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TOXIC EFFECT OF EXPIRED PESTICIDES ON *CATLA CATLA* OF THE GAULA STREAM, INDIA

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Summary

Twelve widely used pesticides were selected to investigate possible chemical, biological and toxicological effects of expired pesticides that may enter into our ecosystem. The selected compounds were tested for acute toxicity to *Catla catla* under static condition. Five to six concentrations (ranges from 0.005 – 80 mg L^{-1}) of both expired and unexpired pesticide formulations were tested along with controls under similar conditions. The results of the LC50 (Lethal Concentration) demonstrated lower LC50 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.

Key words: *Catla catla*, lakes, pesticides

INTRODUCTION

The United Nations Food and Agriculture Organization (FAO) defines 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). The FAO estimates that half a million tones of obsolete pesticides are in storage world-wide (Carey and Bryant, 1995). 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 (Arnold, et al. 1995). 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

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on the possible altered toxicological effects and since considerable amount of expired pesticides do exist in our society, there is a need to characterize the risk of such products. India has at least 200 tones of obsolete pesticides (Statham and Lech, 1975). In some situations, it was observed that the dealers gave those expired pesticides to farmers. The Pesticide Dealers trapped most of the farmers who have insufficient knowledge about the selection of proper pesticides and no money to buy them. They bought pesticides on credit (Kim, 1998). Unfortunately, farmers may not be aware of the source of the pesticides sold to them and unwittingly introduce dangerous chemicals into the environment (Catherine and Gloria, 2000). When croplands are treated, some impacts of pesticides occur on non-target terrestrial and aquatic ecosystems, as well as on adjoining agro ecosystems (Goodbred, 1997). Surface water also can be contaminated directly by pesticide spray drift 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 (Jobling, 1998). As pesticides degrade, they may leach into soil and water, or they may be windswept or volatilized reaching neighboring, or far away areas (Haider and Inbaraj, 2008). Pregnant women who consume fish from contaminated waters have shown elevated rate of mental, developmental and behavioral disorders (Tilak et al., 2004) in infants. The effects on fish occurred largely during the reproductive cycle (i.e. at the time that the yolk sac was absorbed) (Singh and Agarwal, 2009). 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) (Rao and Rao, 2006). 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.

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. Environment Impact Quotient was calculated by Researchers at Cornell University (Rao and Rao, 2001) by combining information on dermal toxicity, chronic toxicity, 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 (Rao and Rao, 2001). Hence, present study was focused on toxicity effects of expired pesticides on fish.

Catla catla is a prime cultured and important staple freshwater fish generally found in rivers, ponds and reservoirs (Murty, 2010) and popular in Thailand, Bangladesh and northern India. Hence, we investigated the effect of certain expired pesticides to *Catla catla* in acute exposure test.

The objective of this investigation was to evaluate the toxicity effect of expired pesticide formulations to freshwater fish, *Catla catla,* using a standard test guidelines OECD # 203 and Gaitonde Committee Guidelines (6.4.0.D.ii) to determine 96 hour LC50 and NOEC (No Observed Effect Concentration).

MATERIALS AND METHODS

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

The above products were purchased from market and they were all in within the mandatory 2-year shelf life. The same 12 products in expired were also obtained (expiry ranged between 10 months to 24 months after 2-year shelf life) and were tested simultaneously as given in Table 1.

Catla catla used in this study were procured from the Gaula stream, Near Haldwani, Uttarakhand, India. The size of the fish was in the range of 15 to 20.5 cm and weight of fish was around 1.0 kg/fish. Fish were acclimatized to the laboratory conditions for 7 days and kept in starvation for 24 hours prior to the commencement of the study. The acute toxicity study to the fresh water fish, *Catla catla,* 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 of light and 12 hours of darkness, 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 at a temperature of 25°C.

The study was performed with 4 groups of (twelve) 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 and fish were exposed for 96 hours. 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.

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

Tablica 1: Podaci o formulacijama pesticida korištenim u ovom istraživanju

Test substance name/ Naziv testne tvari	**Concentration exposed / Izloženost koncentraciji (mg L^{-1})**	**Date of manufacture / Datum proizvodnje**	**Date of expiry / Rok trajanja**
Insecticide / Insekticidi			
Dichlorvos 76% EC (Fresh/ Valjan)	0.63, 1.25, 2.5, 5. and/i 10	April/Travanj, 2010	April/Travanj, 2012
Dichlorvos 76% EC (Expired/Istekao)		November/Studeni, 2007	November/Studeni, 2009
Endosulfan 35% EC (Fresh/ Valjan)	0.5, 1.0, 2.0, 4.0 and/i 8.0	March/Ožujak, 2009	March/Ožujak, 2011
Endosulfan 35% EC (Expired/Istekao)		August/Kolovoz, 2007	August/Kolovoz, 2009

Quinalphos 25% EC (Fresh/ Valjan)	0.3, 0.6, 1.3, 2.5, 5.0 and/i 1.0	November/Studeni, 2009	November/Studeni, 2011
Quinalphos 25% EC (Expired/Istekao)		August/Kolovoz, 2007	February/Veljača, 2009
Pyrethroids / Piretroidi			
Alphacypermethrin 10% SC (Fresh/Valjan)	0.025, 0.05, 0.1, 0.2, 0.4 and/i 0.8	November/Studeni, 2009	November/Studeni, 2011
Alphacypermethrin 10% SC (Expired/Istekao)		May/Svibanj, 2007	April/Travanj, 2009
Fenvalerate 20% EC (Fresh/ Valjan)	0.01, 0.02, 0.04, 0.08 and/i 0.16	April/Travanj, 2009	April/Travanj, 2011
Fenvalerate 20% EC (Expired/Istekao)		September/Rujan, 2007	August/Kolovoz, 2009
Lambda-cyhalothrin 5% EC (Fresh/Valjan)	0.005, 0.01, 0.02, 0.04 and/i 0.08	July/Srpanj, 2008	July/Srpanj, 2010
Lambda-cyhalothrin 5% EC (Expired/Istekao)		July/Srpanj, 2007	July/Srpanj, 2009
Herbicides / Herbicidi			
Fenaxaprop-p-ethyl 9.3% EC (Fresh/Valjan)	1.25, 2.5, 5, 10 and/i 20.0	July/Srpanj, 2009	June/Lipanj, 2011
Fenaxaprop-p-ethyl 9.3% EC (Expired/Istekao)		August/Kolovoz, 2007	July/Srpanj, 2009
Pretilachlor 50% EC(Fresh/ Valjan)	1.0, 2.0, 4.0, 8.0 and/i 16.0	November/Studeni, 2009	November/Studeni, 2011
Pretilachlor 50% EC (Expired/Istekao)		August/Kolovoz, 2007	August/Kolovoz, 2009
2, 4 D sodium salt 80% WP (Fresh/Valjan)	100.0	August/Kolovoz, 2009	August/Kolovoz, 2011
2, 4 D sodium salt 80% WP (Expired/Istekao)		August/Kolovoz, 2007	August/Kolovoz, 2009
Fungicides / Fungicidi			
Tebuconazole 25% EC (Fresh/Valjan)	3.13, 6.25, 12.5, 25.0 and/i 50.0	May/Svibanj, 2007	May/Svibanj, 2010
Tebuconazole 25% EC (Expired/Istekao)		May/Svibanj, 2007	May/Svibanj, 2009
Mancozeb 75% WP (Fresh/ Valjan)	2.0, 4.0, 8.0, 16.0, 32.0 and/i 64.0	December/Prosinac, 2008	December/Prosinac, 2010
Mancozeb 75% WP (Expired/Istekao)		September/Rujan, 2007	September/Rujan, 2009
Hexaconazole 5% EC (Fresh/Valjan)	2.5, 5.0, 10.0, 20.0 and/i 40.0	August/Kolovoz, 2008	July/Srpanj, 2010
Hexaconazole 5% EC (Expired/Istekao)		November/Studeni, 2006	October/Listopad, 2008

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 were at the start and end of the experiment. The pH, temperature, conductivity and dissolved oxygen was analysed using instruments and total hardness (EDTA method) was determined using the methods given by APHA (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 LC50s (with 95% confidence limits), NOEC (Fisher's exact test) were derived.

RESULTS AND DISCUSSIONS

LC50 and NOEC values are given in Table 2 and 3. LC50 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 than unexpired but still they were reported as toxic. 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 (Susan et al., 2001). Pesticides have the property of bioaccumulation and biomagnifications and they will derive long lasting impacts on the environment and human health (Houlahan et al., 2000). Data on pH of expired and unexpired formulations are given in Table 4. Some of the expired pesticides Dichlorvos 76% EC, Fenvalerate 20% EC, Lambda-cyhalothrin 5% EC, Pretilachlor 50% EC, Tebuconazole 25% EC, Hexaconazole 5% EC 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 substances in them due to altered pH, though the nature of these are not studied in this study.

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 (Kegley, et al. 1999). The continual contamination and pollution is faced by the Nigerian Coastal and Marine environment from pesticide run-offs with resultant fish kills and human deaths. Some soluble pesticides are easily leached into streams and lakes (Ewing, 1999). Fish kills occur when pesticides are improperly applied to or otherwise end up in bodies of water through either misapplication or drift (Jana and Bandyopadhyaya, 2005). Accidental killing of fish due to the contamination of the aquatic environment remains among the most frequent poisoning causes. Every year, about 260 to 300 of such accidents are diagnosed (Bishop, 2000).

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 (McDiarmid, 2007). Herbicides and insecticides used in agriculture have been responsible for a number of fish kills. Endosul-

Table 2: LC_{50} value of expired and functional pesticide in Catla catla

Tablica 2: Vrijednost LC_{50} za pesticide kojima je istekao rok trajanja i one kojima nije kod Catla catla

Insecticide/ Insekticidi	LC_{50}(mg L^{-1})		Pyrethroids / Piretroidi	LC_{50}(mg L^{-1})		Herbicides/ Herbicidi	LC_{50}(mg L^{-1})		Fungicides/ Fungicidi	LC_{50}(mg L^{-1})	
	Fresh/ Valjan	Expired/ Istekao		Fresh/ Valjan	Expired/ Istekao		Fresh/ Valjan	Expired/ Isrekao		Fresh/ Valjan	Expired/ Istekao
Dichlorvos 76% EC	8.2	8.4	Alphacypermethrin 10% EC	1.04	0.49	Fenaxaprop-p-ethyl 9.3% EC	14.1	12.7	Tebuconazole 25% EC	38.52	36.86
Endosulfan 35% EC	0.82	1.29	Fenvalerate 20% EC	0.051	0.041	Pretilachlor 50% EC	3.78	5.09	Mancozeb 75% WP	5.32	31.45
Quinalphos 25% EC	2.91	8.71	Lambda-cyhalothrin 5% EC	0.0509	0.0391	2,4 D-sodium salt 80%WP	>100	>100	Hexaconazole 5% EC	18.23	8.34

Table 3: NOEC value of expired and functional pesticide in Catla catla

Tablica 3: Vrijednost NOEC za pesticide kojima je istekao rok trajanja i one kojima nije kod Catla catla

Insecticide/ Insekticidi	NOEC(mg L^{-1})		Pyrethroids/ Piretroidi	NOEC (mg L^{-1})		Herbicides/ Herbicidi	NOEC (mg L^{-1})		Fungicides/ Fungicidi	NOEC (mg L^{-1})	
	Fresh/ Valjan	Expired/ Istekao		Fresh/ Valjan	Expired/ Istekao		Fresh/ Valjan	Expired/ Istekao		Fresh/ Valjan	Expired/ Istekao
Dichlorvos 76% EC	4.0	4.0	Alphacypermethrin 10% EC	0.08	0.3	Fenaxaprop-p-ethyl 9.3% EC	7.0	7.0	Tebuconazole 25% EC	30.0	30.0
Endosulfan 35% EC	0.3	0.4	Fenvalerate 20% EC	0.03	0.02	Pretilachlor 50% EC	3.0	3.0	Mancozeb 75% WP	6.0	6.0
Quinalphos 25% EC	0.8	1.5	Lambda-cyhalothrin 5% EC	0.030	0.020	2,4 D-sodium salt 80%WP	0.020	0.020	Hexaconazole 5% EC	15.0	25.0

phans used in the cotton growing areas have been particularly problematic over the last decade (Kirk, 2008). 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 chronically exposed rainbow trout (Ouellet, 1997).

Table 4: pH of fresh and expired pesticide formulations (1% solution)
Tablica 4: pH valjanih i isteklih formulacija pesticida (1% otopine)

No	Test substance name / Naziv testne tvari	Fresh/ Valjan	Expired/ Istekao
1	Dichlorvos 76% EC	3.86	2.76
2	Endosulfan 35% EC	6.96	6.99
3	Quinalphos 25% EC	7.61	7.63
4	Alphacypermethrin 20% EC	6.95	7.01
5	Fenvalerate 20% EC	6.15	7.85
6	Lambda-cyhalothrin 5% EC	6.09	7.49
7	Fenaxaprop-p-ethyl 9.3% EC	5.4	5.84
8	Pretilachlor 50% EC	7.98	5.92
9	2,4 D sodium salt 80% EC	10.05	9.93
10	Tebuconazole 25% EC	5.44	6.68
11	Mancozeb 75% EC	9.5	9.1
12	Hexaconazole 5% EC	5.75	5.18

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 population had discovered was toxic to fish and had been used by the local population 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 (Wang and Murphy, 2011).

High DDT and dieldrin residues were reported in African fish at levels that could potentially affect their reproduction and have chronic toxic and behavioral effects and even drastically affect their population (Kirk, 2008). 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) (Kim, 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. Studies have found fish in contaminated water with reproductive defects as well as alarmingly high ratios of female to male fish in some locations (Rao and Rao, 2006). It is dangerous to flush toxic chemicals into the ecosystem through municipal sewer systems, and one potentially devastating threat to wild fish populations comes from an unlikely source, estrogen. Male fish exposed to estrogen become femi-

nized, producing egg protein normally synthesized by females. In female fish, estrogen often retards normal sexual maturation, including egg production (Susan, et al. 2001). 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 were more toxic after shelf life expiry and some remained toxic but there may be variation when compared with fresh respective formulation. Based on the observed LC50 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 + Hexaconazole 5% WP. The altered pH may cause by-product formation in expired pesticide which leads to acute toxicity to *Catla catla* 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 chemicals without impairing the aquatic environment is required.

Sažetak

TOKSIČNO DJELOVANJE PESTICIDA KOJIMA JE ISTEKAO ROK TRAJANJA NA VRSTU *CATLA CATLA* IZ RIJEKE GAULI U INDIJI

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Dvanaest često korištenih pesticida je odabrano kako bi se istražili mogući kemijski, biološki i toksikološki učinci pesticida kojima je istekao rok trajanja, a koji mogu ući u naš ekosustav. Odabrani spojevi su testirani na akutnu toksičnost kod *Catla catla* u statičnim uvjetima. Testirano je pet-šest koncentracija (od 0.005 – 80 mg L^{-1}) formulacija pesticida kojima je istekao rok trajanja, kao i onih kojima nije, zajedno s kontrolnim u sličnim uvjetima. Rezultati LC50 (letalna koncentracija) su pokazali niže vrijednosti za pesticide kojima je istekao rok trajanja u usporedbi s odgovarajućim pesticidima kojima nije istekao rok. Evidentno je temeljem ovih podataka da je nužno pažljivo odlaganje formulacija pesticida kojima je istekao rok trajanja te da treba zaustaviti njihovo istjecanje u vodna tijela, pri čemu se sprječava nastajanje štete za ribu.

Ključne riječi: *Catla catla*, jezera, pesticidi

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