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ASSESSMENT AND EVALUATION OF HEXACHLOROCYCLOHEXANE (HCH) AND DICHLORODIPHENYLTRICHLOROETHANE (DDT) RESIDUES AND EXTENT OF DNA DAMAGE IN CATTLE OF KASARGOD DISTRICT, NORTHERN KERALA, INDIA

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ABSTRACT: Pesticide uses pose a serious threat to environment and public health owing to their persistence and tendency to accumulate in animal and plant tissues. The present investigation was conducted to analyze pesticide residues mainly HCH and DDT in field, and biological samples from the plantation areas of Northern Kerala, India and the extent of DNA damage in cattle was measured. Environmental samples mainly water and fodder and biological samples milk and blood were collected from study area. All samples were analyzed using Gas Chromatography with Electron Capture Detector and confirmed by Gas Chromatography with Mass Spectrometry (GC-MS). The extent of DNA damage was analyzed using COMET assay. Among samples analyzed, water samples revealed detectable pesticide residues. One water sample was detected with all HCH isomers, and two samples were detected with *p,p'*-DDD. Mean concentration (ppm) of total HCH and DDT were 8.073×10^{-4} and 3.51×10^{-5} respectively. Pesticide residues in fodder and biological samples were with below detectable level. Comet assay revealed significantly higher ($p < 0.05$) percent DNA in tail, tail length, tail and olive tail moment in study area blood samples than control. Presence of high ratio of β HCH isomer and *p, p'*-DDD in environmental samples reveals the existence of persistent organic pollutants (POPs) in study area. Absence of detectable level of residues in biological samples, necessitate further investigation to corroborate basis for DNA damage detected.

INTRODUCTION: Indiscriminate and disproportionate use of pesticides can lead to their residues getting into the food chain and then resulted in harmful effects in human beings and animals.

Persistent organic pollutants (POPs) are semi-volatile hazardous chemicals having anthropogenic origin and having the persistence, bioaccumulation, long-range transport and toxic characters ^{1, 2}. In recent years, many POPs are believed to be possible carcinogens or mutagens and are of considerable concern to human and environmental health ³.

Organochlorine is used as an insecticide to control the pests and ectoparasites in animals. Hexachlorocyclohexane (HCH) and Dichlorodiphenyltrichloroethane (DDT) are common organochlorines

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found as ubiquitous anthropogenic environmental contaminants. Growing concern over adverse environmental effects, especially on wild birds, led to severe restrictions and bans on DDT in many countries in the early 1970s⁴. But in several tropical countries like India, DDT is still being manufactured and used in various public health programs due to less cost and efficiency. Dichlorodiphenyltrichloroethane (DDT) is a moderately toxic organochlorine insecticide which is slowly metabolized from the body and can act both as an acute and chronic poison. It is absorbed into the body through gastrointestinal tract and by inhalation. It may also be absorbed through intact skin, especially in case of oil-based formulations⁵.

Chemically, technical DDT is a mixture, the main components of which are p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT) (63-77%), o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT) (8-21%) and p,p'-dichlorodiphenyldichloroethylene, (p,p'-DDE) (0.3-4%). It also contains DDE and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) as contaminants; both are breakdown products of DDT⁴.

DDT and especially DDE are highly resistant to metabolism and especially in cold and temperate climates. They tend to be highly persistent in the environment. DDT and DDE have very high octanol-water partition coefficients, meaning that they are very lipid-soluble, and tend to accumulate in lipid compartments in biological systems. DDE has a very long half-life and is of toxicological importance⁴. Half-lives of DDT and DDE in humans have been estimated in ranges between 6 and 10 years⁶ so that even short-term exposures can raise body burdens.

Technical grade HCH consists of 65-70 percent of α -HCH, 7-10 percent of β -HCH, 14-15 percent of γ -HCH, and approximately 10 percent of other isomers and compounds. Lindane contains more than 99 percent of γ -HCH. It is solid, with low vapor pressure and is poorly soluble in water but very soluble in organic solvents such as acetone and aromatic and chlorinated solvents. Lindane is being used as a broad-spectrum insecticide since the early 1950s for agricultural and non-agricultural purposes which include treatment of seeds and soils, application on trees, timber and stored

materials, treatment of animals against ectoparasites and in public health⁷.

HCHs and DDT were classified in group 2B (possibly carcinogenic) by IARC⁸. Genotoxic effect of HCHs and DDT affect human and animal health directly, damaging the genetic material, which is considered to play an important role in oncogenesis. According to Ramirez and Cuenca⁹, alkaline single cell gel electrophoresis (Comet assay) is of value in the genetic monitoring of pesticide exposed populations. The genotoxic potential of HCH and DDT has been studied by Lessa¹⁰, Gopalaswamy and Nair¹¹, Hassoun¹², Rehana¹³, Mattioli¹⁴, Gauthier¹⁵, Kalantzi¹⁶, Ennaceur¹⁷, Garaj-Vrhovac¹⁸ and Zhao¹⁹ observed the potential genotoxic and mutagenic activity of pesticides by using the different models.

Severe health and environmental problems of the Kasargod district, Northern Kerala, India have been attributed to endosulfan use, which was being aerially sprayed by Plantation Corporation of Kerala in the cashew plantation areas for past 24 years. In 2001, Pesticide monitoring Lab of Centre for Science and Environment (CSE) found traces of pesticides in all the samples analyzed from the region ranging from human blood and milk to soil, water, fruits, vegetables, cow's milk and skin tissue, fish and frog. The study area is being reported for unreasonably high proportion of obscure disease among the animals. Analysis of soil samples from northern Kerala, India has revealed total endosulfan residue in the range of 0.001-0.01 $\mu\text{g/g}$ ²⁰. However, data on other pesticide residue levels are remaining unanswered. So, the present study was undertaken to assess the level of HCH and DDT residues in water, fodder and biological samples and to measure the extent of DNA damage in cattle blood from Enmakaje Panchayath of Kasargod, Kerala, India.

MATERIALS AND METHODS: A preliminary survey was conducted among 60 farmers belonging to the study area to identify the water sources, pesticide usage, cattle rearing pattern, prevailing disease pattern in cattle. Standard solutions of HCH (α , β , γ , and δ) and DDT (p , p' DDE, p , p' DDD, p , p' DDT) were bought from Sigma- Aldrich, India.

Sampling Methodology: Representative environmental samples of water and fodder that are consumed by cattle in the study area were collected. Biological samples such as milk and blood were collected from 20 adult cattle which are being maintained for a period of past five years or more in the selected study area.

Environmental / Field Samples: Water and fodder samples were collected as per the method described by Lorgue²¹.

Drinking water sources for cattle in these villages are from open wells, streams, tunnel wells, bore wells, and paddy fields. A total of 20 representative water samples were collected in one liter screw-top labeled sterile glass bottles for analysis. Fodder samples were collected randomly from open grazing lands, local vegetation and dried hay routinely fed to animals in the study area, labeled and stored in polyethylene bags for further analysis.

Biological Samples: Fresh individual milk samples (500 ml) were collected in sterile glass bottles from randomly selected 20 cows inhabiting the area. Each 10 ml of venous blood was collected from 20 cattle. Each five ml of blood was used for comet assay and remaining separated to serum and used for pesticide residues analysis. Blood samples from 20 cattle in the University Livestock Farm (ULF), Mannuthy were collected and used as controls for the comet assay.

Pesticide Residue Extraction: Samples collected were processed using standard clean up techniques to remove impurities and to concentrate the pesticide residue in the sample before injecting into the Gas-Liquid Chromatography (GLC).

Environmental Samples:

Residue Extraction from Water: Clean up procedure given by AOAC²², was followed for processing water samples. 750 ml of water sample along with 150g NaCl was taken in separating funnel and shaken well to dissolve followed by 75 ml of dichloromethane (DCM) was added and shaken well for one minute, and then the pressure was released.

This mixture was again shaken on the mechanical shaker at 250 rpm for 5 min. The separating funnel was then placed on a stand for 5 min to separate the

organic and aqueous layers. The lower organic (DCM) layer was collected into a conical flask. The partitioning was repeated twice using 40 ml of DCM. Again the partitioning was repeated using 50 ml of hexane. The entire organic layer was collected and combined to which 5-10 g of anhydrous Na₂SO₄ was added till free-flowing of organic layer. This was then concentrated on rotary evaporator (Make: Heidolph®) to about 5 ml and to this extract, 20 ml of n-Hexane was added and again concentrated to 2-3 ml. This concentration process was repeated twice. Then the residues were transferred into a test tube and evaporated the contents to dryness on a Turbo vap evaporator (Make: Zymark TurboVap LV). Finally the extract was taken in 1 ml of n-Hexane for injection into GC.

Residue Extraction from Fodder: Clean up was performed by following Dhanya²³, procedure. Fodder samples were taken in an extraction thimble (25g) and introduced into Soxhlet extraction unit. This was then extracted with 200ml solvent mixture of acetone and hexane in 1: 1 combination. The extraction was carried for six siphoning and extract obtained was concentrated using Rotary evaporator (Heidolph®).

Acetonitrile Clean Up: After evaporation, the extract was collected, to which 15 ml of acetonitrile saturated with petroleum ether was added and shaken and repeated thrice. The bottom layers were transferred to one liter separating funnel containing 600 ml of water, 100 ml of petroleum ether and 40 ml of saturated NaCl solution respectively. Shook well and allowed the layers to get separate. The bottom aqueous layer was transferred to another one liter separating funnel containing 100 ml of petroleum ether. Shook well and allowed to separate. The aqueous layers were discarded and petroleum ether layers from two separating funnels were pooled together and washed with 100 ml of distilled water thrice and dried with anhydrous Na₂SO₄ and evaporated using vacuum flash evaporator (Heidolph®).

Florisil Column Chromatography Clean Up: A glass column (30 × 450 mm) was prepared by adding 5 g of anhydrous Na₂SO₄ at the bottom, 25 g of florisil in the middle and 10 g of Na₂SO₄ on the top. This column was wetted with petroleum ether

and allowed to drain. When petroleum ether came down to the surface of Na₂SO₄, added acetonitrile clean up a sample using small quantities of petroleum ether. Then eluted the column with 200 ml of 6 percent diethyl ether in petroleum ether followed by 15 percent diethyl ether in petroleum ether. Both elutes pooled together and vacuum flash evaporated. The residue was dissolved in 5 ml of petroleum ether and injected into GC.

Biological Samples:

Residue Extraction from Milk: Milk samples were analyzed as per the method specified by FDA²⁴ and Sharma²⁵.

Ten gram of milk was weighed in a beaker and to this 5g of florisol and 5g of Na₂SO₄ were added and then mixed well. This mixture was then packed into the column of 25mm × 50cm length. To this packed mixture, 50 ml of acetone was added and kept undisturbed for 30 min. After 30 min, elute was collected and concentrated to 10ml volume by evaporation. This was then transferred to a separating funnel. Into this 100 ml of distilled water and 20g of NaCl was added, mixed thoroughly. This was then eluted thrice with 30ml of hexane each time. The elutes was concentrated to dryness using vacuum flash evaporator and residue was extracted in 2 ml of n-hexane for injection into GC.

Residue Extraction from Blood: Clean up procedure given by Pitarch²⁶ was followed for processing blood samples.

Two ml serum was diluted with 3 ml deionized water and then 5 ml DCM was added and shaken well for five minutes. Repeated this procedure and extract was centrifuged to remove the emulsion obtained. The organic layer formed after extraction was then separated and dried by passing through a column packed with 3 cm layers of anhydrous Na₂SO₄. The extract was evaporated to dryness under a gentle stream of air and residue was dissolved in 1ml of n-hexane for injection into the GC.

Pesticide Residue Analysis on Gas Chromatography (GC): Pesticide residues analysis was performed using SHIMADZU-2010 GC with electron capture detector (ECD) having

⁶³Ni as radioactive source with following operating condition.

Column	30m × 0.25 mm
Injection port ratio	250 °C, split mode, 1: 30 split
Oven temperature	170 °C
Detector	ECD, 300 °C
Carrier	Nitrogen gas
Sampler	Analytical syringe-2µl injection
Analysis	70 min

Pesticide Residue Confirmation on GC-MS:

Pesticide residues were confirmed by using SHIMADZU-2010 GC with Mass Spectrometry detector (GC-MS) - Quadrupole on electron ionization (EI) mode equipped with split/splitless auto-injector model AOC-20i. The non-polar stationary phase used was a fused silica capillary column DB-1 (1 % phenyl polysiloxane) of 30 m, 0.25 mm i.d., and 0.25 µm film thickness.

Measurement of DNA Damage by Comet Assay:

The basic alkaline technique of Singh²⁷ with some modification described by Dhawan²⁸ was followed.

Slide Preparation and Blood Cell Incorporation in the Slide:

Microscope slides were covered with 1.0% Normal Melting Point Agarose (NMPA) in Milli Q water by dipping the slide at about 45°C. Slides were wiped underside; air-dried and kept in the incubator overnight. This layer was used to promote the attachment of the second layer of 0.5% Low Melting Point Agarose (LMPA). Aliquots of 5–10 µl of whole blood containing approximately 10,000 cells were mixed with 200 µl of 0.5% LMPA to form a cell suspension.

The cell suspension was rapidly pipetted onto the first agarose layer, spread the suspension by using a coverslip, and keep the slide on the ice pack for 5 min to solidify. After removal of the coverslip, the third layer of 1% LMPA (200 µl) at 37 °C was added, then spread the suspension as described above. After removal of the coverslip the slides were immersed in prechilled lysing solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris; pH 10) with 1% Triton-X100 and 10% DMSO (added just before immersion) for a minimum of 1 h at 4 °C.

Alkali Treatment and Electrophoresis: The slides were removed from the lysing solution, drained and placed in a horizontal gel electrophoresis tank side by side, avoiding space and with the agarose ends facing each other, nearest the anode. The tank was filled with fresh electrophoresis solution (1 mM Na₂EDTA and 300 mM NaOH, pH >13) to a level approximately 0.25 cm above the slides.

Before doing electrophoresis, the slides were left in the solution for 30 min to allow the unwinding of the DNA and for the expression of alkali-labile site.

Electrophoresis was conducted for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level in the tank. All these steps were conducted under dimmed light to prevent additional DNA damage. After electrophoresis, the slides were neutralized by Tris buffer (0.4 M Tris, pH 7.5) for 5 min. The neutralizing procedure was repeated three times.

Staining: To each slide, 40 µl Ethidium bromide (20 µg/ml) was added. The slides were covered with a coverslip and analyzed within 3–4 h after staining.

Scoring of DNA Damage: For analyzing DNA damage slides were observed at 40X magnification on a fluorescent microscope (Make: ZEISS LAB.A1 AX10). Images of 100 randomly selected lymphocytes were analyzed from each sample, and the DNA damage was scored visually. The quantification of the DNA strand breaks of the stored images was done by the imaging software CASP using this percentage DNA in tail, tail length, tail moment, and the olive tail moment were obtained. The distribution of DNA between the tail and head of the comet was used to evaluate the degree of DNA damage.

Statistical Analysis: The results are expressed as mean with standard deviation. Results were analyzed using Statistical Package for the Social Sciences (SPSS) 17 version. Statistical comparison of Comet assay results from the study area and control samples was performed using the student t-test (unpaired, two-tailed). Type I error alpha level was set at 0.05 and data with p-value ≤ 0.05 considered as significantly different.

RESULTS: According to the preliminary survey, most of the animals are allowed for grazing in open fields. Major source of drinking water for animals is tunnel wells (65%) and also have easy access to water from open ponds (30%) and canals (5%) which may be contaminated with agricultural runoff. Main type of feed given to cattle is paddy straw (74%), fodder (15%), concentrate (5%) and miscellaneous (6%). Most of the farmers in the study area recently started to follow organic farming, and in some places they relied on spraying of copper sulfate solution for agricultural practice. Disease prevailing are indicated in **Fig. 1**.

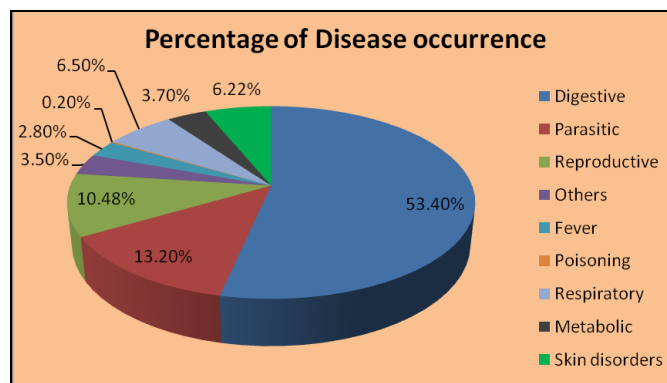


FIG. 1: PERCENTAGE OCCURRENCE OF DISEASE IN THE STUDY AREA

Recovery Study: Extraction and clean up procedures of field and biological samples were tested for recovery percentage by fortifying the samples with a known amount of HCH and DDT standard. Recovery studies revealed the recovery about 70-80 percent is satisfactory to carry out sample analysis. **Fig. 2** showing the Chromatogram of HCH and DDT Standard and their retention time.

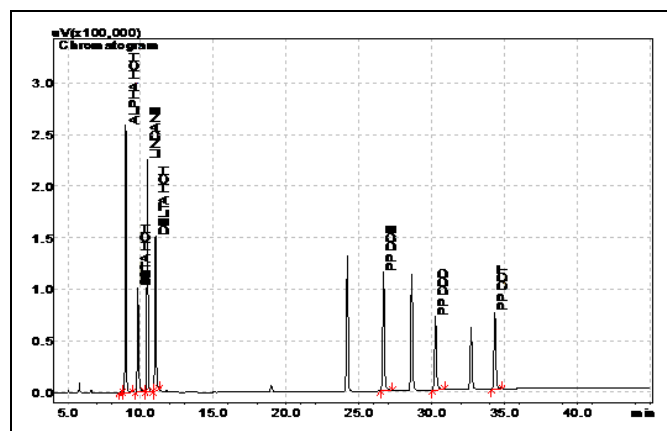


FIG. 2: CHROMATOGRAM OF HCH AND DDT STANDARD AND THEIR RETENTION TIME

HCH and DDT Residues in Environmental Samples:

Water: Among the all analyzed water samples, one sample was detected with residues of all the isomers of HCH and the mean concentration (ppm) of total HCH was $8.073 \times 10^{-4} \pm 8.073 \times 10^{-4}$. The chromatogram of the water sample detected with HCH pesticide residues from the study area is

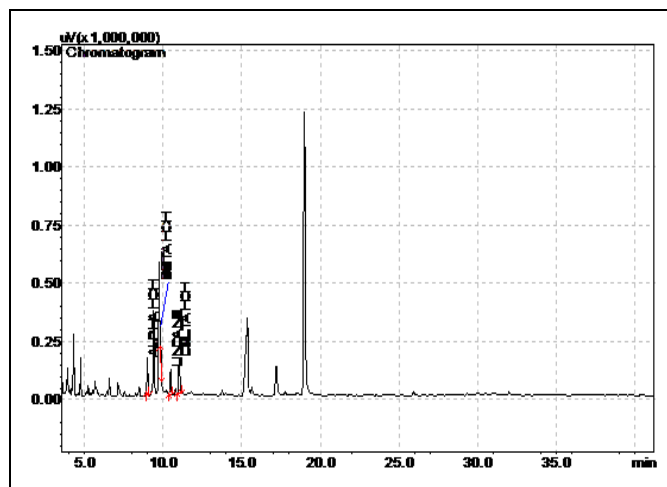


FIG. 3: CHROMATOGRAM OF WATER SAMPLE WITH PRESENCE OF ISOMERS OF HCH

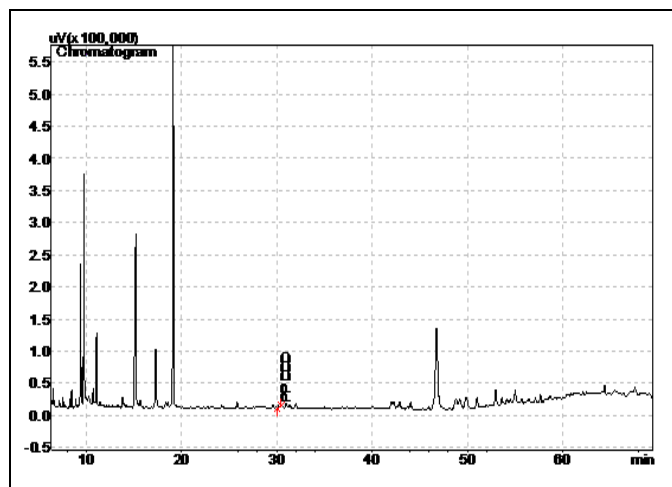


FIG. 4: CHROMATOGRAM OF WATER SAMPLE 1 DETECTED WITH P,P'-DDD FODDER

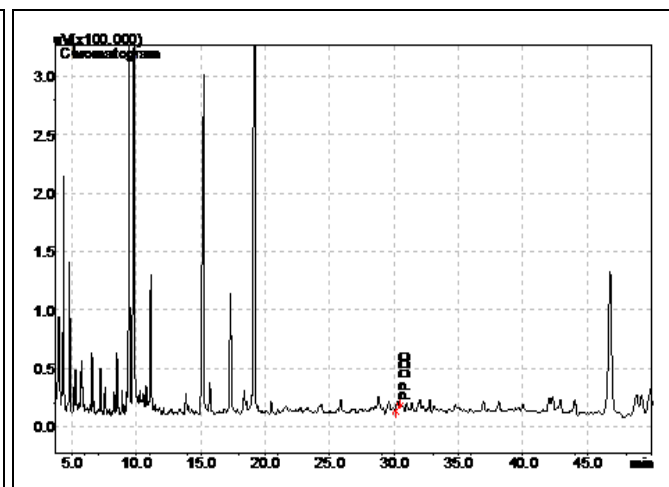


FIG. 5: CHROMATOGRAM OF WATER SAMPLE 2 DETECTED WITH P,P'-DDD

Residues of HCH and DDT were with below detection limit in all analyzed fodder samples (data not shown).

HCH and DDT Residues in Biological Samples:

Milk and Blood: HCH and DDT residues were below detection limit in all of the milk and blood samples collected from the study area (data not shown).

Single-Cell Gel Electrophoresis (Comet Assay):

The mean values of percent DNA in tail, tail

length, tail moment and olive tail moment of blood samples were estimated, and the values are presented in Fig. 6. Comets of study sample are shown in Fig. 7 and 8 and control samples are shown in Fig. 9 and 10. The values of percent DNA in tail, tail length, tail moment and olive tail moment in blood samples of cattle from study area were significantly higher ($P < 0.05$) than the corresponding values of blood samples of control animals which indicates higher level of DNA damage in the study area.

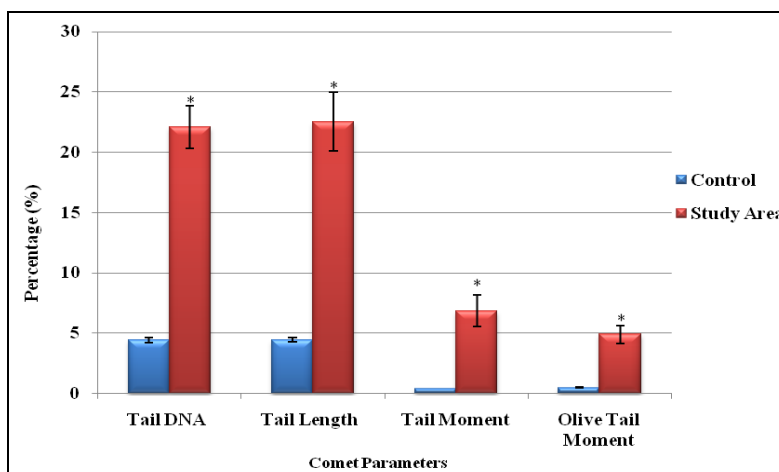


FIG. 6: COMET PARAMETERS OF BLOOD SAMPLES FROM THE CONTROL AND STUDY AREA
Results are expressed as Mean ± SD. P value ≤ 0.05 considered as significant.

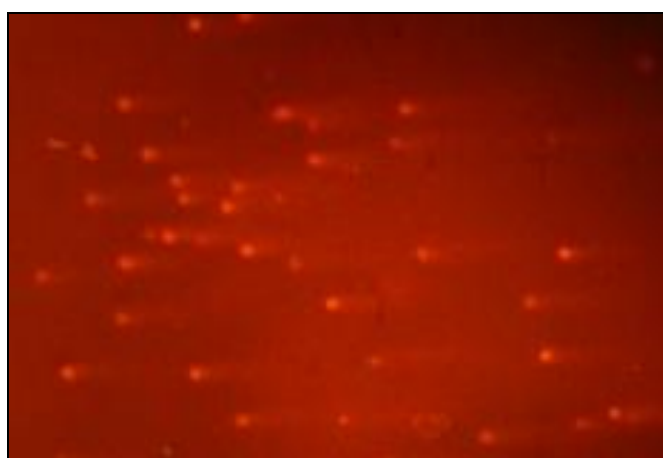


FIG. 7: COMET CELLS FROM STUDY AREA (10X)

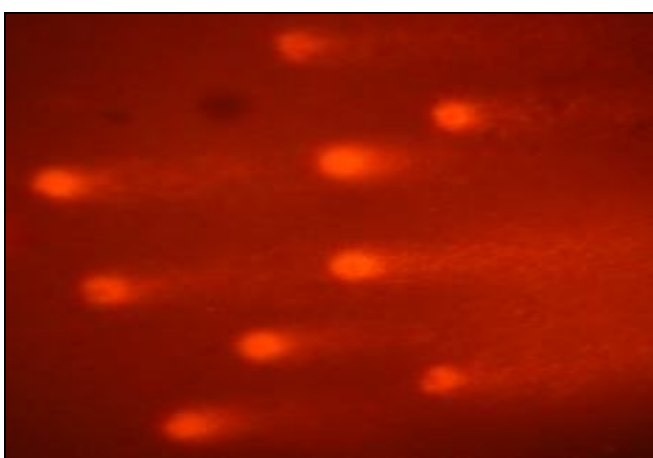


FIG. 8: COMET CELLS WITH VARYING INTENSITY OF DNA DAMAGE FROM STUDY AREA (40X)

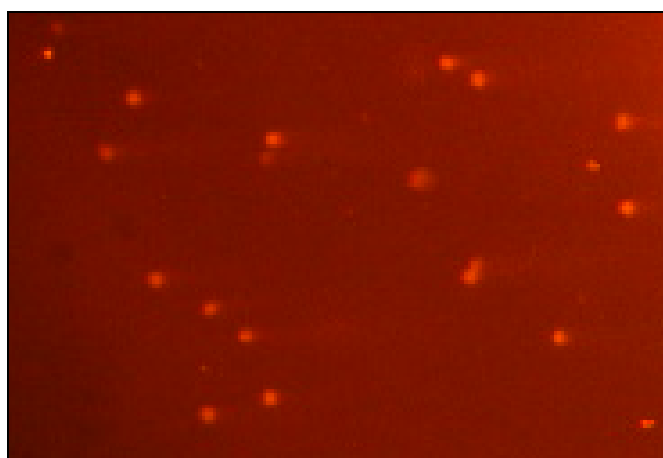


FIG. 9: COMET CELLS OF CONTROL SAMPLES FROM ULF (10X)

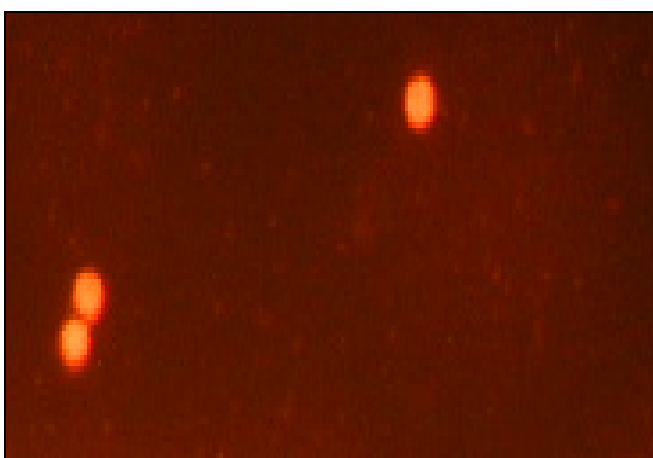


FIG. 10: COMET CELLS OF CONTROL SAMPLES FROM ULF (40X)

The values of percent DNA in tail, tail length, tail moment and olive tail moment in blood samples of cattle from study area were significantly higher ($P < 0.05$) than the corresponding values of blood samples of control animals which indicates higher level of DNA damage in the study area.

DISCUSSION: The study area was selected due to its pesticide affliction in the past, predominant agrarian economy established in the district and presence of a substantial resident cattle population. The study area is being reported for unreasonably high proportion of obscure animal diseases.

The survey conducted revealed the major type of illness being reported are digestive disorders followed by parasitic diseases and reproductive disorders. Highly prevalent digestive disorders might be due to fodder as main type of feed being fed to cattle is paddy straw (74%), fodder (15%), concentrate (5%) and miscellaneous (6%) and water since major source of drinking water for animals was tunnel wells (65%) and also had easy access to water from open ponds (30%) where contamination with agricultural runoff are highly possible. Two water samples were positive for *p,p'*-DDD residues and one sample was contaminated with all isomers of HCH. In all other samples, pesticide residue levels were below the detection limit (BDL). This indicated that the HCH and DDT were of limited contamination in the study area as these pesticide usages were restricted in the study area and agrarian communities started following organic farming.

In accordance with the European Economic Commission (EEC) Directive, the sum of pesticide level in surface water should not exceed 1 µg/L. In the current study the residues were within the EEC limit. Among the HCH residues in analyzed samples, delta HCH was found in high concentration. Relative proportions of α , β , γ , and δ HCH were 22, 13, 20 and 45 percent, respectively with greater proportion of δ -HCH. This might be due to the longest half-life of HCH isomers²⁹.

Two water samples were contaminated with *p, p'*-DDD, which is the product of anaerobic degradation of *p, p'*-DDT. Presence of *p, p'*-DDD indicates agricultural runoff in the past use of DDT in the study area. Similar types of observation were reported by Kumar², Ize-Iyamu³⁰, Kaushik³¹ and Mudiam³² in different areas of India and another part of the world.

Among 20 analyzed water samples, one sample was found to be contaminated with HCH and two samples were contaminated with *p, p'*-DDD. The estimation revealed that the presence of HCHs was lower than DDTs. This might be due to their differences in physiochemical and biological properties since HCHs have higher water solubility, vapor pressure and biodegradability, lower lipophilicity and particle affinity compared to DDT³³.

Although the level of contamination of water in the present study was relatively much less, the presence of HCH and DDT residues in water is important matter of concern because consumption of contaminated water by milch animals in the long run might lead to accumulation of lipophilic DDT and HCH residues in the animal body which ultimately gets secreted in milk^{34,35}.

The major route of pesticide entry into the animal body is through the contaminated feed and fodder. Pesticide residues may be found in fodder plants from accidental contamination, volatilization or spoilage of the residues from contaminated soil, through wind, dust or by direct absorption via roots and leave³⁶. The level of total HCH and total DDT in fodder samples (ppm) collected from study area were below the detection limit which is in consistent with findings of Deka³⁷, who analyzed a total of 15 feed and fodder samples in Assam state, India and no pesticide residues were found in the fodder samples. The current limited pesticide usage in agriculture area and short time span of taking for fodder growth might be the reason for the below detectable level of pesticide residues in fodder samples in the study area.

Animal-derived products, however, are likely to carry a greater load of pesticides due to their higher lipid content. Milk, being a fat-rich food it is an important source of OCP accumulation and hence one of the convenient foodstuff for measuring the persistent OCPs³⁸. Milk can be considered as a suitable indicator for monitoring the burden of persistent lipophilic chlorinated insecticides in the environment & human body³⁵. According to EU pesticide residue legislation the MRL for α , β and δ HCH in milk were 0.004, 0.003 and 0.001 ppm respectively⁸. Whereas for DDT, it is 0.04ppm³⁹.

However, no residues of HCH and DDT were detected in milk samples. So milk from the study area is safe for consumption as far as pesticide residue level is concerned. Pesticide residue in human and animal serum is a biological index of pesticide exposure and studies on blood can be used to assess the total body burden of pesticides in the general population. Residues of HCH and DDT in serum samples of cattle from the study area were below the detection level.

Saxena⁴⁰, found very high level of DDT and its metabolites in occupationally unexposed population which might be due to ingestion of DDT through food contaminated as a result of general environmental contamination. However, in present study, there was no residue in feeds which could be correlated with absence of residues in serum.

Significantly high-level Comet parameters were observed in study samples when compared to the control samples, is indication of the extent of DNA damage. However, HCH and DDT residues in the biological samples of cattle were below detection level still a significant amount of DNA damage in the samples of study area was observed. Presence of substance like hexachlorobenzene and pentachlorophenol¹¹, chlordane and endrin¹², aldrin, dieldrin and organophosphorus pesticides like dimethoate and methyl parathion¹³, toxaphene¹⁵, benzopyrene⁴¹ have been linked to DNA damage.

Analysis of soil samples from northern Kerala, India has revealed endosulfan residue contamination²⁰. However, there were no previous studies reported for DDT and BHC pesticide contamination in the selected area, and to our knowledge this is the first study revealed the presence of persistent organic pollutant with higher DNA damage parameters. Presence of detectable levels of pesticide residues in environmental samples along with significant level of DNA damage in absence of detectable level of HCH and DDT residues in biological samples, need further exploration to uphold the causes for the DNA damage.

CONCLUSION: Presence of a high ratio of β HCH isomer and *p, p'*-DDD in environmental samples reveals the presence of persistent organic pollutants (POPs) in the study area. Presence of significant level of DNA damage, which was indicated by higher number of comet parameters, in absence of detectable level of HCH and DDT residues in biological samples, calls for further investigation to corroborate the grounds for the DNA damage. Although, the level of pesticide residues in environmental and biological samples in the study area is not enough to cause health hazards, in long run, presence of very low levels of HCH and DDT pesticide residues may continue to

accumulate in the living system can cause major health hazards which need to be monitored regularly.

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CONFLICT OF INTEREST: Nil

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