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# Excretion of DDT-Residues in Milk of Indian Buffaloes *Bubalus bubalis* (L.) Following Oral and Dermal Explosure to Technical DDT.

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The excretion of DDT-residues in milk of Indian buffaloes after oral and dermal exposure to technical DDT was studied. Equilibrium conditions of residue levels of DDT were attained in milk of animals fed on contaminated ration for 100 days. The transfer coefficient of technical-DDT in milk at 'plateau' levels showed an average value of about 12%. Half-life values for the rate of decline of DDT residues during the post-dosing period, ranged from 3 to 4 days during rapid-phase and from 5 to 24 days during slow-phase of decline. Dermal application of technical DDT also resulted in significant residues in milk. TDE analogues were the predominant compounds present in milk following oral ingestion of technical-DDT, whilst DDT analogues were present in greater quantity than the metabolites when animals were treated dermally.

Feeding of DDT and its excretion in milk of dairy cows have been studied extensively [1-8]. Recently, Kalra *et al.*[9] showed that buffaloes excreted more of DDT residues in their milk than cows, when fed on feed contaminatied with the *p,p'*-DDT. However, technical-grade DDT used for the control of insect pests is comprised of *p,p'*-DDT, *o,p'-DDT* and other compounds. According to Fries *et al.*[8] the excretion pattern of these compounds in milk by dairy cows has been found to be different from that of *p,p'*-DDT. The present studies were, therefore, undertaken to determine the pattern of excretion of technical grade-DDT into the milk of buffaloes following its oral administration and dermal exposure.

## MATERIALS AND METHODS

Fifteen buffaloes similar in body weight and stage of lactation were assigned randomly to five groups. The buffaloes were fed daily an average ration of 45 kg of green fodder, 4 kg of wheat bran and 2 to 6 kg of feed concentrate. The dry matter content of total feed was approximately 15 kg. The intake of total DDT through normal feed ranged from 0.1 to 2.5 mg per day. Four groups of 3 buffaloes each were fed daily with 10, 25, 50 and 100 mg of technical-DDT for 100 days following the already described procedure[9]. At the end of the period of compound intake, the animals were placed on normal feed.

For dermal application six buffaloes similar in body weight and stage of lactation, were assigned to two groups. Using a hand compression sprayer, each of the three buffaloes of the first group were completely drenched with 700 ml of 0.7% technical-DDT aqueous suspension prepared from 50% water dispersible powder (Hindustan Insecticides Ltd.) twice at 50 days interval. The animals of the second group were sprayed with water. The animals were prevented from licking their bodies during the first six hours following treatment, after which they were thoroughly bathed with clean water.

Milk samples (each measuring 200 ml) were collected at periodic intervals from morning milkings of each of the experimental buffaloes during the compound intake period as well as after the termination of intake of contaminated ration. In the case of buffaloes which received dermal application, milk samples were collected from morning/evening milkings at periodic intervals before and after treatment. The method described by Kapoor *et al.*[10] was followed for the estimation of DDT-

residues. The nature of the residues were confirmed by TLC on $AgNO_3$ incorporated alumina-G coated glass-plates[11] and alkali-dehydrohalogenation[12]. Average recoveries of *p,p'*-DDT, *p,p'*-TDE, *p,p'*-DDE, *o,p'*-DDT, *o,p'*-TDE and *o,p'*-DDE from spiked samples were above 80%. Fat content of milk samples was determined by Gerber's method[13].

## RESULTS AND DISCUSSION

DDT-residues (DDT-R) in milk were comprised of *p,p'*-DDT, *p,p'*-TDE, *p,p'*-DDE and their *o,p'*-DDT analogues. The normal feed of the animals contained unavoidable contamination which produced back-ground level ranging from 0.50 to 1.02 mg $kg^{-1}$ of DDT-R in milk fat at different intervals. These values were substracted while calculating the residue levels in the milk of treated animals. The changes in the average concentration of DDT-R in the milk fat with time, based on the analysis of milk from individual bufalloes in a group, are presented in Fig. 1. Following the intake of technical-DDT, the concentration of DDT-R in milk fat increased rapidly during the first 10 days and thereafter slowly attained a steady state during the dosing period. A similar trend in the daily excretion of DDT-R in milk was observed (Fig. 2).

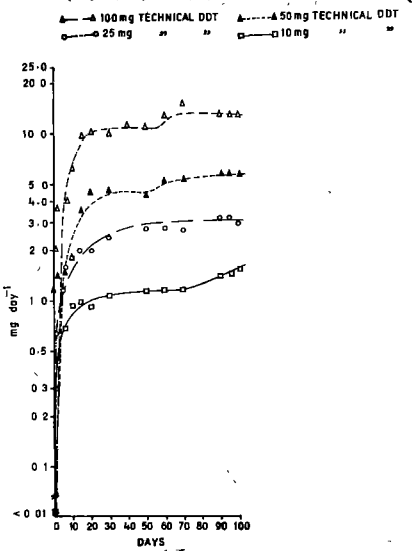


Figure 1 Changes in concentration of DDT-R in milk fat during the dosing period (Treatment: Oral administration of technical DDT)

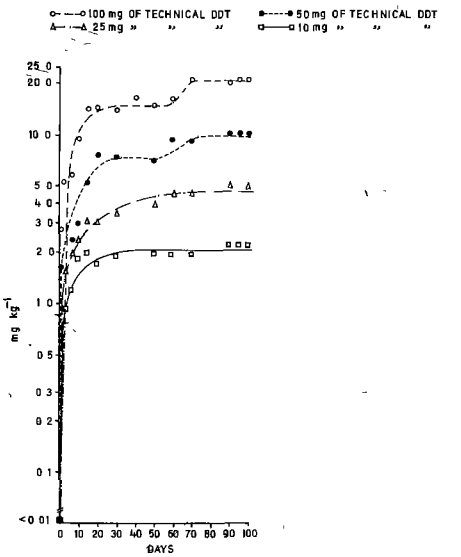


Figure 2 Changes in the daily excretion of DDT-R in milk during the dosing period (Treatment: Oral administration of technical DDT)

The transfer coefficient of technical-DDT (Table 1) ranged from 10.95 to 15.75 with an average value of 12.67. This value is greater than 5% or less reported for dairy cows[5,8] and is comparable with the value reported by Kalra *et al.*[9] for buffaloes fed on ration contaminated with *p,p'*-DDT. The average 'plateau' level concentration of DDT-R in milk fat when plotted against the corresponding daily intake level of technical-DDT, fell along a straight line (Fig. 3), that on extrapolation to the extraneous residue limit of 1.25 mg $kg^{-1}$ of DDT in milk fat established by FAO/WHO, corresponded to a daily intake of 6 mg of technical-DDT. On the other hand, Fires *et al.*[8] reported that the daily intake of 25 mg of *p,p'*-DDT by dairy cows resulted in DDT-R less than 1.25 mg $kg^{-1}$ in milk fat. The amount of 10, 25, 50 and 100 mg of technical DDT, when present in daily ration of 15 kg dry weight, resulted in contamination of feed at levels of 0.67, 1.67, 3.33 and 6.67 mg $kg^{-1}$ (dry weight basis), respectively. Thus, a 1:3 relationship between concentration of technical-DDT in feed and the concentration of DDT-R in milk fat was observed, as against approximately 1:1 relationship reported in the case of dairy cows [7,14,15]. The results further showed

**Table 1 Values of milk fat concentration, daily excretion, transfer coefficient and accumulation coefficient of DDT-R at 'plateau' for individual buffaloes in different groups of animals following oral administration of technical-DDT**

| Daily intake level (mg) | Replicates | Milk yield (kg) | Fat content (%) | DDT-R in milk fat (mg $kg^{-1}$) | DDT-excretion (mg $day^{-1}$) | Transfer coefficient (%) | Accumulation coefficient |
|---|---|---|---|---|---|---|---|
| 10 | 1 | 8.48 | 7.28 | 1.785 | 1.102 | 11.020 | 2.664 |
| | 2 | 11.76 | 6.90 | 1.942 | 1.575 | 15.750 | 2.898 |
| | 3 | 8.10 | 7.06 | 2.903 | 1.660 | 16.660 | 4.229 |
| | Mean | 9.45 | 7.08 | 2.210 | 1.445 | 14.457 | 3.298 |
| 25 | 1 | 9.63 | 7.83 | 4.086 | 3.081 | 12.324 | 2.447 |
| | 4 | 5.40 | 8.10 | 4.580 | 2.000 | 8.000 | 2.742 |
| | 3 | 9.78 | 6.72 | 5.773 | 3.794 | 15.176 | 3.457 |
| | Mean | 8.27 | 7.55 | 4.813 | 2.958 | 11.883 | 2.882 |
| 50 | 1 | 10.00 | 8.30 | 7.613 | 7.610 | 15.220 | 2.747 |
| | 2 | 4.53 | 8.40 | 9.728 | 4.036 | 8.072 | 3.193 |
| | 3 | 7.10 | 7.36 | 10.000 | 5.616 | 11.232 | 3.228 |
| | Mean | 7.18 | 8.02 | 9.113 | 5.754 | 11.508 | 3.056 |
| 100 | 1 | 11.50 | 6.84 | 16.980 | 14.866 | 14.866 | 2.612 |
| | 2 | 5.93 | 8.50 | 16.923 | 10.952 | 10.952 | 3.361 |
| | 3 | 6.30 | 8.77 | 23.295 | 12.870 | 12.870 | 3.942 |
| | Mean | 7.91 | 8.03 | 19.066 | 12.896 | 12.896 | 3.137 |

**Table 2 Correlation between residue level of DDT in milk (mg kg-1) and milk yield (kg) and fat content (%) of milk**

| Daily intake level (mg) | No. of observations | Correlation coefficient (r) | |
|---|---|---|---|
| | | DDT-R and milk yield | DDT-R and fat content |
| 10 | 12 | -0.599 ($p < 0.05$) | 0.508 ($p < 0.05$) |
| 25 | 18 | -0.235 ($p < 0.20$) | 0.346 ($p < 0.10$) |
| 50 | 20 | -0.723 ($p < 0.01$) | 0.757 ($p < 0.01$) |
| 100 | 23 | -0.889 ($p < 0.01$) | 0.799 ($p < 0.01$) |

that the residue concentration of DDT in milk at steady state was affected by the changes in milk yield and fat content. In general, the residue level showed an inverse correlation with milk yield and direct correlation with milk fat content (Table 2). The higher residue level of DDT found in buffaloe's milk than that in cow's milk may, therefore, be attributed to a lower output of milk with higher fat content by buffaloes as compared to cows. Zweig *et al.*[5] also reported that the cows which produced milk with a higher fat content had a higher concentration of DDT residues in milk.

The limiting value of DDT-R in animal feed (dry weight basis) derived by dividing the estimated acceptable level of intake (6 mg) by the average daily ration (15 kg) is 0.4 mg $kg^{-1}$. As the highest accumulation coefficient of technical-DDT was observed to be 4.69, the total content of DDT in feed must, therefore,be kept less than one-fifth of the extraneous residue limit of DDT in milk fat. In this way the limiting value of DDT in feed works out to be 0.25 mg $kg^{-1}$ on dry weight basis.

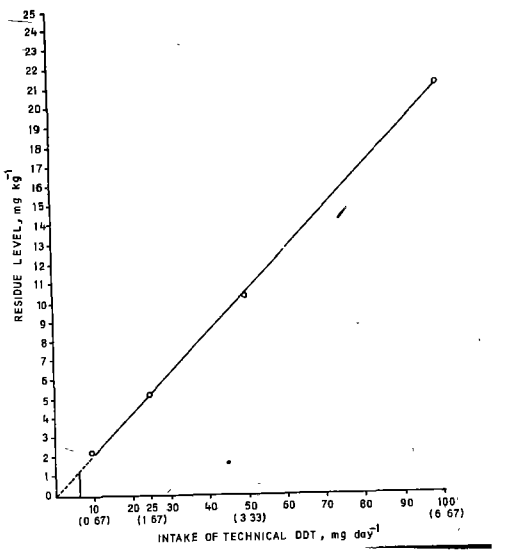


Figure 3 Relationship between the concentration of DDT residues in milk fat and its daily intake by buffaloes (Values in Parantheses give the concentration of pesticide in feed mg $kg^{-1}$ dry weight basis)

The data on rate of decline of DDT-R in milk fat after the end of the intake period of the compound revealed that there was an initial fast rate of decline followed by a slow decline (Fig. 4). Half-life values ($T_{1/2}$) for the rapid decline phase were 1.9, 1.8, 2.8 and 3.1 days for dose level of 10,25,50 and 100 mg technical-DDT, respectively (Table 3). The corresponding values for slow-decline phase were 4.8, 11.4, 22.4 and 23.6 days, respectively. The residue levels of DDT-R in milk reached the background level in 30, 50, 80 and 100 days after the termination of feeding ration contaminated with 10, 25, 50 and 100 mg of technical-DDT, respectively (Fig. 4). Thus, the dosage of technical-DDT, was found to affect significantly the rate of slow decline in milk. These results are in accordance with those obtained by Kalra *et al.*[9] using *p,p'*-DDT. Bruce *et al.*[16] reported the half-life of DDT to be as high as 300 days when cows were fed on ration contminated with concentration of 100 mg $kg^{-1}$ of DDT. However, Whiting *et al.*[17] had reported faster rate of decline at higher dosage of DDT. The overall results suggest that the decline of DDT-R in milk of buffalloes was more rapid than that in cow-milk at comparable levels and duration of intake. The two distinct dissipation rates of DDT-R in buffalo milk suggest two compartment storage having

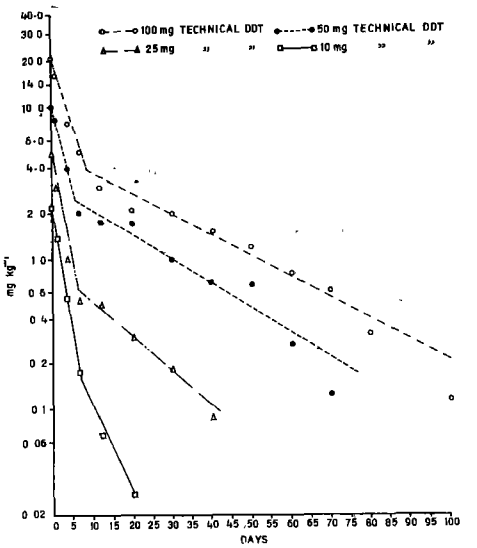


Figure 4 Changes in concentration of DDT-R in milk fat during the post-dosing period (Treatment: Oral administration of technical DDT)

**Table 3 Half-life and residues in milk fat of buffaloes during post-dosing period of technical-DDT**

| Daily intake level, (mg) | Residue level (mg kg$^{-1}$) | Fast-decline phase | | Slow-decline phase | |
|---|---|---|---|---|---|
| | | $m_1$ | $T_{1/2}$ (days) | $m_1$ | $T_{1/2}$ (days) |
| 10 | 2.204 | -0.154 | 1.94 | -0.062 | 4.84 |
| 25 | 5.076 | -0.171 | 1.75 | -0.026 | 11.41 |
| 50 | 10.33 | -0.109 | 2.76 | -0.014 | 22.35 |
| 100 | 21.123 | -0.097 | 3.09 | -0.013 | 23.57 |

different rates of excretion[18]. Evidently, the time required for the concentration of DDT-R in milk to decline below the tolerance level will depend upon the 'plateau' concentration attained. If the concentration of residues attained is low, it will decrease to the acceptable level in few days after the cessation of the feeding of contaminated ration, otherwise greatly extended periods will be required.

Dermal application of aqueous suspension of water dispersible DDT powder also resulted in a rapid increase of DDT residues in milk fat (Fig. 5). The first spray produced a maximum residue level of 11.9 mg kg$^{-1}$ DDT-R in milk fat one day after the treatment. During the next 45 days, there was a decrease in the concentration of DDT residues to a level of 0.06 mg kg$^{-1}$. The second application of DDT made 50 days after the first application resulted in the maximum level of 6.8 mg kg$^{-1}$ of DDT residues in milk fat after 30 h of treatment. Thereafter, the residues declined but did not attain the background level even after 45 days of treatment. The data on the rate of decline of DDT-R in milk fat showed an initial rapid decline which progressively decreased. Half-life values for the first and second dermal applications, respectively, were 1.25 and 1.98 days for fast-decline and 6.56 and 10.56 days for slow-decline phases.

Following oral administration and dermal appliation of technical DDT to buffaloes, six compounds, namely *p,p'*-DDT, *p,p'*-TDE, *p,p'*-DDE, *o,p'*-DDT, *o,p'*-TDE and *o,p'*-DDE were detected in milk. However, their proportions were different

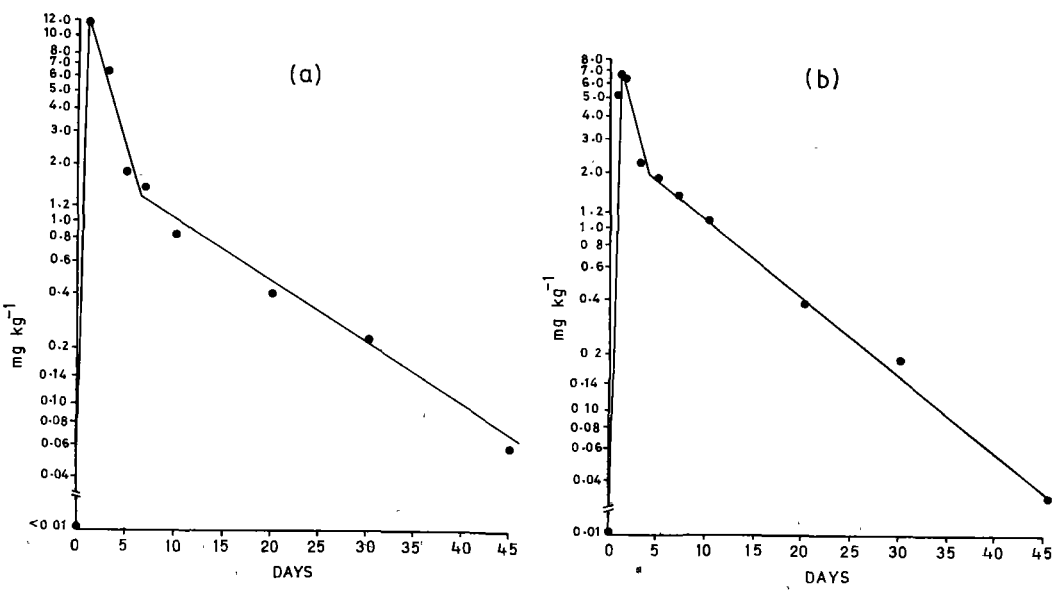


Figure 5 Changes in concentration of DDT-R in milk fat following dermal application of technical DDT. (a) First spray (B) Second spray

(Fig. 6). DDT residues in milk from buffaloes fed with technical-DDT were mainly in the form of *p,p'*-TDE. This was followed by *p,p'*-DDT. The high proportion of *p,p'*-TDE found in milk has been ascribed to the reductive dechlorination of *p,p'*-DDT by rumen microorganisms[19,20]. Witt *et al.*[14] considered that the routes of exposure which by-pass the rumen would cause little conversion of DDT. Thus, the information on the relative proportion of DDT and TDE in samples of milk in commerce, may prove useful in predicting the sources of residues. The sum total of *o,p'*-TDE and *o,p'*-DDE did not exceed 5% of the total DDT residues, though *o,p'*-DDT constituted about 20% of technical-DDT used in the present study. The presence of low levels of *o,p'*-DDT analogues is due to its low transference from feed to milk[21].

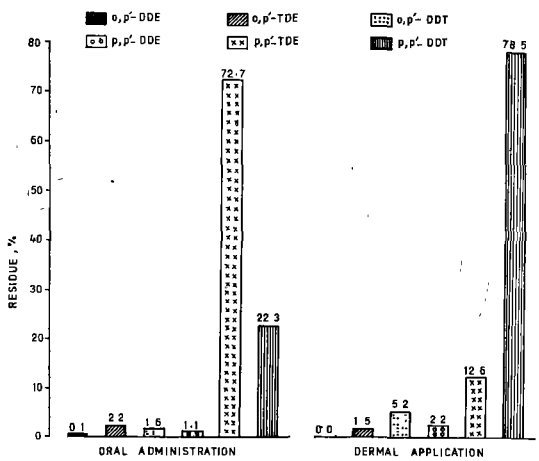


Figure 6 Nature of DDT residues in milk of buffaloes following different routes of exposure to DDT.

## ACKNOWLEDGMENT

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