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Studies on the Accumulation, Distribution, and Degradation of ^{14}C-DDT in Marine *Tilapia* sp *

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Abstract Tilapia could rapidly accumulate and degrade ^{14}C-DDT. Within 24 hours, the accumulation of DDT in almost all the organs of the fish had reached the peaks and the radioactivity declined from then on. At the 24th hour, the concentration ability of organs to DDT were in the descending sequence as follows: liver, bile, intestine, brain, gill, caudal fin, eye, muscle and scale. The relative radioactivity of liver is more than 57 times of that of muscle. The metabolites could be detected in viscera in the 2nd hour. In the 6th hour, the percentage of extractable residues of viscera were: DDT 31.05%, DDD 43.52%, DDE 5.60%, DDMU 3.30%, DDA 2.32%, unknown compound at initial point 14.21%. Radioactivity in water was declined within 48 hours, and then increased slowly. DDT residues in water were analyzed by TLC and the results showed that in the later period of the experiment, the radioactivity in water was almost not DDT. On the 26th day, the radioactive compounds in water were: DDT 2.93%, DDD 6.25%, DDE 1.95%, DDMU 0.98%, DDA 1.76%, unknown compound at initial point 86.13%. Concentration factors and their limitations were also discussed.

Keywords ^{14}C-DDT, *Tilapia* sp, accumulation, distribution, degradation

Classificational number X 171

Pesticides have become an integral part of modern agriculture systems, contributing significantly to improved crop yields and enhanced food production[1]. However, It has been estimated that less than 1% of the pesticides applied to crops attain the target pest species. Excess pesticide moves throughout the environment and may enter marine ecosystems by a number of pathways, provided the environmental persistence of the compound is sufficiently long. The spread of pesticide residues in the environment has created several current or potential future problems. The pesticide residues introduced into aquatic ecosystems may ultimately have a major impact on fishery resources, biological diversity and the functional equibrium of ecosystems[2].

The concentration of DDTs in marine biota are generally higher than the non-DDT compounds, which is a result of the more intensive worldwide use of DDT than of any other pesticide, combined with the long environmental persistence and high bioaccumulation of these compounds. As one of the major pesticides used in the 1950's ~1960's, DDT was first introduced to China in 1951. Up to its use banned in 1983, the total production of DDT was about 327 GT. It was estimated that an accumulated residue of DDT was

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(0.394 1±0.088 3) μg/g in the cultivated layer of soil in 1980[3]. These residues may be brought to marine biota by rainfall and other routes. As the result, residues of DDT were detected in Bohai[4], Yellow Sea[5], and South China Sea[6] in 1980's. Even in recent years, DDTs were detected not only in sediment (4.13～83.83 g/kg), but also in biota (1.770～207.60 g/kg) in Pear River mouth[7]. However, the above works were only focus on the field investigation. Few literatures concerning simulate experiment were reported. We have been carrying out systematic experiments studying the behavior of DDT in marine microcosm from 1994, the present research is to study the nature of DDT in a marine fish, *Tilapia* sp, in simulation conditions.

1 Materials and Methods

1.1 Materials

(1) ^{14}C-DDT and cold standards, provided by the IAEA.

(2) Thin layer plates: The thin layer plates were prepared in laboratory with silicon gel HF254.

(3) Fish: Tilapia were hatched in our university. When the body weight was 2～3 gram, the fishes were climated in salt water by continue salinization until the salinity was 30.

(4) Scintillation cocktail: PPO 4 g, Triton X-100 333 mL, toluene 667 mL.

(5) Liquid Scintillation Spectrum Analyzer, Packard Trib CA 2 000/LL.

(6) Sea water: the salinity was 30, made in the laboratory from artificial salt. Filtered before used in the experiment.

(7) Aquarium: pure glass, volume 20 L.

1.2 Methods

(1) Accumulation of ^{14}C-DDT in Tilapia: Experiment was carried out in a 20 L pure glass aquarium, the specific radioactivity of ^{14}C-DDT was 0.5 μCi/L sea water. After the labeled DDT was added into the aquarium, the sea water was stirred. Then 36 fishes were cultured in the aquarium and aerated. In the different time interval, 3 fishes were sampled. After washed with clean sea water, the fishes were dissected, organs were separated and weighted. The samples were put into vials and digested with $HClO_4$ at 70℃ for 2 h for radioactivity measurement. Water samples were also collected at the same time intervals.

(2) Extraction of DDT and its metabolites in viscera: After dissected, the viscera of the fishes was extracted directly with acetone in vials at 40℃ for 2 h. The extracts were concentrated and analyzed by thin layer chromatography. The total radioactivities were also measured by liquid scintillation spectrum analyzer.

(3) Extraction of DDT and its metabolites in water: The DDT residues in water was collected with carbon column absorption method (Zhong C G and Shi J, in press).

(4) Analysis of DDT and its metabolites: ^{14}C-DDT and its metabolites were analyzed by co-chromatography with standards of DDT, DDE, DDMU, DDD, DDA on a 20 cm × 20 cm thin layer plates coated with silicon gel HF254.

2 Results and Discussions

2.1 The Accumulation of ^{14}C-DDT in Tilapia

Results showed that DDT might rapidly accumulated in Tilapia. Because caudal fin is

composed of skin and born, the high concentration of DDT in caudal fin implied that skin was as important in absorbing DDT as gill (Fig .1, caudal fin and gill). For most organs, the peaks appeared within 24 h. Peak in caudal fin appeared at 2nd hour, while intestine, brain and muscle at 6th hour, liver and gallbladder at the 24th hour. Then gill and eye appeared at 48th hour. After the peak, the concentration of radioactivities in all the organs declined rapidly, as shown in Fig .1. It was notable that the decline of radioactivity in brain was much slower than other organs. On the 31th day, the radioactivity in brain was 30 .77% of the highest at 6th hour, while in liver, the radioactivity on the 31th day was only 4 .55% of the peak at 24th hour. A reasonable explain was higher fat content in brain than in other organs.

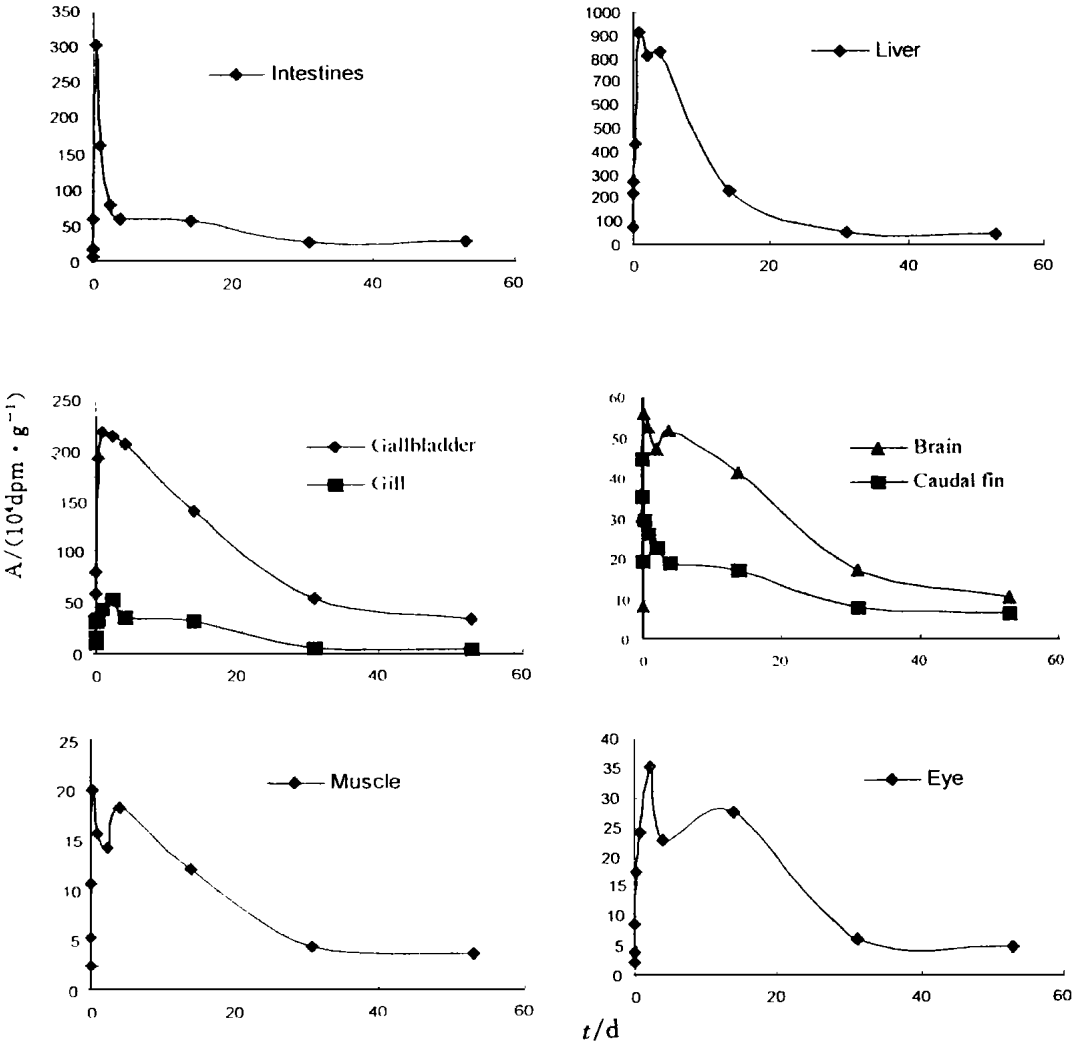

Fig .1 Accumulation of ^{14}C-DDT in organs of Tilapia

2 .2 The distribution and concentration factors of DDT in organs of Tilapia

The distribution of DDT in organs of Tilapia at the 24th hour of the experiment were shown in Tab .1. The radioactivities in organs ranked in descendant sequence were: liver,

Tab .1　The distribution and concentration of ^{14}C-DDT in organs of Tilapia, at the 24th hour

	Radioactivity (DPM g^{-1})	Concentration Factor		Radioactivity (DPM g^{-1})	Concentration Factor
Liver	9 113 690	48 477	Caudal fin	264 550	1 407
Gull bladder	2 194 670	11 674	Eye	241 790	1 286
Intestine	1 634 620	8 695	Muscle	158 900	686
Brain	528 520	2 811	Scale	145 640	775
Gill	438 370	2 332			

gallbladder, intestine, brain, gill, caudal fin, eye, muscle and scale. DDT and its metabolites were mainly accumulated in the digestive system. The relative radioactivity in liver was 57 times of that of muscle. However, because of the biodegradation, these radioactivity did not represent only DDT, as shown in Tab .2.

Tab .2　The Distribution of DDT metabolites in organs of tilapia on the 31th day, in percentage

	Muscle	Brain	Liver	Intestine	Bile
DDT	40 .54	11 .36	8 .42	5 .64	5 .78
DDE	11 .48	15 .92	17 .87	12 .94	9 .48
DDMU	4 .05	7 .38	3 .14	1 .41	4 .39
DDD	27 .02	51 .13	38 .74	45 .17	21 .80
DDA	2 .70	0	5 .48	6 .11	4 .55
unknown	14 .18	14 .21	26 .37	28 .70	54 .01

Concentration factor (CF) is widely used as index of the bioaccumulation capability of the aquatic organisms to pollutants. With traditional methods such as GC, the concentration factor of DDT are always expressed as the concentration of total DDT (*pp'*-DDT, *op'*-DDT, *pp'*-DDE, *pp'*-DDD) of organisms divided by the concentration of total DDT of the water[4]. The calculation of CF in this way is absolute acceptable because the exact amount of DDTs are used.

Concerning about the radioactive components of the organs and water, significant part of the radioactivities was not DDT and its non-polar metabolites any more, especially at the later stage of the experiment. For example, on the 5th day of the experiment, 56% of the radioactivity in water had been break down to polar compound (s) other than that of DDTs (Fig .3). Thus we strongly suggested that it should be cautious to calculate concentration factor simply by radioactivity contents of organisms and water, especially for some unstable labeled compounds. We still used the concept of concentration factors here so as to made it possible for a comparison with our previous studies and with the work of other authors. In our previous studies, DDT was proved to be persistent and much more stable in some organisms such as clam, muscles, and some other fishes. In these cases, concentration factors might be used after carefully analyzing the radioactive components and most of the radioactivities were proved to be DDT.

2 .3 Variation of Radioactivity in Water

Fig .2 showed the variation of radioactivity in water. Result showed that ^{14}C-DDT might be be rapidly uptaken by fish and the content of ^{14}C-DDT in water declined significantly within 6 hours. At the 6th hour, the radioactivity was only 11 .25% of the total radioactivity applied in the experiment. Then the radioactivity in water increased slowly. By TLC analysis, DDT residues in water on the 5th day of the experiment showed that

56.42% of the metabolites were polar unknown compound (s) other than DDTs. On the 26th day, the radioactive compounds in water were: DDT 2.93%, DDD 6.25%, DDE 1.95%, DDMU 0.98%, DDA 1.76%, unknown compound (s) 86.13%. Since significant parts of the radioactivity in the digestive system were polar unknown compound (s) (Tab.2), it was reasonable to believe that the increase of radioactivity in water was the result of the excretion of the metabolites from fishes.

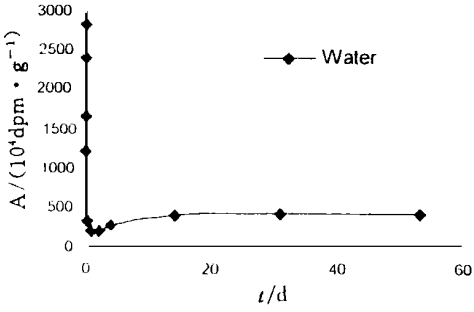

Fig.2 Variation of radioactivity in water

2.4 The DDT metabolites in viscera of Tilapia

The extractable metabolites of DDT in viscera of Tilapia were shown in Fig.3. Results showed that Tilapia could rapidly degrade DDT, at 2nd hour of the experiment, DDD and DDA were detectable, which were 6.82% and 8.69% of the total extractable component. At 6th hour, the percentage of DDD was 43.52%, higher than DDT, and then declined. The ratio of DDD/DDT and DDE/DDT were 56.43% and 3.50% respectively at the 24th h. The amount of DDD was around 16 times of DDE at that time. These results demonstrated that the degradation path of DDT in Tilapia is through DDD.

Hassall has summarized the tree major pathways by which DDT is degraded[8]. One involves dehydrochlorination to DDE, a second proceeds via reductive dechlorination to DDD and a third is oxidative and leads to dicofol. DDE is the major DDT-derived residue normally found in autopsy or biopsy samples of animal tissues. The enzyme catalyzing its formation has been termed DDT dehydrochlorinase or DDTase. The development of resistance to DDT in some species of insets is closely correlated with the greater capacity of resistant organisms to convert DDT to the less insecticidal DDE. Reductive dechlorination of DDT occurs widely in nature and DDD is an almost ubiquitous residue in animal fat. However the bulk of this residue may not be formed under normal conditions by enzymes located in vertebrate liver for DDD can be readily produced by bacterial living anacrobically in the gut[8]. The present result is difference from our previous studies which showed that the dominant metabolite in most of the marine organisms was DDE. Le et al[5] studied the residues of DDT in 13 species of fishes and 3 bivalves in Zhoushan Fishery field. There were 10 species in which the concentration of DDE were higher than DDD, with the DDE/DDD ratios ranging 1.2～2.5. In the rest 6 species, the ratios of DDD/DDE were in the range of 1.05～1.72. Similar results were also observed in Bohai Bay[4]. The rations of DDE/DDD were in the range of 0.6～2.78 in 11 species of fishes, among them, 8 species' concentration of DDE were higher than that of DDD. In our present study, the ratio of DDE/DDD (0.062) was extremely lower than reported in the literatures. This difference might be the result of species specifity and the sample of Tilapia for analysis being viscera.

2.5 Distribution of DDT Metabolites in Organs of Tilapia

Distribution of DDT metabolites in organs of Tilapia on the 26th day of the experiment were showed in Tab.2. The composition of DDT metabolites in organs was rather different. In muscle, 67.56% of the labeled compound were DDT and DDD, while in brain, DDD was the dominant metabolic product. In organs of the digestive system, DDD and polar unknown compound (s) were the majorities, unknown compound (s) possessed

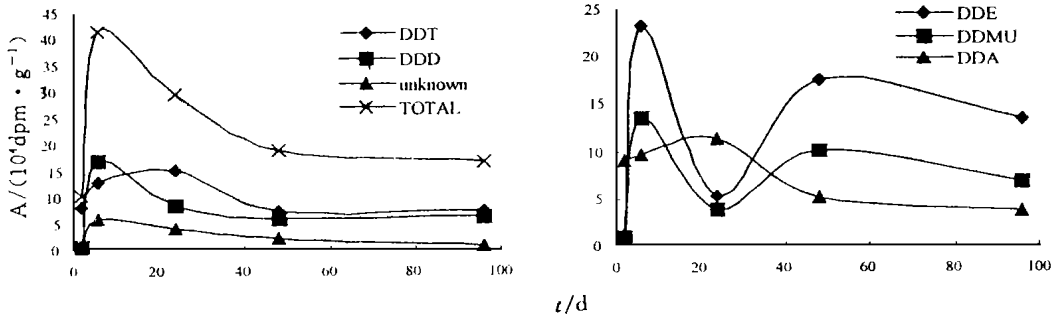

Fig .3　Variations of DDT metabolites in viscera of Tilapia

more than 50% of the total radioactivity. The results demonstrated again that DDT might be degraded to DDD and/or break down into smaller molecular in liver and excreted via bile through intestine to outside of the body.

References

1 Alexandratos N. World agriculture towared 2000, FAO. London: Rome and Belhaven Press, 1988

2 Carvalho F P, Fowler S W, Villeneuve J P, et al. Pesticide residues in the marine environment and analytical quality assurance of the results. In: Environmental behaviour of crop potection chemicals. Vienna: IAEA, 1996, 35～57

3 Xu B J, Zhang Y X. Behavior of ^{14}C-DDT in subtropical flooded soil. Nucl Sci Tech, 1993, 4: 199～203

4 Chen S, Zhang Y Q, Chen Z Q. Distribution and tranformation of HCH and DDT in fishes of Baohai Bay. Mar Environ Sci, 1983, 2 (3): 14～19

5 Le Z, Liu Z G. Studies on HCH and DDT residues in seawater, sediment and organisms in Zhoushan fishing field. Mar Environ Sci, 1985, 3 (3): 69～75

6 Liao Q. A sevey report of HCH and DDT residues in seawater, sediment and organisms in sea costa of East Guangdong. Mar Environ Sci, 1986, 5 (2): 8～14

7 Cai F, Lin Z, Chen Y, et al. The content and distribution of HCH and DDT in dry season in Zhujian River mouth area, China. Report of 2nd RCM, 1995

8 Hassal K A. The biochemistry and uses of pesticides. Houndmills: Macmillan Press Ltd, 1990

^{14}C-DDT 在罗非鱼体内的积累、分布及降解

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摘　要　用示踪法研究海水中^{14}C-DDT 在罗非鱼中的积累分布和降解. 结果表明, 24 h 内鱼体所有器官几乎都达到积累高峰, 随后缓慢下降. 在第24 h, 鱼体各器官对 DDT 的积累能力依次为: 肝脏, 胆囊, 肠, 脑, 鳃, 尾鳍, 眼, 肌肉和鳞片. 肝脏单位重量放射性活度为肌肉的57倍. 对 DDT 降解物的 TLC 分析发现, 内脏团中在2 h 内已经有 DDT 的降解物出现. 第6 h 内脏团中可提取物中 DDT 及其残留物的比例分别为: DDT 31.05%, DDD 43.52%, DDE 5.60%, DDMU 3.30%, DDA 2.32%, 原点未知物14.21%. 提示 DDT 能在罗非鱼中很快的降解, 主要降解产物为 DDD. 这和以往在其它鱼类得出的研究结果不同. 水中的放射性活度在48 h 内快速减少, 此后缓慢上升. 对水中标记残留物的 TLC 分析发现, 在实验后期水中放射性残留物已基本不是 DDT. 第26 d 水样的放射性残留物中, DDT 只占2.93%, 而原点未知物则占83.13%.

关键词　^{14}C-DDT, 罗非鱼, 积累, 分布, 降解

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