28-day inhalation toxicity of 3-methoxybutyl chloroformate in rats

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ABSTRACT

The 28-day repeated inhalation study was applied for hazard assessment of 3-methoxybutyl chloroformate (3-MBCF) in Sprague Dawley rats. Groups of five rats per sex were exposed 6 h/day, 5 days per week for 4 weeks to test substance concentration (ranging from 3 to 12 ppm) using a whole-body exposure system. At the terminal sacrifice, following blood collection and gross pathological examination, organ weights were determined and fixed organs were examined. The micronucleus test was performed using bone marrow cells. Exposure of 3-MBCF induced mortality at concentrations above 6 ppm. Decreases in body weight and food intake, hemato logical alterations, organ weight changes, and gross and microscopic findings were seen even at the lowest concentrations of 3 ppm. Histopathology revealed principal test substance exposure correlated with lesions in the respiratory tract in both male and female rats above 3 ppm. Groups of male rats exposed above 6 ppm show microscopic lesions in spleens, livers, testes and epididymides; however, the micronucleated polychromatic erythrocytes frequency in bone marrow cells was not changed. Based on histopathology of the respiratory tract and other organs, the no observed adverse effect level (NOAEL) of 3-MBCF in the present study was less than 3 ppm.

1. Introduction

South Korea is one of the biggest chemical-producing countries in the world, and the 3rd largest producer in Asia after China and Japan [1]. In addition to producing large amounts of chemicals, hazardous chemicals are often found in workplaces, and the number of such chemicals has been steadily increasing. In South Korea more than 300 chemicals are newly registered each year, and there are presently approximately 43,500 types of chemicals used in Korean workplaces [2].

Recently, EU regulation no.1907/2006 on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) is the main basis for the environmental hazard assessment of industrial chemicals in each country [3]. In South Korea, The Ministry of Environment (MOE) reinforced the Registration, Evaluation, Authorization and restriction of Chemicals (K-REACH) to manage and inform the matters concerning the registration of chemical substances, as well as the review and assessment of the toxicity, hazards, and risks of chemical substances and products containing hazardous chemical substances [4]. MOE published a draft list of 518 existing chemical substances for registration under the Act on the Registration and Evaluation of Chemicals [5]. According to this list, manufacturers and importers who administer new chemicals or chemicals exceeding 1 ton per year should register it within 3 years after the publication date. The amount of ecotoxicological data requested depends on the production or import tonnage: higher tonnages require the provision of more extensive datasets, such as acute and chronic tests with fish and aquatic invertebrates, or reproduction studies.

3-Methoxy butyl chloroformate (3-MBCF; CAS No. 75032-87-0; Synonyms: 3-Methoxybutyl chloroformate; 3-methoxybutyl carbonochloridate; EINECS 278-058-3; AC1MI6SD) is a clear to light-yellow in color, water insoluble, and possesses a severe, pungent odor. 3-MBCF is used as a reactive chemical intermediate, especially for any chemical compound containing carbonate, pyrocarbonate, carbamate, urethane, and others, and may be used in organic chemical and plastics manufacturing. (PubChem Compound Database, 2005). 3-MBCF is a harmful material (Globally Harmonized System of Classification and Labelling of Chemicals; GLP, Good Laboratory Practice; HCT, hematocrit; HGB, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MNPCe, microneucleated polychromatic erythrocytes; MOE, The Ministry of Environment; NCE, normochromatic erythrocytes; NOAEL, no observed adverse effect level; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocytes; PLT, platelets; RBC, red blood cell counts; RDW, red cell distribution width; REACH, Registration, Evaluation, Authorization and Restriction of Chemicals; SD, Sprague-Dawley; SPF, specific-pathogen-free; WBC, white blood cell counts; 3-MBCF, 3-methoxy butyl chloroformate

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of Chemicals (GHS). Acute toxicity, oral category 4) and causes skin irritation, serious eye irritation, and respiratory irritation (GHS skin corrosion/irritation-category 2; GHS serious eye damage/eye irritation-category 2A; GHS specific target organ toxicity, single exposure; respiratory tract irritation-category 3) (ECHA, 2017).

3-MBCF is included in the 518 existing chemical substances registered for their hazard to humans, animals, and the environment according to K-REACH [5], but there is little information associated with the biological hazard of 3-methoxybutyl chloroformate (3-MBCF). Only a single study reported about the pulmonary effects, including tracheal and nasal irritation. In acute inhalation studies, 3-MBCF was shown to cause respiratory irritation at concentrations of 2000 ppm and higher (MNPCE) in the Asian Institute of Medical Sciences (AIMS) rat model.

2. Materials and methods

2.1. Animals

Male and female specific-pathogen-free (SPF) SD rats aged 6–7 weeks were purchased (Orient Bio Inc., South Korea) and acclimatized for 11 days in polycarbonate cage with SPF conditions before the grouping. Exposures were conducted in inhalation chambers (Model No. SIS-20RG, Shibata Co., Japan) with individual wire mesh cages. Each group consisted of 10 rats, except for the 12 ppm group, which consisted of 5 rats. The rats were exposed to 3-MBCF for 6 h/day, 5 days/week for four weeks. Body weight data were collected twice a week for the first 2 weeks and then at least once per week after. Individual food consumptions were also collected once a week for 4 weeks. Clinical observations were recorded twice a day during exposure periods. At the end of the experiment all animals fasted for 12 h and were then anesthetized with pentobarbital (30 mg/kg, JW Pharmaceutical, South Korea). Blood samples were then collected from the abdominal aorta. Then animals were sacrificed by exsanguination from the abdominal aorta and necropy, including gross findings and organ weight determinations, was performed on all animals.

2.2. Chemicals and inhalation exposure

3-Methoxybutyl chloroformate (99.5% pure, CAS No.:75032-87-0) was purchased from Sekiato Chemicals (GHS). Acute toxicity, oral category 4) and causes skin irritation, serious eye irritation, and respiratory irritation (GHS skin corrosion/irritation-category 2; GHS serious eye damage/eye irritation-category 2A; GHS specific target organ toxicity, single exposure; respiratory tract irritation-category 3) (ECHA, 2017).

2.3. Experimental design

Twenty rats of each sex were randomly assigned to four groups (n = 5); control (filtered air), 3, 6, and 12 ppm and were exposed to 3-MBCF for 6 h/day, 5 days/week for four weeks. Body weight data were collected twice a week for the first 2 weeks and then at least once per week after. Individual food consumptions were also collected once a week for 4 weeks. Clinical observations were recorded twice a day during exposure periods. At the end of the experiment all animals fasted for 12 h and were then anesthetized with pentobarbital (30 mg/kg, JW Pharmaceutical, South Korea). Blood samples were then collected from the abdominal aorta. Then animals were sacrificed by exsanguination from the abdominal aorta and necropy, including gross findings and organ weight determinations, was performed on all animals.

2.4. Hematology

Hematologic examination was performed using a hematology analyzer (CL-7200, Shimazu, Japan). Whole blood samples were collected in sample tubes containing ethylenediamine tetraacetic acid (EDTA-A2K) and examin...
concentrations of 3, 6 or 12 ppm for 6 h/day exposure, respectively.

3.2. In-life parameters

During the exposure, difficulty in respiration, epistaxis, and conditioned gaping (a reflection of nausea) were observed in 6 and 12 ppm groups of rats. On the second exposure day, one male animal exposed to 12 ppm was found dead; at the end of the exposure, all male rats and four female rats were dead. In the 6 ppm group, three male and two female rats were found dead from the seventeen day to the end of the study (Table 2). The mucous exudate from trachea, congestion and discoloration of lung and excess intestinal gas were noted at the dead animals and the observations in respiratory organs with dyspnea were thought to be linked to death of animals. Mean body weights of both male and female rats in 3-MBCF treated groups were generally reduced during 3-MBCF exposure compared to those in the control group (Fig. 1) until exposure Day 26. The body weights showed slight increase on Day 28 in all 3-MBCF inhaled rats. Food consumptions showed more reduction in 3-MBCF exposed rats compared to the control group than in female rats (Table 3, Data of 12 ppm groups not shown due to the high mortality).

3.3. Hematology

Data for hematology was summarized in Table 4 (Data of 12 ppm groups not shown due to the high mortality). In male rats there were dose-dependent, significant decreases of WBC and PLT and an increase of HGB. In female rats, dose-dependent increases of HGB and RDW were found. In addition, significant increase of RBC and RDW were observed in the 6 ppm female group.

3.4. Urinalysis

Only the ketone bodies were increased in male groups compared to control males, but were not in a dose-related manner. No other changes of urinalysis parameters were observed in male and female rats (data not shown).

3.5. Organ weights

Data for absolute and relative organ weights are shown in Tables 5 and 6 (Data for 12 ppm groups not shown due to the high mortality). In male rats, absolute weights of thymus, heart, testes, kidneys, spleen, and liver decreased and lung weights increased in a dose-dependent manner. But the relative weights showed the increased weight of some organs such as heart, testes, lung, kidneys and brain with a dose-dependent manner. In female rats, absolute weights of thymus, heart, kidneys, spleen, and liver decreased and lung weights increased in a dose-dependent manner. As well as, the relative weights were shown the increase of weight in some organs such as heart, lung, kidneys, liver and brain in a dose-dependent manner.

3.6. Histopathological findings

Microscopically, there were pathological changes in lung, trachea, spleen, testes, epididymis, kidney and liver (Fig. 2). In males, inflammatory exudate and enlargement of the alveolar septum, pulmonary edema, phagocyte infiltration, and obstructive bronchitis in the lung were observed in test substance inhaled groups. The incidence and severity of lung lesions were increased with dose-dependent manner compared to control group. In addition, the incidence of trachea-bronchial lesions – including epithelial hyperplasia, epithelial cell atrophy, and squamous metaplasia – was increased compared to control group. Also congestion of the spleen, atrophy of seminiferous tubules, exfoliation of germ cells of testes, and degeneration of germ cells in epididymal ducts were observed in the 6 and 12 ppm groups with increased incidence and dose-dependent manner. Additional observations included obstructive bronchitis in the lung, centrilobular cell necrosis in the liver and one case of myocardial degeneration of 12 ppm group (Table 7). In female rats, lesions were found in the respiratory tracts – similar to those in males – with dose-responsive incidence and severity; however, unlike in male rats, there were no liver lesions. Only minimal to mild congestion of the kidneys was noticed in 6 and 12 ppm groups. Also, the pathologic findings of unscheduled dead rats, from expose day

![Fig. 1. Mean body weight of SD rats inhaling 3-MBCF for 28-days. (A) male rats; (B) female rats.](image-url)
edema with hydrothorax in acute inhaled studies. The oral LD50 values for respiratory distress including dyspnea, pulmonary emphysema and lung in animal studies [13,12]. Similarly, butyl chloroformate induced respiratory distress such as dyspnea in human subjects [12]. In animal studies, the LC50 of methyl chloroformate, induce cough, lacrimation, cocking, headache, and nausea in male and female rats, and main target organ was known as nasal turbinate, trachea and lung for systemic diseases, due to the high mortality. The MNPCE frequency was not changed significantly in 3 or 6 ppm groups of male rats, but increased in 3 and 6 ppm groups of female rats. However, decreases in the proportion of PCEs to total erythrocytes were observed in males, and were significant in female rats.

### 3.7. Bone marrow micronucleus test of 3-MBCF

Incidence of micronucleus formation, distributions of micronuclei found in PCE and NCE, and the ratio of PCE/NCE in femurs of male and female rats are shown in Table 8 (Data for 12 ppm groups not shown due to the high mortality). The MNPCE frequency was not changed significantly in 3 or 6 ppm groups of male rats, but increased in 3 and 6 ppm groups of female rats. However, decreases in the proportion of PCEs to total erythrocytes were observed in males, and were significant in female rats.

### 4. Discussion

The inhalation of chemicals in many industrial workplaces can be a trigger of respiratory tract disorders as well as systemic diseases, depending on the type of inhaled substance and exposure time [10,11]. Among industrial chemical substances, chloroformate derivatives used as intermediates in the synthesis of materials have a higher probability of trigger of respiratory tract disorders as well as systemic diseases, due to the increase in distribution and prevalence in industrial use, 3-MBCF, one of the chloroformate derivatives, has been examined in order to obtain more toxicological data for health management of industrial workers, but little data have been released to date.

In the present study we confirmed the clear evidence of toxicity of 3-MBCF through the mortality, clinical observations, body weight loss, hematological data, and pathological observation using laboratory animals. In preliminary inhalation studies for substance level determination, 3-MBCF levels of 10, 30, 90, and 180 ppm were used on using SD rats (Fig. 3); a level of 10 ppm was considered the highest substance level required for the main study due to the observed fatality. In both males and females, body weight gain was significantly reduced in all groups of rats that inhaled 3-MBCF; half of the animals died in the 6 ppm group and only one rat survived in the 12 ppm group. The extreme decrease in food consumption is considered to be associated with the pain and stress caused by irritation of the skin, mucus membranes, and/or eyes. Also, some chloroformate derivatives induced respiratory distress such as dyspnea, which is considered to be associated with low food consumption [12].

Several changes were observed in hematology and urinalysis (data not shown), and the WBC counts were significantly decreased in males but not in females. Increased levels of HGB and HCT were observed in both genders. Hematological indices including HGB and HCT are influenced by various factors. Several studies indicated that pulsed electric field exposure [14], herbal extracts [15,16], and chemicals [17] can influence hematological parameters, but in this study, alteration ranges were minimal and within the normal range, thus it is difficult to judge directly by test substance [18]. Generally, increased RDW indicates the presence of cell of different sized and related with iron deficiency anemia if accompanied by a decrease of MCV. The significant increase of RDW in females was not considered to be substance-related change because it was minimal and shown no other hematological changes related. Also, WBC and PLT were significantly decreased in male rats compared to control groups, but these alterations, observed only male

| Table 3 |
| --- |
| Total mean food consumptions of the SD rats with inhaled with 3-methoxybutyl chloroformate. |
| Sex | Groups | Food consumption (g/day/rat) |
| --- | --- | --- |
| Male | Control | 27.17 ± 1.37 (5/5) |
| 3 ppm | 14.85 ± 4.28 (5/5) |
| 6 ppm | 8.50 ± 3.17 (5/5) |
| Female | Control | 21.30 ± 3.40 (5/5) |
| 3 ppm | 16.50 ± 5.83 (5/5) |
| 6 ppm | 10.65 ± 4.52 (5/5) |
| 1 week | 2 weeks | 3 weeks | 4 weeks |
| --- | --- | --- | --- |
| Male | | | | |
| Control | 29.07 ± 0.97 (5/5) |
| 3 ppm | 14.26 ± 2.03 (5/5) |
| 6 ppm | 11.11 ± 3.06 (5/5) |
| Female | | | | |
| Control | 22.44 ± 2.04 (5/5) |
| 3 ppm | 15.48 ± 2.43 (5/5) |
| 6 ppm | 12.02 ± 2.17 (5/5) |

Data were expressed as mean ± S.D.

\* Numbers in parenthesis are the numbers of alive/total animals.

2, were more severe and frequently compared to live animals.

### Table 4

Hematological data in SD rats inhaled with 3-methoxybutyl chloroformate.

| Parameters | Male | Female |
| --- | --- | --- |
| | Control | 3 ppm | 6 ppm | Control | 3 ppm | 6 ppm |
| WBC | 4.58 ± 0.91 | 2.64 ± 0.70* | 1.60 ± 0.74* | 2.50 ± 0.59 | 2.61 ± 0.55 | 3.10 ± 1.16 |
| RBC | 7.48 ± 0.27 | 7.55 ± 0.63 | 8.20 ± 0.23 | 7.03 ± 0.15 | 6.80 ± 0.20 | 7.74 ± 0.26* |
| HGB | 14.30 ± 0.31 | 15.30 ± 0.42* | 16.2 ± 0.71* | 13.82 ± 0.52 | 14.12 ± 0.44 | 15.03 ± 0.38* |
| HCT | 41.92 ± 1.14 | 41.28 ± 3.18 | 45.8 ± 1.56 | 40.18 ± 1.15 | 39.36 ± 1.64 | 44.97 ± 2.78* |
| MCV | 56.04 ± 0.52 | 54.72 ± 0.92 | 55.9 ± 0.35* | 57.16 ± 1.57 | 57.84 ± 1.43 | 58.07 ± 1.76 |
| MCH | 19.14 ± 0.88 | 20.36 ± 1.59 | 19.8 ± 0.35 | 19.66 ± 0.43 | 20.78 ± 1.12 | 19.43 ± 1.12 |
| MCHC | 34.14 ± 1.32 | 37.20 ± 2.58 | 35.4 ± 0.35 | 34.40 ± 1.41 | 35.92 ± 1.89 | 33.53 ± 2.78 |
| RDW | 14.94 ± 0.40 | 15.48 ± 0.89 | 16.0 ± 0.50 | 13.86 ± 0.30 | 15.14 ± 0.55* | 15.30 ± 0.26* |
| PLT | 916.6 ± 100.80 | 693.8 ± 52.47* | 680.5 ± 17.68* | 833.0 ± 102.24 | 778.6 ± 54.09 | 953.3 ± 86.12 |

All values are expressed as mean ± SD.

HCT, hematocrit (%); HGB, hemoglobin (g/dl); MCH, mean corpuscular hemoglobin (pg); MCHC, mean corpuscular hemoglobin concentration (%); MCV, mean corpuscular volume (u²); PLT, platelet (10^9/u²); RBC, red blood cell count (10^6/mm³); WBC, white blood cell count (10^3/mm³); RDW, red cell volum distribution.

Significant differences as compared with control: * p < 0.05.
rats, were considered to be due to extreme body weight loss and reduced feed intake rather than specific organ toxicity by the test substance directly.

The weight reductions identified in absolute weights of organs such as heart, testes and kidney were inconsistent with the relative weights of organs and thought to be linked with body weight changes. But the increase of absolute and relative lung weights in male and female rats were considered to be related to the inflammation of lung confirmed in Table 5:

**Table 5**

| Organ Weights (mg) | Male | Female |
|--------------------|------|--------|
|                    | Control 3 ppm | 6 ppm |
| Thymus             | 606.4 ± 128.9 | 305.6 ± 110.8* | 198.5 ± 146.4 |
| Heart              | 1432.6 ± 70.3 | 1024.2 ± 144.3** | 907.5 ± 72.8* |
| Testis (Ovary)     | 3237.2 ± 154.8 | 2938.0 ± 232.8 | 2392.0 ± 115.0 |
| Lung               | 1240.2 ± 130.8 | 1757.6 ± 243.6* | 1681.0 ± 181.0 |
| Kidney             | 2664.4 ± 174.9 | 1949.8 ± 111.1* | 1587.5 ± 204.4 |
| Spleen             | 671.8 ± 95.2 | 447.2 ± 126.2* | 266.0 ± 53.7** |
| Liver              | 12093.6 ± 609.1 | 6681.8 ± 431.4*** | 5830.0 ± 166.9*** |
| Brain              | 1495.4 ± 72.2 | 1747.6 ± 63.9 | 1579.0 ± 76.4 |

All values are expressed as mean ± SD. Significant differences as compared with control: * p < 0.05; ** p < 0.01; *** p < 0.001.

**Table 6**

| Organ Weights (%) | Male | Female |
|-------------------|------|--------|
|                    | Control 3 ppm | 6 ppm |
| Thymus             | 136.8 ± 27.4 | 101.9 ± 30.1 | 88.4 ± 58.5 |
| Heart              | 323.7 ± 20.6 | 347.6 ± 41.1 | 417.7 ± 8.3* |
| Testis (Ovary)     | 733.3 ± 56.6 | 1020.0 ± 113.5** | 1091.6 ± 167.9 |
| Lung               | 281.1 ± 35.5 | 601.5 ± 113.9* | 781.1 ± 161.4 |
| Kidney             | 603.7 ± 56.6 | 663.5 ± 44.4 | 729.0 ± 21.1* |
| Spleen             | 151.5 ± 17.6 | 150.0 ± 31.4 | 121.7 ± 12.5 |
| Liver              | 2733.9 ± 99.2 | 2272.3 ± 138.2** | 2690.6 ± 192.4 |
| Brain              | 339.1 ± 154.3 | 595.0 ± 35.0* | 731.5 ± 198.3 |

All values are expressed as mean ± SD. Significant differences as compared with control: * p < 0.05; ** p < 0.01; *** p < 0.001.

**Fig. 2.** Light micrograph of lower respiratory tract, testis, epididymis, and liver sections in rats that inhaled 6 or 12 ppm of 3-MBCF for 4 weeks. (A) Alveolar edema and mononuclear cell infiltration in lung of female rat that inhaled 6 ppm of 3-MBCF; (B) Atrophy and exfoliation of epithelial cells in bronchus of male rat that inhaled with 6 ppm of 3-MBCF; (C) Obstructive bronchitis in lung of male rat that inhaled 12 ppm of 3-MBCF; (D,E) lesions in male reproductive organs including germ cell atrophy after inhalation of 6 ppm of 3-MBCF; (F) Centrilobular liver cell necrosis in a male rat that inhaled 12 ppm of 3-MBCF. Hematoxylin and eosin stain (A–F), x100(A–D), x200(E), x400(F).
histopathology. In the histopathology of respiratory tract indicated that the fatal relation of findings with dose dependent manner between control and substance inhaled groups in both gender. Other findings of spleens, testes, epididymides, and livers also showed test substance-related lesions including epithelial damage with inflammation of tracheobronchial lesions, degeneration and atrophy of male reproductive organs and congestions and necrosis of the spleen and liver, respectively. The germ cell degeneration is common in spermatogenesis, but increased degenerating germ cells that were slugged, unable to determine the stage, were observed in epididymides. Also atrophy of testes is known to be accompanied with reduction of organ weight according to the severity, but the relative weights of testes were increased in our study. It is thought to be associated with the minimal to mild severity of atrophy including absence of germ cells and segmental distribution in some testicular tubules in testes. Also there were a limit to determine the organ weight of accessory glands such as prostate and seminal vesicle and dead animals.

Bone marrow micronucleus assays, which measure substance-related chromosomal or mitotic damage in vivo, provide more insight into factors for genotoxicity of chemicals in vitro [19,20]. In the present study, with little modification from OECD guideline 474 [7,8], we show the results of a genotoxicity test of 3-MBCF. At the middle and high substance levels mortality was elevated: at 6 ppm, three male and two female rats died, while at 12 ppm all male and four out of five female rats died. Substance levels over the tested doses were unavailable for the micronucleus test. In addition, there was no positive control material that induced DNA damage (such as mitomycin C or cyclophosphamide; [21]). Although the MNPCE frequency was increased in 3-MBCF inhaled female groups, 3-MBCF was not considered to be a clastogen because MNPCE frequency was not altered in male rats and there were no dose-related increase. Nevertheless the PCE/PCE + NCE ratio was decreased in male and female rats, suggesting that 3-MBCF may be cytotoxic in bone marrow. Further in vivo or in vitro experiments to confirm the genetic toxicity of 3-MBCF are required.

5. Conclusion

The number and amount of chemicals used in workplace is increasing, and more information is needed to protect workers from

| Table 7 | Histopathological findings for SD rats inhaled with 3-methoxybutyl chloroformate. |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Groups** | **control** | **3 ppm** | **6 ppm** | **12 ppm** | **control** | **3 ppm** | **6 ppm** | **12 ppm** |
| **Organs and findings** | **No. of animals** | | | | | | | | | | |
| Lung | Inflammatory exudate | | | | | | | | | | |
| | Total | 5 | 5 | 5(3)**,# | 5 | 5(2)**,# | 5 | 5(4)**,# |
| | ± | 5 | 4 | 1 | | | | | | | |
| | + | 1(1) | 1 | 1 | 1(1) | 1(1) | 1 | 2 | 2(2) | 2(2) |
| | ++ | 1(1) | 3(3) | 2(2) | 3(2) | | | | | |
| Enlargement of alveolar septum | Total | 0 | 1 | 4(3)**,# | 0 | 0 | 4(2)**,# | 5(4)**,# |
| | ± | 1 | 2(1) | 2(1) | 2(1) | | | | | |
| | + | 2(2) | 1(1) | 1(1) | | | | | | |
| | ++ | 2(2) | 2(2) | 2(2) | 3(2) | | | | | |
| Phagocyte infiltration | Total | 0 | 4** | 5(3)**,# | 0 | 3* | 4(2)**,# | 5(4)**,# |
| | ± | 3 | 1(1) | 1(1) | 1 | 1 | 1 | 1(1) | 1 | 2(2) |
| | + | 1(1) | 2(2) | 2(2) | 2(1) | | | | | |
| | ++ | 2(1) | 2(2) | 2(2) | | | | | | |
| Edema | Total | 0 | 3* | 4(3)**,# | 0 | 3* | 5(2)**,# | 5(4)**,# |
| | ± | 3 | 1 | 1(1) | 3 | 3 | 3 | 3 | 2(2) | 2(2) |
| | + | 1(1) | 2(2) | 2(2) | 2(1) | 2(1) | | | | |
| | ++ | 2(2) | 2(2) | 2(2) | 1(1) | | | | | |
| Obstructive bronchitis | Total | 0 | 0 | 0 | 4(4)**,# | 0 | 0 | 0 | 2(2) | | |
| | ± | 1(1) | 1(1) | 1(1) | | | | | | |
| | + | 2(2) | 2(2) | 2(2) | | | | | | |
| | ++ | 1(1) | 1(1) | 1(1) | | | | | | |
| Trachea with bronchi | Epithelial hyperplasia | Total | 0 | 0 | 2(1) | 4(4)**,# | 0 | 0 | 3(2)** | 2(2) |
| | ± | 0 | 0 | 0 | 0 | | | | | |
| | + | 0 | 2(2) | 2(2) | 2(1) | 2(1) | 2(1) | 2(1) | 2(1) | 2(1) |
| | ++ | 0 | 2(2) | 2(2) | 2(2) | 2(1) | 2(1) | 2(1) | 2(1) | 2(1) |
| Spleen | Congestion | Total | 0 | 0 | 3(3)** | 5(5)** | 0 | 0 | 2(2) | 4(4)** |
| | ± | 0 | 0 | 0 | 0 | | | | | |
| | + | 0 | 0 | 0 | 0 | | | | | |
| | ++ | 0 | 0 | 0 | 0 | | | | | |
| | +++ | 0 | 0 | 0 | 0 | | | | | |
| Liver | Necrosis, centrilobular, ± / + / ++ | Total | 0 | 0 | 0 | 5(5)** | 1/3/1 | 0 | 0 | 0 | 0 |
| | ± | 0 | 0 | 0 | 0 | | | | | |
| | + | 0 | 0 | 0 | 0 | | | | | |
| | ++ | 0 | 0 | 0 | 0 | | | | | |
| Kidneys | Congestion | Total | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ± | 0 | 0 | 0 | 0 | | | | | |
| | + | 0 | 0 | 0 | 0 | | | | | |
| | ++ | 0 | 0 | 0 | 0 | | | | | |

Values in basket are numbers of un-scheduled dead rats with lesions. Grade of change; ±, minimal; +, mild; ++, moderate; ++++, severe.

Table 8 | Incidence (%) of micronucleated polychromatic erythrocyte (MNPCE) and PCE/NCE (polychromatic/normochromatic erythrocyte) ratio in SD rats inhaled with 3-methoxybutyl chloroformate.

| **Sex** | **0 ppm** | **3 ppm** | **6 ppm** |
| --- | --- | --- | --- |
| MNPCE (%) | Male | 0.16 + 0.08 | 0.13 + 0.10 | 0.17 + 0.15 |
| | Female | 0.04 + 0.05 | 0.08 + 0.09 | 0.08 + 0.10 |
| PCE/PCE + NCE (%) | Male | 59.38 + 7.61 | 57.49 + 9.28 | 53.91 + 7.39 |
| | Female | 60.28 + 9.75 | 48.75 + 6.99 | 41.49 + 3.91 |

All values are expressed as mean ± SD.

Significant differences as compared with control: * p < 0.05.

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occupational disease. In light of this, we focused on the inhalation toxicity of 3-MBCF using experimental rats. In summary, mortality occurred at 6 and 12 ppm of 3-MBCF during a 28-day repeated inhalation study, but the genotoxicity of 3-MBCF was not confirmed via bone marrow micrornucleus test. Based on the body weight decreases, organ weight changes of lung, and incidence of histopathological findings of lung, liver, and testes of male, and lung lesions of female, the low observed adverse effect level (LOAEL) of 3-MBCF was estimated to be 3 ppm and the no observed adverse effect level (NOAEL) of 3-MBCF is proposed to be less than 3 ppm following 28-day repeated inhalation to male and female SD rats. Further studies should be considered to confirm the specific organ toxicity mechanism or genotoxicity and their human relevance.

Conflicts of interest

The authors declare that there is no conflict of interest.

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