The Journal of Veterinary Medical Science

NOTE

Toxicology

Dichlorodiphenyltrichloroethane (DDT) levels in rat livers collected from a malaria vector control region

Kodai MOTOHIRA[1)], Yoshinori IKENAKA[1,2)], Yared Beyene YOHANNES[1)], Shouta M. M. NAKAYAMA[1)], Victor WEPENER[1,2)], Nico J. SMIT[2)], Johan H. J. VAN VUREN[2)], Ana Catarina SOUSA[1,3)], Alex Ajeh ENUNEKU[4)], Emmanuel Temiotan OGBOMIDA[5)] and Mayumi ISHIZUKA[1)]*

[1)]Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Laboratory of Toxicology, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan
[2)]Water Research Group, Unit for Environmental Sciences and Management, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom, South Africa
[3)]CICECO, Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal
[4)]Department of Environmental Management and Toxicology Faculty of Life Sciences, University of Benin, Benin City, P.O. Box 1154, Nigeria
[5)]Ecotoxicology and Environmental Forensic Unit National Centre for Energy and Environment (Energy Commission of Nigeria), NCEE Administrative Block Complex, University of Benin, Ugbowo Campus, Benin City, Edo State, Nigeria

J. Vet. Med. Sci.
81(11): 1575–1579, 2019
doi: 10.1292/jvms.19-0168

Received: 26 March 2019
Accepted: 28 May 2019
Advanced Epub: 23 August 2019

ABSTRACT. Dichlorodiphenyltrichloroethane (DDT) is an organochlorine insecticide that has been used for indoor residual spraying for the control of mosquito-borne diseases including malaria. However, due to its toxicity and environmental persistence, there are concerns about its potential deleterious effects in humans and wildlife. Therefore, the current study aimed to monitor and estimate the level of DDTs in human communities. The accumulation of DDT and its metabolites was evaluated in house rat (as sentinel) livers collected in an area where DDT was sprayed. DDTs were measured using a gas chromatography / Electron Capture Detector. The results revealed high concentrations of DDTs in the rat livers and the levels of DDTs were similar to findings reported from the same area in 2014.

KEY WORDS: dichlorodiphenyltrichloroethane, house rat, indoor residual spraying, malaria vector control, South Africa

Dichlorodiphenyltrichloroethane (DDT) is a well-known organochlorine pesticide that has been widely used for vector control and agriculture. However, due to its toxicity and environmental persistency, the use of DDT was banned in many countries since the 1970s. Furthermore, DDT is regulated under the Stockholm convention and thus its use is banned on a global scale with the exception of some countries in which malaria and other vector borne diseases are still a public health concern (UNEP, https://www.unenvironment.org/). In animals and the environment, DDT is metabolized into DDE (1,1,-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene) and DDD (1,1,-dichloro-2,2-bis (*p*-chlorophenyl)-ethane). All these metabolites are biologically active and accumulate in animals and the environment. Due to its toxicity, its use is restricted for indoor residual spraying (IRS). However, inhabitants of the sprayed houses are increasingly exposed to DDT and its metabolites (DDTs). In humans, the half-life values of *p,p′*-DDT and *p,p′*-DDE are estimated to be 6 years and approximately 10 years, respectively [3]. The concentrations of *p,p′*-DDT and *p,p′*-DDE in the sera of mothers living in houses sprayed with DDT were approximately 5 to 7 times higher than the ones found in women that lived in a house never sprayed with DDT [4]. Epidemiological studies derived from the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) cohort reported several deleterious health effects on women and their children from IRS areas in South Africa due to exposure to DDTs [2, 5, 6]. In this sense, the use of sentinels that are exposed to the same contaminants as humans is a suitable alternative. In rural regions of South Africa, rodents such as rats roam and feed around homesteads, therefore these animals can be considered as indicators of human exposure to DDTs.

In a previous study, DDT and its metabolites were detected in the liver of rats which were collected in South Africa and the total

*Correspondence to: Ishizuka, M.: ishizum@vetmed.hokudai.ac.jp
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

concentrations of DDTs range from 127 to 7,334 μg/kg wet weight [11]. Since DDT is used almost every year for malaria control as IRS, the current study aimed to monitor DDT exposure in the Phongolo River floodplain, South Africa (Fig. 1), and compare the exposure of DDTs with previous study. Additionally, this study evaluated the influence of sex and age on the accumulation of DDT and its metabolites.

In the current study, 37 house rats were collected using baits by setting cage traps inside houses in communities around the Phongolo River floodplain in February 2017. Animal ethics were upheld according to the recommendations of the Hokkaido University Manual for Implementing Animal Experimentation for animal welfare. Captured rats were collected every morning and transported to the field laboratory. Rats were euthanized and necropsied. Liver samples were collected from each rat and frozen in liquid nitrogen and kept at −20°C. The eyes were collected and preserved in 10% formalin for age determination.

Species identification was conducted by nucleotide sequence analysis of *cytochrome b* (*cyt-b*) gene from synthesized cDNA. RNA was extracted from the liver samples using TRI reagent (Sigma-Aldrich, St. Louis, MO, U.S.A.) and Nucleospin RNA kit (Macherey-Nagel, Düren, Germany). Next, the extracted RNA was reverse-transcribed to cDNA using ReverTra Ace qPCR RT Kit according to the manufacturer's specifications (TOYOBO, Osaka, Japan). *Cyt-b* gene was amplified using forward and reverse primers (5′-CCATGAGGACANATATCNTT-3′, 5′-GGGTGTTCTACTGGTTGGCC-3′), respectively. The amplified products were purified by QIAquick® PCR Purification kit (Qiagen, Hilden, Germany), and DNA sequence analysis was conducted by FASMAC Industrial Inc. (Atsugi, Japan). Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Molecular Evolutionary Genetics Analysis software (MEGA) (https://www.megasoftware.net/) were used for the identification of the species from the sequence data.

All rats collected in the current study were *Rattus tanezumi*. The bibliometric data for the wild rodents is summarized in the supplementary data.

The eye lens was retrieved from formalin preserved samples and dried for 48 hr at 50°C and weighed to estimate the age according to the protocol developed by Tanikawa [8]. This protocol was developed for *Rattus rattus* aged between 20 to 1,121 days.

The age of the rats was calculated by Eq.1 and Eq. 2:

Males: Log Y=1.00 + 0.023 X (Eq. 1),

Females: Log Y=1.05 + 0.023 X (Eq. 2),

where, Y is the age (days) and X is the weight of lenses in mg.

The age of *R. tanezumi* estimated using eye lens weight ranged from 24 to 289 days in the current study.

The majority of the rats were older than 8 weeks, i.e., sexually mature (62.2%), and only 18.9% were juveniles (younger than 5 weeks). The remaining rats (18.9%) were aged between 5 and 7 weeks. Generally, rats become sexually mature at about the sixth week; however, the exact period of maturity for this species and for this habitat is unknown, and therefore, these animals were included in the transition zone category. There were more female rats (n=27) than male rats (n=10).

The determination of DDTs in liver samples was performed as described by Yohannes *et al*. [11] with slight modifications. Approximately 500 mg of liver sample was homogenized with anhydrous sodium sulfate, spiked with an internal standard (PCB 77) and extracted with hexane/acetone (3:1, v/v) in a Soxtherm apparatus (S306AK Automatic Extractor, Gerhardt, Germany). The extracts were rotary evaporated to 2 m*l* and passed through a 5 g activated florisil column, eluted with hexane/dichloromethane (7:3, v/v). The eluate was finally concentrated to near dryness under a gentle nitrogen gas flow and redissolved in 100 μl n-decane. DDTs were quantified with a Shimadzu gas chromatography equipped with a 63Ni μ–electron capture detector and an ENV-

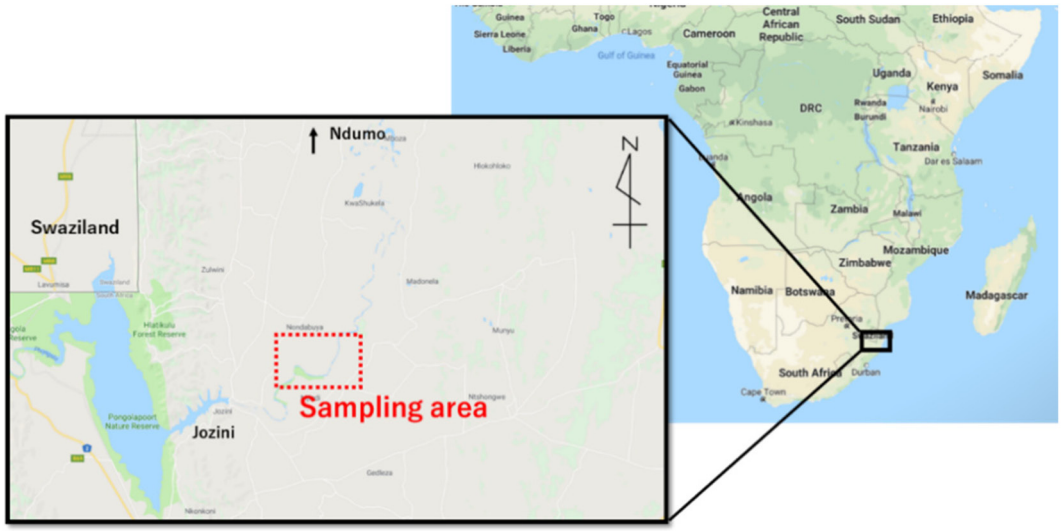

Fig. 1. Map of the sampling area in KwaZulu-Natal, South Africa. Maps were obtained from Google Earth.

8MS capillary column (30 m × 0.25 mm × 0.25 μm). Multilevel calibration curves were created for quantification, and linearity (r^2>0.995) was achieved. The concentrations of DDTs are shown in wet weight (ww). Quality control (QC) and quality assurance (QA) were performed based on analyses of procedural blanks, spiked blanks, and blind duplicate samples with a daily check of the calibration curves. The quality of analytical data was investigated by comparing the peak area values of the standard DDTs mixture and the internal standard.

The concentrations of DDTs in the rat livers are shown in Table 1. Among the analysed compounds, *p,p′*-DDE was the most predominant and detected at the highest concentration (median 706 *ng*/g ww, range 32–2,423 *ng*/g ww in female rat livers) followed by *p,p′*-DDT. And the oldest rats had the highest mean ΣDDTs concentration; 1,961 *ng*/g ww (Fig. 2D). Overall, DDTs were detected in all rat livers and ranged from 59–5,237 *ng*/g ww.

No significant differences in DDTs concentrations (*ng*/g, ww) were observed between sexes and among phases of sexual development (immature, transition, and mature) (Table 1, Fig. 2).

DDE was the most predominant compound in all groups, except for the youngest group. In fact, these animals tended to have the lowest concentrations of *p,p′*-DDE compared with the other age groups (Fig. 2A), but these differences were not significant. The Steel-Dwass test was used to evaluate the differences among the values in the multiple groups and Wilcoxon's test was used for performing a comparative analysis between the two groups. The *P*-value was set to 0.05 for detecting significant differences. The higher levels of DDTs in older animals could be attributed to their longer exposure time to DDTs from diet and environment. Comparing the ratio between DDT and the ΣDDTs revealed that the ratio of DDT was higher in females (33%) than in males (25%) and it was also higher in younger animals (43%) than the transition and matured groups (22 and 30%, respectively) (Figs. 3 and 4). *p,p′*-DDT and *p,p′*-DDE were main compounds detected in rat livers. Previous reports from a nearby region in South Africa also detected these compounds in the liver of wild rodents; however, in that study conducted in 2014, *p,p′*-DDD was predominant and *p,p′*-DDT was much lower than the other compounds [11]. DDT disappears in a much shorter time period compared with DDE in the environment. In an Australian study, the half-life of DDE exceeded 15 years, even though that of DDT was only 3–4 years [7]. Monitoring of both DDT and its metabolites should elucidate a more accurate exposure level in the environment. The difference in the DDTs ratio seen in the current study might be a consequence of the periods after spraying, and the age difference among the rats. Nevertheless, the ranges of the concentrations of *p,p′*-DDE and sum of DDTs were similar between the current study and the previous study conducted in 2014 (Table 1).

The contamination of terrestrial species by DDTs living in Phongolo region, our study area, has also been reported. High levels of DDT (36−14,398 *ng*/g ww) were detected in the livers of free ranging chickens [10] and in chicken eggs (5,200–48,000 *ng*/g ww DDTs) [9]. High concentrations of DDT in rat liver samples were also recorded in Ethiopia, where a similar study was conducted [11] in regions where DDT was being used for IRS for malaria vector control (Table 1). In both regions, DDTs were reported in different fish species (0.3−81.49 mg/kg fat, 0.77−61.9 *ng*/g ww) in South Africa and Ethiopia, respectively [9]. This is indicative of DDT exposure and subsequent bioaccumulation in aquatic species, terrestrial species and food products from these malaria vector control IRS regions.

After the toxicity of DDTs was revealed, many countries banned or reduced the use of DDTs in pest control e.g. malaria vector control programmes. Even though the global use of DDTs is decreasing, DDTs accumulation in rat livers did not change in the three years between the current study and our previous report sampled in 2014 [11]. Additionally, the current study revealed possible DDT exposure in humans after IRS with DDT as high concentrations of DDTs were detected in the collected house rats. However, it is very difficult to withdraw DDTs for many African countries because of the high incidence of malaria and the consequent fatalities. For instance, malaria cases in South Africa markedly increased after the discontinuation of DDT [1]. Currently, World Health Organization (WHO) recommends regulated usage of DDT with some regulation including IRS for the

Table 1. Comparison of concentration of Dichlorodiphenyltrichloroethane (DDT) and its metabolites (in *ng*/g ww) detected in the liver of wild rats between this study and previous studies

	Sex	N		*p,p′*-DDE (*ng*/g ww)	*p,p′*-DDD (*ng*/g ww)	*p,p′*-DDT (*ng*/g ww)
South Africa 2017	Male	10	Range	67−2,074	ND−2,461	15−1,389
			Median	600	301	407
	Female	27	Range	32−2,423	ND-1,152	18−1,452
			Median	706	300	504
Ethiopia 2014[a)]	Male	7	Range	206−1,666	343−7,005	49–413
			Median	737	752	98
	Female	14	Range	216−3,746	365−5,268	24−1,165
			Median	914	1249	168
South Africa 2014[a)]	Male	12	Range	67−4,427	42−4,733	ND−1,278
			Median	399	246	51
	Female	12	Range	14−2,296	42−4,828	24−1,165
			Median	610	882	74

a) Data extracted from Yohannes *et al.* (2017) [11].

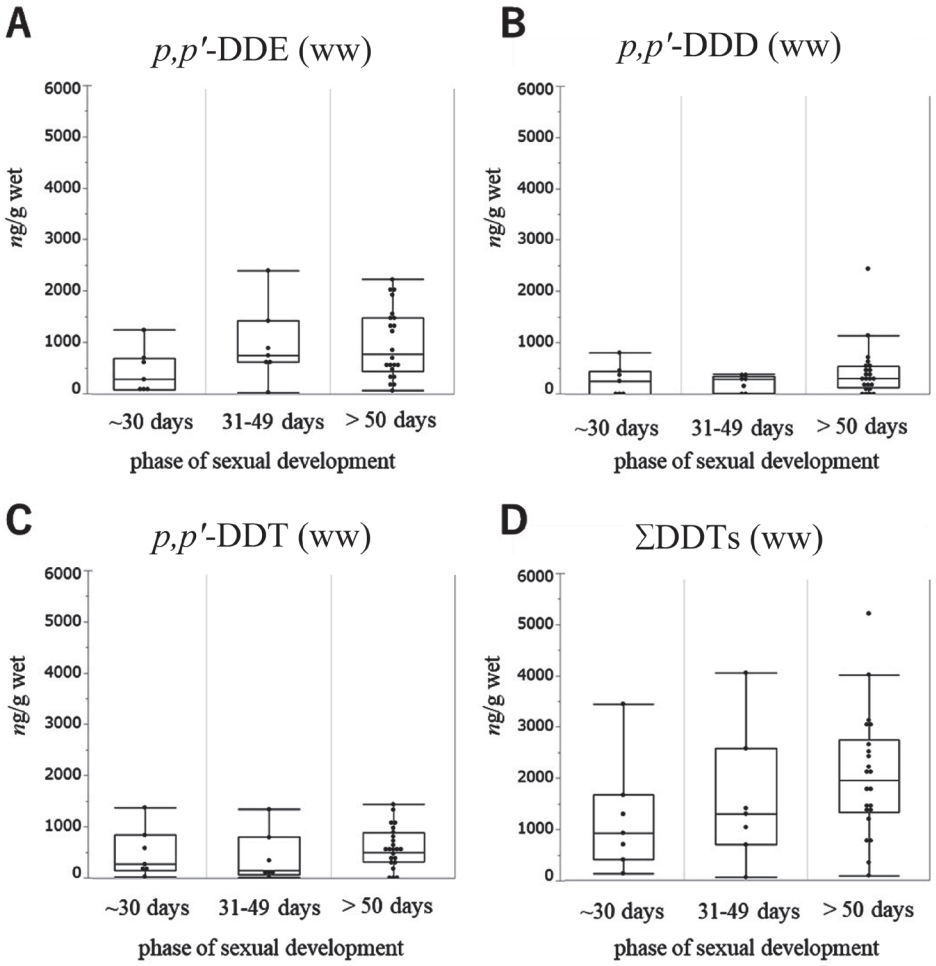

Fig. 2. Box plots depict levels of Dichlorodiphenyltrichloroethane (DDT) and its metabolites in the liver at each phase of sexual development (A: *p,p′*-DDE, B: *p,p′*-DDD, C: *p,p′*-DDT, D: ΣDDTs). No significant difference was seen between each phase at any level of DDTs (Steel-Dwass test, $P<0.05$).

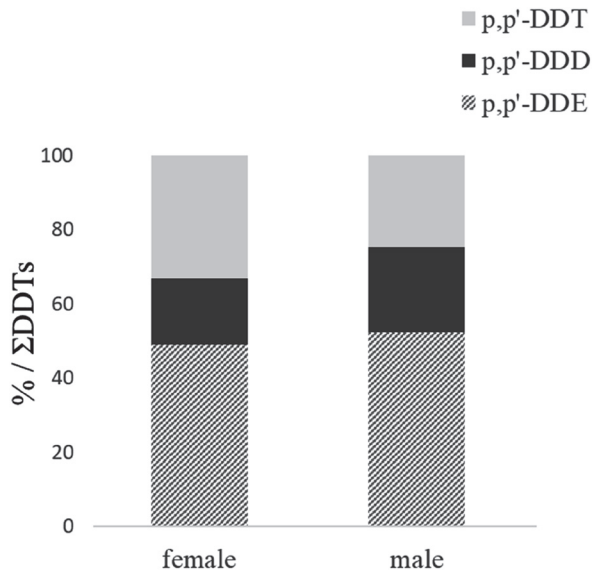

Fig. 3. Ratio of Dichlorodiphenyltrichloroethane (DDT) and its metabolites in male and female rats.

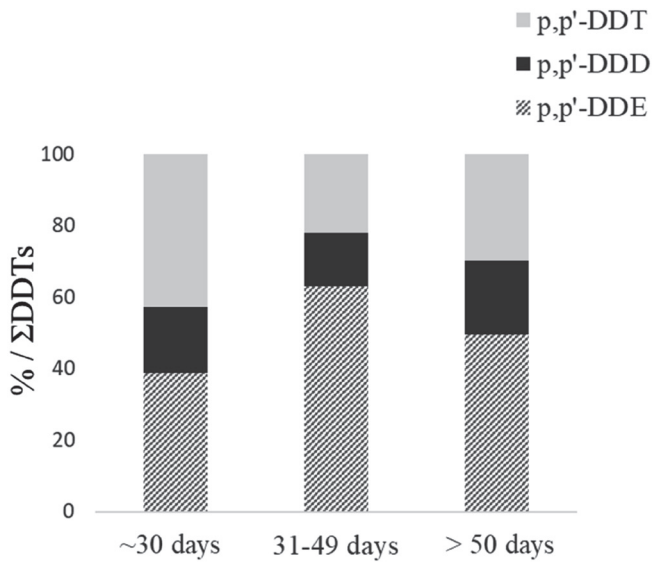

Fig. 4. Ratio of Dichlorodiphenyltrichloroethane (DDT) and its metabolites in each development phase.

control of malaria. Therefore, continuous monitoring campaigns should be performed to assess the toxic effect of DDTs on the environment as mosquitoes have shown resistance to other insecticides.

ACKNOWLEDGMENTS. The current study was financially supported by Grants-in-Aid for Challenging Exploratory Research (No. 17K20038, No. 18K1984708) and Scientific Research (A) (No. 16H01779, 18H0413208) from the Japan Society for the Promotion of Science and partly supported by the Ministry of Education, Culture, Sports, Science, and Technology, Japan (MEXT). We also gratefully acknowledge our contacts in the KwaZulu-Natal region of South Africa, without whom it would not have been possible to collect samples. In particular, we would like to thank Samwel Menyuka and Nelson Nkwanyana for guidance and interpretation, and Dr. Ntantiso at the Jozini Agriculture Office. From the KwaZulu-Natal Health Department, Bruce Margot, Bheki Owabe, Phineas Zikhali, and various other guides at the health camps facilitated the current study extensively. Laboratory assistance given by Takahiro Ichise in Hokkaido University is appreciated. The contribution number from the NWU-Water Research Group is #349 of the NWU_WRG.

REFERENCES

1. Channa, K., Röllin, H. B., Nøst, T. H., Odland, J. Ø. and Sandanger, T. M. 2012. Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non-malaria coastal regions of South Africa. *Sci. Total Environ.* **429**: 183–190. [Medline] [CrossRef]
2. Coker, E., Chevrier, J., Rauch, S., Bradman, A., Obida, M., Crause, M., Bornman, R. and Eskenazi, B. 2018. Association between prenatal exposure to multiple insecticides and child body weight and body composition in the VHEMBE South African birth cohort. *Environ. Int.* **113**: 122–132. [Medline] [CrossRef]
3. Eskenazi, B., An, S., Rauch, S. A., Coker, E. S., Maphula, A., Obida, M., Crause, M., Kogut, K. R., Bornman, R. and Chevrier, J. 2018. Prenatal exposure to DDT and pyrethroids for malaria control and child neurodevelopment: The VHEMBE cohort, South Africa. *Environ. Health Perspect.* **126**: 047004.
4. Gaspar, F. W., Chevrier, J., Quirós-Alcalá, L., Lipsitt, J. M., Barr, D. B., Holland, N., Bornman, R. and Eskenazi, B. 2017. Levels and determinants of DDT and DDE exposure in the VHEMBE cohort. *Environ. Health Perspect.* **125**: 077006. [Medline] [CrossRef]
5. Huang, J., Eskenazi, B., Bornman, R., Rauch, S. and Chevrier, J. 2018. Maternal peripartum serum DDT/E and urinary pyrethroid metabolite concentrations and child infections at 2 years in the VHEMBE birth cohort. *Environ. Health Perspect.* **126**: 067006. [Medline] [CrossRef]
6. Murray, J., Eskenazi, B., Bornman, R., Gaspar, F. W., Crause, M., Obida, M. and Chevrier, J. 2018. Exposure to DDT and hypertensive disorders of pregnancy among South African women from an indoor residual spraying region: The VHEMBE study. *Environ. Res.* **162**: 49–54. [Medline] [CrossRef]
7. Shivaramaiah, H. M. and Odeh, I. O. A. Kennedy, Ivan. R. and Skerritt, John H. 2006. Mapping the Distribution of DDT Residues as DDE in the Soils of the Irrigated Regions of Northern New South Wales, Australia Using ELISA and GIS. 5360–5367.
8. Tanikawa, T. 1993. An Eye-Lens Weight Curve for determining age in black rats, Rattus rattus. *J. Mammal. Soc. Jpn.* **18**: 49–51.
9. Thompson, L. A., Darwish, W. S., Ikenaka, Y., Nakayama, S. M. M., Mizukawa, H. and Ishizuka, M. 2017. Organochlorine pesticide contamination of foods in Africa: incidence and public health significance. *J. Vet. Med. Sci.* **79**: 751–764. [Medline] [CrossRef]
10. Thompson, L. A., Ikenaka, Y., Darwish, W. S., Yohannes, Y. B., van Vuren, J. J., Wepener, V., Smit, N. J., Assefa, A. G., Tharwat, A., Eldin, W. F. S., Nakayama, S. M. M., Mizukawa, H. and Ishizuka, M. 2018. Investigation of mRNA expression changes associated with field exposure to DDTs in chickens from KwaZulu-Natal, South Africa. *PLoS One* **13**: e0204400. [Medline] [CrossRef]
11. Yohannes, Y. B., Ikenaka, Y., Ito, G., Nakayama, S. M. M., Mizukawa, H., Wepener, V., Smit, N. J., Van Vuren, J. H. J. and Ishizuka, M. 2017. Assessment of DDT contamination in house rat as a possible bioindicator in DDT-sprayed areas from Ethiopia and South Africa. *Environ. Sci. Pollut. Res. Int.* **24**: 23763–23770. [Medline] [CrossRef]