

RESEARCH ARTICLE

28-day subchronic toxicity studies of 2, 2-dichlorovinyl dimethyl phosphate in albino rats

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Abstract

Sixty adult *Rattus norvegicus* (both sexes) of mean body weight of 150.0 ± 3.5 g was distributed into 6 groups of 10 rats per group (same sex). The groups A, B, C, D, E and F were exposed to adjusted dosages of 0%, 20%, 40%, 60%, 80% and 100% of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) respectively by inhalation from day 1 to day 28. At $p < 0.05$, DDVP caused significant decrease in GPT activity, WBC and lymphocytes levels in exposed groups while GOT activity, creatinine, haemoglobin, PCV and neutrophil levels were significantly increased. Increase in GOT activity and creatinine level was dose-related. ALP activity was unchanged in groups exposed to 20%; decreased in groups exposed to 40%, 60% and 80% while significant increase was observed in group exposed to 100% DDVP. Albumin was also significantly increased in groups exposed to 80% and 100% DDVP. Groups exposed to 0%, 20% and 40% DDVP showed GOT/GPT ratios < 1 while > 1 was the value obtained for groups exposed to 60%, 80% and 100%. Groups exposed to 0%, 20% and 40% DDVP showed weight gain while weight loss was recorded in group exposed to 100% DDVP throughout the experimental period. Investigated parameters were generally higher in group exposed to 100% than other groups. Severe toxicity was observed in group exposed to 100% DDVP after 21 d of continuous exposure. Cholinergic signs observed in severely affected group that was exposed to 100% DDVP included anorexia, muscular tremors, mucous nasal discharges, increased frequency of salivation and urine staining, decreased body weight gain and diarrhoea. No adverse effects or cholinergic signs were observed in the functional observational battery and locomotor of the rats exposed to 0% to 80%; the NOEL was determined to be 20%. No mortality was recorded and none was killed because of intoxication. The leukopenia, lymphocytopenia, neutrophilia, increased haemoglobin, albumin and creatinine established in this study resulted from increased DDVP concentration and were attributed to malnutrition from low feed intake and dehydration.

Keywords: *Rattus norvegicus*, DDVP, creatinine, haemoglobin, neutrophil, leukopenia, lymphocytopenia.

Introduction

In Nigeria, 2, 2-dichlorovinyl dimethyl phosphate (DDVP or Dichlorvos) is one of the classes of insecticide referred to as organophosphate which is commonly used as insecticide in agriculture and also used to control household and stored products insects. It is effective against mushroom flies, aphids, spider mite, caterpillars, thrips, and white flies in greenhouse, outdoor fruits, and vegetable crops (Murphy, 1986) it is traded under the brand name 'sniper' or 'ota piapia' or 'ogun efo say yanmuyanmu'. According to Tomlin (1997) dichlorvos is produced as a metabolite of the insecticide named 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate. Dichlorvos may also be produced as an initial degradation product of the insecticide trichlorfon (dimethyl 2, 2, 2-trichloro-1-hydroxyethylphosphonate) under neutral to alkaline conditions and may in fact be responsible for the insecticidal effect of trichlorfon. Howard (1991) commented that dichlorvos is one of the more volatile organophosphates.

Formulations in which dichlorvos has been available therefore include a pressurised gas formulation with CO₂ propellant, ready to use liquid formulations, plastic or naphthalene matrices providing sustained release as well as emulsifiable concentrate and liquid concentrate formulations. Dichlorvos has been commercially manufactured and used throughout the world since 1961 (WHO, 1993). Dichlorovinyl dimethyl phosphate and about 40 other organophosphate insecticides such as parathion, methyl parathion is available nationally in Nigeria. Organophosphates are potent toxicants, the potential toxicity to humans and other animals include abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems. Additionally, many studies have indicated that pesticide exposure is associated with long-term health problems such as respiratory problems, memory disorders, dermatologic conditions, cancer, depression, neurological deficits, miscarriages, and birth defects (Gupta *et al.*, 1991).

There are also benefits to the use of pesticides in the organophosphate class such as 2, 2-dichlorovinyl dimethyl phosphate (DDVP) which has saved 7 million human lives since 1945 by preventing the transmission of diseases such as malaria, bubonic plague, sleeping sickness, and typhus. Dichlorvos exhibits respiratory, contact and stomach action and gives rapid knockdown; it acts by inhibiting the activity of cholinesterase enzymes which normally block nerve transmissions after they are sent, its vapour pressure is sufficiently high to give it high insecticidal activity in the vapour phase and its main importance is based on its fumigant-type action and knockdown ability (Brimijoin, 1992). In Nigeria, DDVP is commonly produced and used as an effective and potent insecticide, its effectiveness and potency surpassed other competitive household insecticides (such as mosquito coil and flints) in Nigeria, the use and handling of DDVP as a household insecticide by Nigerians are indiscriminate. Keeping in view the problem of DDVP high acute toxicity to man and non-target organisms, this study aims at carrying out subchronic toxicity studies by exposing albino rats to different concentrations of DDVP for 28 d via inhalation and also to determine the effects of exposure (subchronic toxicity) on some biochemical parameters in exposed rats.

Materials and methods

Sources and management of experimental animals: Sixty male and female albino rats (*Rattus norvegicus*) with a mean body weight of 150 ± 3.5 g were obtained from the small animal holding unit of the Department of Science Laboratory Technology, the Federal Polytechnic Bida, Niger state, Nigeria-West Africa. They were allowed free access to food and water.

Collection of DDVP: Three bottles (100 mL each) of Sniper (2, 2-dichlorovinyl dimethyl phosphate-DDVP) was purchased from a standard agro-allied store in Minna city, Niger state, Nigeria.

Animal grouping and DDVP administration: This study was carried out after approval from the Departmental Ethical Committee on the use and care of experimental animals. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes-ETS-123 (ETS, 2005). Sixty albino rats of both sexes were distributed into 6 groups (A to F) of 10 rats per group. Group A rats were control group that were exposed to 0% DDVP solution. Groups B to F animals were exposed to different amounts of DDVP as indicated below:

| | |
|------------------|---|
| Group A: Exposed | to 0% (0 mL DDVP/100 mL distilled water- v/v) |
| Group B: Exposed | to 20% (20 mL DDVP/80 mL distilled water- v/v) |
| Group C: Exposed | to 40% (40 mL DDVP/60 mL distilled water- v/v) |
| Group D: Exposed | to 60% (60 mL DDVP/40 mL distilled water- v/v) |
| Group E: Exposed | to 80% (80 mL DDVP/20 mL distilled water- v/v) |
| Group F: Exposed | to 100% (100 mL DDVP/0 mL distilled water- v/v) |

Exposure of animals to DDVP solutions: Twenty mL of 20% DDVP solution was poured into a sealed container that was perforated by the side for easy diffusion of its odour in and out of the container and inhalation by the animals. The container was fixed in a corner in group B cage in such a way that the animals were unable to pour away the content of the container in order to prevent body contact. This procedure was repeated for other groups using the same volume (20 mL). All the rats were allowed free food (rat chow) and water throughout the 28 d of experiment in the sealed wooden cages of netted doors.

At the end of 28th d, rats were sacrificed by cutting their jugular veins to collect blood. Some of the blood was collected in plain bottles for the preparation of serum while some other part was collected into EDTA coated bottles and refrigerated immediately. Liver was also extracted for liver function test.

Preparation of serum: The blood sample in a clean, dry centrifuge tube was allowed to clot and centrifuged at $224 \text{ g} \times 10 \text{ min}$ (Ajiboso, 2000). The clear supernatant (serum) was separated from the pellet, kept frozen and used within 24 h.

Preparation of liver homogenate: The extracted liver was suspended in ice-cold 0.25 M sucrose solution (Ajiboso, 2000) buffered with Tris pH 7.4, and was cut into tiny pieces and homogenized until a fine homogenate was produced using Teflon homogenizer.

Biochemical analyses

Transaminases and alkaline phosphatase activities: The transaminases and alkaline phosphatase activities were determined in the serum and liver homogenate according to the methods described by Bergmeyer (1974).

Albumin: The method of Bromocresol Green (BCG) was used in determining serum albumin level as described by Webster (1974).

Creatinine: Creatinine was determined according to Jaffe's reaction method described by Ferry *et al.* (1996).

Determination of body weight: The method described by Ajiboso *et al.* (2007) was used to determine body weight of experimental rats. Individual rat was monitored for weekly gain in body weight using digital electronic balance (Gilbertini, Italy). Gain in weight was obtained from the relationship given below:

Weekly gain in weight = Final week weight – Initial week weight

Determination of haematological indices: The methods described by Akpanabiatu *et al.* (2012) and Uboh *et al.* (2010) were used to determine the haematological indices of experimental rats.

Statistical analysis: Data are presented as Means \pm SD and analyzed using ANOVA and Duncan post hoc test and significance was determined at $p < 0.05$.

Table 1. Toxicity studies (28 d) of DDVP inhalation on some liver enzymes of rats.

| Treatment group | ALP (U/L) | GOT (U/L) | GPT (U/L) | GOT/GPT |
|-----------------|--------------------------|-------------------------|-------------------------|--------------------------|
| A | 165.6 ± 3.4 ^a | 8.4 ± 0.2 ^a | 34.0 ± 0.4 ^a | 0.2 ± 0.0 ^a |
| B | 165.6 ± 2.3 ^a | 14.1 ± 0.7 ^b | 32.2 ± 2.0 ^b | 0.4 ± 0.0 ^a |
| C | 165.4 ± 5.0 ^a | 19.0 ± 1.0 ^c | 20.3 ± 1.7 ^c | 0.9 ± 0.0 ^a |
| D | 138.2 ± 3.0 ^b | 19.0 ± 0.3 ^c | 17.0 ± 0.1 ^d | 1.1 ± 0.0 ^{a,b} |
| E | 138.0 ± 4.2 ^b | 27.0 ± 1.1 ^d | 17.1 ± 0.0 ^d | 1.6 ± 0.0 ^b |
| F | 207.0 ± 8.3 ^c | 52.6 ± 6.4 ^e | 20.0 ± 0.4 ^c | 2.6 ± 0.3 ^c |

Results are mean values of triplicate determinations and expressed as Mean ± SEM;

At $p < 0.05$, same letter across the column shows no significant difference;

A= Exposed to 0%, B= Exposed to 20%, C= Exposed to 40%, D= Exposed to 60%, E= Exposed to 80% and F= Exposed to 100%

Table 2. Toxicity studies (28 d) of DDVP inhalation on some selected serum enzymes and biomolecules of rats.

| Treatment group | ALP (U/L) | GOT (U/L) | GPT (U/L) | ALB (mg/L) | Creatinine (mg/L) |
|-----------------|-------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| A | 36.3 ± 5.1 ^a | 21.3 ± 0.7 ^a | 32.0 ± 4.0 ^a | 27.0 ± 0.5 ^a | 59.1 ± 3.1 ^a |
| B | 36.0 ± 0.0 ^a | 31.0 ± 1.0 ^b | 27.3 ± 0.3 ^b | 39.0 ± 2.1 ^b | 75.9 ± 7.8 ^b |
| C | 34.2 ± 4.6 ^b | 57.1 ± 7.3 ^c | 33.0 ± 3.2 ^a | 48.1 ± 0.0 ^c | 126.4 ± 9.2 ^c |
| D | 27.0 ± 0.1 ^c | 105.7 ± 6.1 ^d | 34.1 ± 3.5 ^a | 48.0 ± 0.6 ^c | 170.5 ± 8.6 ^d |
| E | 32.8 ± 2.8 ^d | 108.3 ± 9.7 ^e | 43.7 ± 3.1 ^c | 50.2 ± 4.2 ^d | 190.0 ± 5.0 ^e |
| F | 53.0 ± 7.8 ^e | 130.0 ± 9.1 ^f | 83.5 ± 4.7 ^d | 50.0 ± 0.8 ^d | 220.0 ± 9.1 ^f |

Table 3. Toxicity studies (28 d) of DDVP inhalation on haematological indices of rats.

| Treatment group | Hb | PCV | WBC X 10 ³ | Neut | Lymph | Mono | Eos | Baso | RBC Morphology |
|-----------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------------------|------------------------|-------------------|
| A | 10.0 ± 0.1 ^a | 13.3 ± 1.0 ^a | 20.0 ± 3.4 ^a | 50.3 ± 2.5 ^a | 74.0 ± 5.6 ^a | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | Normal |
| B | 10.3 ± 0.3 ^a | 23.0 ± 0.0 ^b | 10.0 ± 0.0 ^b | 70.0 ± 1.3 ^b | 44.0 ± 1.0 ^b | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | Normal |
| C | 11.0 ± 1.3 ^a | 27.0 ± 0.5 ^c | 9.0 ± 0.1 ^b | 73.1 ± 4.7 ^c | 43.0 ± 1.4 ^b | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | Normal |
| D | 12.0 ± 1.0 ^b | 30.0 ± 0.0 ^d | 9.0 ± 0.7 ^b | 75.0 ± 3.0 ^d | 40.0 ± 3.2 ^c | 1.0 ± 0.0 ^b | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | Normal |
| E | 15.3 ± 1.2 ^c | 33.0 ± 3.0 ^e | 7.0 ± 1.4 ^c | 81.5 ± 1.0 ^e | 37.0 ± 0.1 ^d | 1.0 ± 0.0 ^b | 0.0 ± 0.0 ^a | 0.0 ± 0.0 ^a | Slightly crenated |
| F | 37.1 ± 2.9 ^d | 41.7 ± 1.3 ^f | 2.3 ± 0.7 ^d | 97.0 ± 5.1 ^f | 13.3 ± 0.6 ^e | 2.0 ± 0.0 ^c | 2.0 ± 0.0 ^b | 2.0 ± 0.0 ^b | Highly crenated |

Table 4. Toxicity studies (28 d) of DDVP inhalation on mean body weight of rats (g).

| Treatment group | 0 d | 7 d | 14 d | 21 d | 28 d |
|-----------------|--------------|---------------------|---------------------|---------------------|---------------------|
| A | 151.3 ± 9.2 | 156.2 ± 6.0 (3.2%) | 163.0 ± 7.3 (4.4%) | 168.4 ± 2.0 (3.3%) | 175.1 ± 4.3 (4.0%) |
| B | 152.7 ± 13.0 | 156.3 ± 7.1 (2.4%) | 161.7 ± 2.3 (3.5%) | 165.9 ± 4.1 (2.6%) | 171.2 ± 9.0 (3.2%) |
| C | 150.1 ± 11.3 | 155.0 ± 1.3 (3.3%) | 160.1 ± 5.0 (3.3%) | 165.7 ± 6.3 (3.5%) | 170.5 ± 8.3 (2.9%) |
| D | 147.1 ± 12.1 | 151.1 ± 0.7 (2.7%) | 153.3 ± 4.7 (1.5%) | 150.8 ± 7.2 (-1.6%) | 147.0 ± 6.4 (-2.5%) |
| E | 150.3 ± 9.1 | 153.5 ± 1.1 (2.1%) | 154.2 ± 8.4 (1.1%) | 147.1 ± 9.3 (-2.7%) | 142.2 ± 5.6 (-3.3%) |
| F | 150.9 ± 6.7 | 150.2 ± 7.4 (-0.5%) | 143.5 ± 3.1 (-4.5%) | 136.0 ± 4.0 (-5.2%) | 127.1 ± 8.3 (-6.5%) |

Results

A 28 d sub-chronic toxicity study of DDVP was carried out in *Rattus norvegicus*. The mean results obtained are shown in Tables 1 to 4.

Liver enzymes: As shown in Table 1 at $p < 0.05$, exposure of groups B and C to 20% and 40% respectively of DDVP solutions did not alter the alkaline phosphatase activity of the groups while 60% and 80% DDVP solutions caused significant decrease in exposed groups D and E respectively. However, there was a significant increase in alkaline phosphatase activity of group F that was exposed to 100% DDVP. GOT and GPT were significantly increased and decreased respectively in exposed groups B to F, increased GOT and decreased GPT activity observed in exposed groups were dose related.

However, there was no significant difference in GPT activity of groups exposed to 40% and 60%; 80% and 100% DDVP solutions. The GOT/GPT ratios revealed values that were less than 1 in groups A, B and C and greater than 1 in groups D, E and F.

Serum enzymes and selected biomolecules: As shown in Table 2, at $p < 0.05$ activity of serum alkaline phosphatase was unchanged in group B that was exposed to 20% DDVP solution; serum ALP activity was significantly decreased in groups C, D and E (that were exposed to 40%, 60% and 80% respectively of DDVP solutions) while significant increase in serum ALP activity was observed in group F. Serum GOT activity, albumin and creatinine were significantly increased in exposed groups B to F; serum GOT activity was 5 and 6.5 times of control group A values in groups E and F respectively.

Serum GPT activity was significantly increased in groups C to F and significantly decreased in group B. Activity of serum GPT in group F was 3 times of control group A value and there was significant difference in ALP activity of groups D, E and F. There were no significant differences in the albumin values of group C and D; E and F, higher albumin values were recorded for groups E and F. The significant increase of creatinine was dose-related since increase in DDVP dosage (concentration) resulted in increase in creatinine level. Creatinine level of Group F was 4 times of control group A value. The liver and renal functions markers investigated were generally higher in group F than other groups.

Haematological indices: As shown in Table 3, at $p < 0.05$ there was a significant increase in haemoglobin, PCV and neutrophil levels of exposed groups B to F. Haemoglobin was higher in group F than other groups. Haemoglobin level of group F was 3.7 times of control group A value. PCV and neutrophil levels of group F were 4 times and 2 times respectively of control group A values. WBC and lymphocytes were significantly decreased in exposed groups B to F with marked decreased of about 9 times and 7 times respectively of control group A values in group F. Monocytes were absent in groups A to C; eosinophil and basophil were also absent in groups A to E.

However, monocytes levels were the same in groups D and E while 2 times amount of monocytes in groups D and E was seen in group F. Eosinophil and basophil were also detected high in group F. The results of microscopic examination of red blood cell morphology revealed normal shape of red blood cells in groups A, B, C and D (0%, 20%, 40% and 60% DDVP solutions); red blood cells of group D (exposed to 80% DDVP solution) were slightly crenated while group F (exposed to 100% DDVP) showed highly crenated red blood cells.

Body weight: As shown in Table 4, 147.1 g to 152.7 g body weights range of rats were used for this study on d 0. From computed percentages on gain and loss in body weights of experimental rats on d 7, 14, 21 and 28. 2.1% to 3.3% of d 0 body weights were gained by group A to E while 0.5% body weight loss was recorded in group F on d 7, least body weight gain of 2.1% was recorded in group E. On d 14, 1.1% to 4.4% of d 7 body weights were gained by groups A to E, least body weight gain of 1.1% was observed in group D while 4.5% body weight loss was also recorded for group F. Weight gain of 2.6% to 3.5% of d 14 body weights was observed in groups A to C on d 21. Loss in body weight of 1.6% to 5.2% was also recorded for groups D to F. On d 28, weight gain of 2.9% to 4.0% of d 21 body weights was observed in groups A to C, loss of 2.5% to 6.5% body weights was also recorded for groups D to F. General gain in body weights was recorded in groups A to C throughout the experimental period.

However, change in body weights of experimental rats was dose related in which increase in DDVP concentration resulted into loss in body weights.

Discussion

The liver has a variety of transaminases to synthesize and break down amino acids and to interconvert energy storage molecules. The concentrations of these in the serum (the non-cellular portion of blood) are normally low. However, if the liver is damaged, the hepatocyte cell membrane becomes more permeable and some of the enzymes leak out into the blood stream. The two transaminases commonly measured are alanine transaminase (ALT) and aspartate transaminase (AST). These levels were also called the serum glutamate-pyruvate transaminase (SGPT) and the serum glutamate-oxaloacetate transaminase (SGOT). GPT is not commonly found outside the liver; GOT too is most commonly found in the liver. In general, very high elevations of the transaminases suggest severe liver damage, such as viral hepatitis, liver injury from lack of blood flow, or injury from drugs or toxins. Most disease processes cause GPT to rise higher than GOT; GOT levels double or triple that of GPT are consistent with alcoholic liver disease (Gimson, 1996). The increase in activity of liver and serum GOT observed with increased DDVP concentration in this study suggests toxic and harmful effects of DDVP on the liver.

This effect was more pronounced on the groups that were exposed to higher DDVP concentrations indicating that GOT activity in exposed groups was dose-dependent in this study. The increase in GOT activity of groups exposed to DDVP in this study agrees with earlier investigation of Atef (2010) on effects of a class of organophosphate insecticide on physiological and histopathological investigations in rats. High levels of GPT usually indicate a damaged liver while most low GPT level indicates malnutrition as a major cause of low blood GPT levels (Gimson, 1996). The mild decrease in activity of liver GPT in groups D to F is due to malnutrition from withdrawal from food by groups exposed to higher concentration. According to Lum (1995) malnutrition is one of the conditions that lead to low serum ALP.

The decreased liver and serum activity of ALP in this study may be attributed to malnutrition that resulted from withdrawal from food by groups D to F due to continuous and prolonged exposure to DDVP. ALP activity was not altered in the liver of groups B and C and serum of group B. Impairment in alkaline phosphatase of rats exposed to other classes of insecticides such as DDT, malathion, phosalone, and elsan as reported by Saigal *et al.* (1982) was also observed in this study on altered activity of both liver and serum ALP of groups exposed to 20% and 40% DDVP solutions. Albumin is the main protein of plasma; its main function is to regulate the colloidal osmotic pressure of blood.

The increased albumin observed in the exposed groups may be attributed to dehydration (Sugio *et al.*, 1999) from the withdrawal from food by exposed groups. The kidney maintains the blood creatinine in a normal range, creatinine has been found to be a fairly reliable indicator of kidney function and elevated creatinine level suggests impaired kidney function or kidney disease (Atef, 2010).

Increase in creatinine levels of exposed groups shows the toxic and idiosyncratic properties of DDVP, the increase in creatinine levels of groups exposed to DDVP in this study agrees with the result obtained by Atef (2010) on effects of malathion on creatinine levels of rats. According to Gimson (1996) GOT/GPT ratio is useful in determining extent of damage to liver cell integrity and also for differentiating between causes of liver damage (hepatotoxicity). The GOT/GPT ratio <1 of groups B and C indicates the non-toxic effects of DDVP solutions on the exposed groups B and C while the GOT/GPT ratio value <1 of unexposed group A also indicates normal liver cell integrity. Groups D, E and F GOT/GPT ratio >1 suggests damaged liver, this may be attributed to increased DDVP concentration in groups D, E and undiluted DDVP solutions.

Blood analysis is crucial in many fields of toxicology research and in environmental monitoring as possible indicator of physiological or pathological changes in diseases investigation. In warm-blooded animals, changes in the blood parameters occur because of injuries or infections of some tissues or organs, leading to the dysfunction or injuries of organs or tissues (Folmar, 1993). According to Gu (1998) dehydration is an important factor that results in increase in hemoglobin and PVC levels while seizure leads to neutrophilia (increased neutrophil). Leukopenia (decreased WBC) and lymphocytopenia (reduced lymphocytes) recorded in this present study may be attributed to destruction of WBC and lymphocytes cells (Gu, 1998) due to continuous and prolonged exposure to DDVP. Absence of monocytes prevent the occurrence of atherosclerosis (Gu, 1998) while presence of monocytes in groups exposed to increase concentration of DDVP suggests alteration in lipid metabolism particularly in group F that was exposed to 100% DDVP. Increased eosinophilia and basophilia suggest tissue inflammation in group exposed to 100% DDVP suggest tissue inflammation in the group.

Significant increase in body weights of the unexposed and exposed groups to 20% and 40% DDVP solutions throughout the experimental period may be attributed to an increase in the feed intake of the groups. The decrease in body weights observed in groups exposed to 60% and 80% DDVP on d 21 to 28 and the weekly decrease in body weights of the groups exposed to undiluted DDVP indicate decrease in feed intake, this observation confirms the report of El-Hilaly *et al.* (2004) on changes in body weight and feed intake as an indicator of the adverse effects of drugs and chemicals.

Volatilisation of DDVP solutions established in this study from the observed toxic effects on exposed groups via inhalation was in accordance with the earlier reports of Mensink *et al.* (1995) on the volatility of DDVP (dichlorvos) from dry surfaces, making dichlorvos one of the more volatile organophosphates and also on Howard (1991) report of higher mobility of highly water soluble metabolites of dichlorvos than the parent substance.

Spray Drift Task Force (1997) also reported the potential of Dichlorvos to reach non-target areas directly via sprayed dichlorvos solutions as vapours or indirectly through movement of directly released vapours or dichlorvos volatilising from sprayed surfaces in spray drift. However, the methods of use employed in this study involved neither direct spray nor release from sprayed surfaces but release from non-contact perforated containers. Thus, an indication that DDVP has mobility potential whether sprayed or not (in use and not in use) and this mobility is dose-related (concentration dependent). Severe toxicity was observed after the 21st d of continuous exposure of group F rats to 100% DDVP. Cholinergic signs observed in group F rats included anorexia, muscular tremors, mucous nasal discharges, increased frequency of salivation and urine staining, decreased body weight gain and diarrhoea. No adverse effects or cholinergic signs were observed in the functional observational battery and locomotor of the exposed rats to 20% to 80% DDVP, the NOEL was determined to be 20% diluted DDVP.

Conclusion

The 28 d subchronic toxicity studies of continuous exposure of rats to different concentrations of DDVP showed dose-related toxic effects on the investigated parameters. DDVP showed high mobility potential as a highly volatile compound in both diluted state and parent concentrate whether sprayed or in containing container. 100% DDVP showed severe toxicity while no observed effect level (NOEL) in this study was 20% (20 mL DDVP in 80 mL water, v/v). Based upon the conclusion, the following recommendations are suggested from the study:

1. The use of DDVP in diluted form (by mixing 20 mL DDVP with 80 mL water) as household insecticide.
2. When not in use, it should always be kept in tight, sealed and non-perforated container.
3. It should be kept in a safe place that is out of reach of children.
4. It should be kept in a cool and not hot place to avoid explosion being a volatile compound.
5. It should be applied in corners of room away from food stuffs.
6. Avoid usage in kitchen and contact with drinking water tank and other food items.
7. Hand gloves should be worn during preparation of diluted solutions.
8. Hands should be washed after DDVP application.

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