Table 1: Details of pesticide formulations used in the present study

Test substance name	Concentrations exposed (mg/L)	Date of Manufacture	Date of Expiry
Insecticides			
Dichlorvos 76% EC (Fresh)	0.63, 1.25, 2.5, 5 and 10 mg/L	Apr, 2010	Apr, 2012
Dichlorvos 76% EC (Expired)		Nov, 2007	Nov, 2009
Endosulfan 35% EC (Fresh)	0.5, 1, 2, 4 and 8 mg/L	Mar, 2009	Mar, 2011
Endosulfan 35% EC (Expired)		Aug, 2007	Aug, 2009
Quinalphos 25% EC (Fresh)	0.3, 0.6, 1.3, 2.5, 5 and 10mg/L	Nov, 2009	Nov, 2011
Quinalphos 25% EC (Expired)		Aug, 2007	Feb, 2009
Pyrethroids			
Alphacypermethrin 10% SC (Fresh)	0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/L	Nov, 2009	Nov, 2011
Alphacypermethrin 10% SC (Expired)		May, 2007	Apr, 2009
Fenvalerate 20% EC (Fresh)	0.01, 0.02, 0.04, 0.08 and 0.16 mg/L	Apr, 2009	Apr, 2011
Fenvalerate 20% EC (Expired)		Sep, 2007	Aug, 2009
Lambda-cyhalothrin 5% EC (Fresh)	0.005, 0.01, 0.02, 0.04 and 0.08 mg/L	Jul, 2008	Jul, 2010
Lambda-cyhalothrin 5% EC (Expired)		Jul, 2007	Jul, 2009
Herbicides			
Fenaxaprop-p-ethyl 9.3% EC (Fresh)	1.25, 2.5, 5, 10 and 20 mg/L	Jul, 2009	Jun, 2011
Fenaxaprop-p-ethyl 9.3% EC (Expired)		Aug, 2007	Jul, 2009
Pretilachlor 50% EC(Fresh)	1, 2, 4, 8 and 16 mg/L	Nov, 2009	Nov, 2011
Pretilachlor 50% EC (Expired)		Aug, 2007	Aug, 2009
2, 4 D sodium salt 80% WP (Fresh)	100 mg/L	Aug, 2009	Aug, 2011
2, 4 D sodium salt 80% WP (Expired)		Aug, 2007	Aug, 2009
Fungicides			
Tebuconazole 25% EC (Fresh)	3.13, 6.25, 12.5, 25 and 50 mg/L	May, 2007	May, 2010
Tebuconazole 25% EC (Expired)		May, 2007	May, 2009
Mancozeb 75% WP (Fresh)	2, 4, 8, 16, 32 and 64 mg/L	Dec, 2008	Dec, 2010
Mancozeb 75% WP (Expired)		Sep, 2007	Sep, 2009
Hexaconazole 5% EC (Fresh)	2.5, 5, 10, 20 and 40 mg/L	Aug, 2008	Jul, 2010
Hexaconazole 5% EC (Expired)		Nov, 2006	Oct, 2008
Combinational Fungicides			
Captan 70% + Hexaconazole 5% WP (Fresh)	0.3, 0.6, 1.3, 2.5, 5 and 10 mg/L	Jul, 2009	Jul, 2011
Captan 70% + Hexaconazole 5% WP (Expired)		Feb, 2007	Feb, 2009
Carbendazim 12% + Mancozeb 63% WP (Fresh)	1, 2, 4, 8 and 16 mg/L	Feb, 2010	Feb, 2012
Carbendazim 12% + Mancozeb 63% WP (Expired)		Apr, 2007	Apr, 2009
Metalaxyl 8% + Mancozeb 64% WP (Fresh)	5, 10, 20, 40 and 80 mg/L	Sep, 2009	Sep, 2011
Metalaxyl 8% + Mancozeb 64% WP (Expired)		Aug, 2006	Aug, 2008

Fish (*Labeo rohita*) used in this study were procured from commercial fish farm (Tamil Nadu Fish Seed Farm, Poondi, Thiruvallur dist., Tamil Nadu, India). The size of the fish was in the

range of 5 to 7.5 cm and weight of fish was around 2g/fish. Fish were acclimatized to the laboratory conditions for 7 days and fish were kept in starvation for 24 hour prior to the commencement of the study.

The acute toxicity study to the fresh water fish, *Labeo rohita* was conducted as per OECD 203 testing guidelines (OECD, 1992) and Gaitonde Committee Guidelines (6.4.0.D.ii).

The test room was maintained with 12 hours light and 12 hours darkness controlled by an automatic timer and test temperature was maintained between 21 - 25°C. Glass aquaria (25 L capacity) were used as test Chamber. Exposure medium was blended water (well water and reverse osmosis water in the mixture of 1:1 ratio). pH of both expired and unexpired products was determined by making 1% solution using pH meter (Eutech pH Testr 30) at temperature 25°C.

The study was performed with 5 groups of (fifteen) expired pesticides and fish were exposed to five to six test concentrations and blended water as a control for each test substance (Table 1). The test concentrations were fixed for each test substance based on reference literature (BCPC, 2003). The fish were exposed for 96hours. One test aquarium (contains 20 L of exposure medium) was used per test concentration for each test substance with ten fish in each chamber. Observations for mortality were made twice on the first day (3 hours and 6 hours) of the exposure, thereafter at the end of every 24 hours until 96 hours, when the experiment was terminated.

During the experiment, physico-chemical parameters (pH, temperature, dissolved oxygen and hardness) were determined in the exposure medium for each test substance in control and highest concentrations at the start and end of the experiment. The pH, temperature, conductivity and dissolved oxygen was analysed using instruments (Eutech pH Testr 30 and Lovibond-Sensodirect Oxi200) and total hardness (EDTA method) was determined following the methods given by APHA et al. (1975).

Mortality data of fish at the end of 96 h exposure were analysed using software- TOXSTAT 3.5 version (West Inc and Gulley, 1996) and LC_{50}s (with 95% confidence limits), NOEC (Fisher's exact test) were derived.

RESULTS AND DISCUSSIONS

LC_{50} and NOEC values are given in Table 2. LC_{50} values of expired Fenvalerate 20% EC, Lambda cyhalothrin 5% EC, Fenoxaprop p ethyl 9.3% EC, Tebuconazole 25% EC and Hexaconazole 5% EC were lower to unexpired, meaning they are toxic as against to the unexpired. EC based expired formulations were more toxic as compared to other types of formulations -WP and SC, suggesting that organic solvent based formulations could be more toxic. The impurities may contribute to the toxicity of the pesticide or may alter the physical properties of the product (Ambrus et al., 2003). Pesticides have the property of bioaccumulation and biomagnifications and they will derive long lasting impacts on the environment and human health (Shah and Devkota, 2009). Data on pH of expired and unexpired formulations are given in Table 3. Some of the expired pesticides Dichlorvos 76% EC, Fenvalerate 20% EC, Lambda-cyhalothrin 5% EC, Pretilachlor 50% EC, Tebuconazole 25% EC, Hexaconazole 5% EC and Captan 70% + Hexaconazole 5% WP tested exhibited major change in pH. Although when the study was performed the pH of the exposure medium did not differ, the decreased LC_{50} of expired pesticides could be due to degrading substance/s in them due to altered pH, though the nature of these are not studied in this study.

Table 2: LC_{50} and NOEC values of expired and fresh pesticide formulations against *Labeo rohita*

LC_{50}

Insecticides	LC_{50} (mg/L)		Pyrethroids	LC_{50} (mg/L)		Herbicides	LC_{50} (mg/L)		Fungicides	LC_{50} (mg/L)		Combinational fungicides	LC_{50} (mg/L)	
	Fresh	Expired		Fresh	Expired		Fresh	Expired		Fresh	Expired		Fresh	Expired
Dichlorvos 76% EC	7.5	7.5	Alphacypermethrin 10% SC	0.09	0.24	Fenaxaprop-p-ethyl 9.3% EC	12.0	10.56	Tebuconazole 25% EC	33.75	31.88	Captan 70% + Hexaconazole 5% WP	0.88	4.34
Endosulfan 35% EC	0.75	1.05	Fenvalerate 20% EC	0.033	0.022	Pretilachlor 50% EC	2.92	4.07	Mancozeb 75% WP	3.90	26.39	Carbendazim 12% + Mancozeb 63% WP	5.40	7.20
Quinalphos 25% EC	2.66	7.54	Lambda-cyhalothrin 5% EC	0.0409	0.0215	2, 4 D- sodium salt 80% WP	>100	>100	Hexaconazole 5% EC	15.00	6.00	Metalaxyl 8% + Mancozeb 64% WP	24	30.72

NOEC

Insecticides	NOEC (mg/L)		Pyrethroids	NOEC (mg/L)		Herbicides	NOEC (mg/L)		fungicides	NOEC (mg/L)		Combinational fungicides	NOEC (mg/L)	
	Fresh	Expired		Fresh	Expired		Fresh	Expired		Fresh	Expired		Fresh	Expired
Dichlorvos 76% EC	5.0	5.0	Alphacypermethrin 10% SC	0.05	0.1	Fenaxaprop-p-ethyl 9.3% EC	5.0	5.0	Tebuconazole 25% EC	25	25	Captan 70% + Hexaconazole 5% WP	0.3	2.5
Endosulfan 35% EC	0.5	0.5	Fenvalerate 20% EC	0.02	0.01	Pretilachlor 50% EC	2.0	2.0	Mancozeb 75% WP	2	16	Carbendazim 12% + Mancozeb 63% WP	4.0	4.0
Quinalphos 25% EC	0.6	2.5	Lambda-cyhalothrin 5% EC	0.020	0.010	2, 4 D- sodium salt 80% WP	-	-	Hexaconazole 5% EC	10.0	2.5	Metalaxyl 8% + Mancozeb 64% WP	10	20

Table 3: pH of fresh and expired pesticide formulations (1% solution)

S.No.	Test substance name	Fresh	Expired
1	Dichlorvos 76% EC	2.56	1.56
2	Endosulfan 35% EC	5.66	5.79
3	Quinalphos 25% EC	6.31	6.43
4	Alphacypermethrin 10% SC	5.65	5.81
5	Fenvalerate 20% EC	4.85	6.65
6	Lambda-cyhalothrin 5% EC	4.79	6.29
7	Fenaxaprop-p-ethyl 9.3% EC	4.10	4.64
8	Pretilachlor 50% EC	6.68	4.72
9	2, 4 D sodium salt 80% WP	8.75	8.73
10	Tebuconazole 25% EC	4.14	5.48
11	Mancozeb 75% WP	8.20	7.90
12	Hexaconazole 5% EC	4.45	3.98
13	Captan 70% + Hexaconazole 5% WP	5.16	2.56
14	Carbendazim 12% + Mancozeb 63% WP	8.13	8.07
15	Metalaxyl 8% + Mancozeb 64% WP	7.91	8.12

Fish and aquatic animals are exposed to pesticides in three primary ways (i) dermally, direct absorption through the skin by swimming in pesticide-contaminated waters, (ii) breathing, by direct uptake of pesticides through the gills during respiration, and (iii) orally, by drinking pesticide-contaminated water or feeding on pesticide contaminated prey (Mathur and singh, 2006). The continual contamination and pollution faced by the Nigerian Coastal and Marine environment from pesticides run-offs with resultant fish kills and human deaths (PAN Nigeria, 2007). Some soluble pesticides are easily leached into streams and lakes (David, 2005). A study was carried out from April 1999 to May 2000 to investigate the extent of pollution of Lake Nakuru with respect to organochlorine pesticide residues. The sampling periods included both dry and rainy seasons. Results revealed that more contaminants are added to the lake during rainy season than dry season (Mavura and Wangila, 2004). Fish kills occur when pesticides are improperly applied to or otherwise end up in bodies of water through either misapplication or drift (Mike, 2007). Accidental killing of fish due to the contamination of the aquatic environment remains among the most frequent poisoning cases. Every year, about 260 to 300 of such accidents are diagnosed (Helena and Zdenka, 2009).

Exposure to toxic substances may not result in immediate fish kills, but may affect fish populations by decreasing fecundity (number of eggs produced), reducing the viability of sperm, eggs and larvae, decreasing life expectancy, increasing the incidence of abnormalities and increasing natural mortality (Allan, 2000). Herbicides and insecticides used in agriculture have been responsible for a number of fish kills. Endosulphans used in the cotton growing areas have been particularly problematic over the last decade (Napier et al. 1998). Atrazine is one of the most widely used herbicides and considered as a common terrestrial and aquatic contaminant. Atrazine at low concentrations caused kidney damage in chronic exposed rainbow trout (Oulmi et al., 1995). The pesticide residues were found to cause impairment in reproductive system and thyroid activity in aquatic fauna, fish

(Great and Mehrle, 1969). In a case in Mozambique, pesticides had been burned on a site which had subsequently been covered up with soil. Residues from the incineration had contaminated the surrounding soil, which local populations had discovered was toxic to fish, and had been used by local populations to catch fish in the local river. The toxic soil would cause fish to die instantly, floating up to the surface, where they would be caught and consumed or sold on the local market (FAO and DINA, 2004). High DDT and dieldrin residues were reported in African fish at levels that could potentially affect their reproduction have chronic toxic and behavioral effects and even drastically affect their population (Paul, 2005). So far there is no fish toxicity data available for expired pesticides.

However, expired drugs and pharmaceuticals release in water lead to biological change in fish which leads to reproductive defects. Like agrochemicals, Pharmaceuticals and Personal Care Products (PPCPs) are disposed or discharged into the environment on a continual basis via industrial and household sewage and waste (many individuals dispose of unwanted and expired drugs directly into the domestic sewage system or garbage) (Kreisberg, 2007). They may not cause acute toxicity in aquatic organisms, they may interfere with endocrine systems, particularly when exposure occurs during sensitive times of development such as before birth (SCBWMI, 2005). Studies have found fish in contaminated water with reproductive defects as well as alarmingly high ratios of female to male fish in some locations (KCT, 2009). Dangers of flushing toxic chemicals into the ecosystem through municipal sewer systems, one potentially devastating threat to wild fish populations comes from an unlikely source, estrogen. Male fish exposed to estrogen become feminized, producing egg protein normally synthesized by females. In female fish, estrogen often retards normal sexual maturation, including egg production (Hogan, 2008). Therefore, expired pesticide formulations must be disposed carefully and care should be taken to avoid their discharge to water bodies and thus prevent loss to fish.

CONCLUSIONS

From the above investigation, we conclude that some of the pesticide formulations (mentioned in results section) were more toxic after shelf life expiry and some remained toxic but there may be variation exists when compared with fresh respective formulation. Based on the observed LC_{50} values in the study, EC formulation may cause more toxicity in expired condition rather than other type of formulation like WP, SC because of impurities. pH alteration was found in Dichlorvos 76% EC, Fenvalerate 20% EC, Lambda-cyhalothrin 5% EC, Pretilachlor 50% EC, Tebuconazole 25% EC, Hexaconazole 5% EC and Captan 70% + Hexaconazole 5% WP. The altered pH may cause by-product formation in expired pesticide which lead to acute toxicity to *Labeo rohita* in the present study. This indicates that there is a requirement of detailed investigation on characterization of the expired pesticides and we suggest that the proper disposal of expired chemical without impairing the aquatic environment is required.

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