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Original Article

Inhalation exposure to 2-ethyl-1-hexanol causes hepatomegaly and transient lipid accumulation without induction of peroxisome proliferator-activated receptor alpha in mice

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Abstract: 2-Ethyl-1-hexanol (2EH) is a volatile organic compound known to cause sick building syndrome. However, 2EH-induced hepatotoxicity has been mainly evaluated in experiments orally administering 2EH as a metabolite of di(2-ethylhexyl) phthalate. To evaluate the hepatotoxicity risk of 2EH as an indoor air pollutant, we exposed 10-wk-old male ICR mice to 2EH by inhalation for 8 h/d, 5 d/wk for 3 months (0, 20, 60, or 150 ppm) or 6 months (0, 0.5, 10, or 100 ppm). In both experiments, relative liver weights significantly increased in the highest exposure groups. The 3-month exposure increased histopathological lipid droplets in the liver in a dose-dependent manner, hepatic triglyceride at all exposure levels, hepatic phospholipid at 150 ppm, and microsomal triglyceride transfer protein at 60 and 150 ppm; however, these changes were not observed following the 6-month of exposure. Following the 3-month exposure, alanine transaminase and peroxisomal bifunctional proteins, known markers of liver injury and peroxisome proliferation, respectively, remained unaltered. Therefore, in the present study, the inhalation concentration range of 2EH induced a toxic hypertrophic change, revealing a limited role of peroxisome proliferator-activated receptor alpha (PPARα). The liver weights may have presumably increased via a mechanism independent of PPARα activation.

Key words: 2-Ethyl-1-hexanol, Volatile organic compound (VOC), Inhalation exposure, Lipid droplet, Hepatic enlargement, Peroxisome proliferator-activated receptor alpha (PPARα)

Introduction

Volatile organic compounds (VOCs) are primary chemicals to which individuals are easily exposed indoors via

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inhalation. Among the several kinds of VOCs, 2-ethyl-1-hexanol (2EH) (CAS No. 104-76-7) has drawn attention for inducing sick building syndrome (SBS)[1–3], with numerous cases reported in office buildings and houses where 2EH was detected in the air[4]. In indoor air, 2EH is generated by the hydrolysis of di(2-ethylhexyl) phthalate (DEHP), a plasticizer of polyvinyl chloride (PVC) flooring, reacting with alkali moisture contained within the concrete in the building[2, 5]. The general population might be exposed to 2EH for several years as the indoor concentration of 2EH can be as high as 0.2 ppm (1,080 μg/m^3) in summer, with concentration fluctuations over a prolonged period depending on the room temperature[6]. Reportedly, high concentrations of 2EH have been detected in buildings more than 10 years after completion[7]. Therefore, the toxicological evaluation of inhaled 2EH is necessary, as reports regarding 2EH toxicity after inhalation exposure remain limited.

To date, most toxicological studies investigating 2EH have been conducted following oral administration[8, 9] from the perspective of potential hepatic carcinogenicity induced by DEHP, which is metabolized to 2EH in the body[10]. Peroxisome proliferator-activated receptor alpha (PPARα), a nuclear receptor, is suspected of playing a role in DEHP-induced hepatocarcinogenicity in rodents[11]. Additionally, 2-ethylhexanoic acid (2EHA), a metabolite of 2EH, is known to act as a PPARα agonist[12]. Numerous studies evaluating the oral administration of 2EH have demonstrated an increase in the relative liver weight[8, 9, 13–17], which is frequently reported following PPARα agonist treatment[18, 19].

Collectively, inhalation experiments evaluating subchronic or chronic 2EH exposure are crucial for clarifying the impact on the liver. Therefore, in the present study, mice were exposed to inhalable 2EH at concentrations of 0, 20, 60, and 150 ppm in the subchronic test, and at concentrations of 0, 0.5, 10, and 100 ppm in the chronic test to elucidate 2EH effects on the liver, as well as the involvement of PPARα activation, both histopathologically and biochemically.

Materials and Methods

Animals and 2EH exposure

The study protocol was approved by the Animal Care and Use Committee (approval numbers: H22M-72 and H28M-015) and performed in accordance with the Guide for Animal Experimentation of Nagoya City University. Nine-week-old pathogen-free male ICR mice were obtained from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan) and acclimatized for one week before experimentation. In this study, we used a whole-body inhalation exposure chamber system (Sibata Scientific Technology Ltd., Soka, Saitama, Japan) maintained under a 12-h light/dark cycle at 23–24°C under relatively constant humidity (55%), and the exposure concentration was monitored by charcoal tube sampling (Sibata Scientific Technology Ltd.). This study is part of a previously reported study, and inhalation exposure conditions and concentration measurements in the chamber have been previously described[20]. The experiment was divided into two types of studies, i.e., subchronic and chronic exposure studies. For the subchronic study, mice were exposed to 0 (fresh air), 20, 60, or 150 ppm of 2EH for 8 h/day, 5 days/week. In the chronic study, mice were exposed to 0 (fresh air), 0.5, 10, or 100 ppm. Body weights were recorded weekly.

In the subchronic study, after the 3-month exposure period, mice in each group were randomly divided into two groups for analysis. For histopathological analyses (n=6–7/group), mice were anesthetized using isoflurane and then transcardially perfused with 4% (w/v) paraformaldehyde phosphate buffer (Wako Pure Chemical Industries Ltd., Osaka, Osaka, Japan), and the liver was stored at 4°C. In the second group used for biochemical analyses (n=6–7/group), mouse blood samples were collected into heparinized tubes by decapitation, and the liver, kidney, testis, and lungs were removed and weighed. The liver was stored at −80°C until use.

In the chronic study after a 6-month exposure (n=6–7/group), mice in each group were anesthetized using isoflurane and then perfused with saline, and a small portion of the liver was removed and stored at −80°C for biochemical analyses. The remaining liver tissue was stored until histopathological analysis after transcardial perfusion was performed as described above.

Histopathological analyses

After overnight fixation in 4% paraformaldehyde, the trimmed livers were dehydrated and embedded in paraffin. Next, 5-μm thick sections were obtained using a microtome, placed on amino silane-coated slides, and subsequently stained with hematoxylin and eosin (H&E) (Mayer's Hematoxylin, Wako Pure Chemical Industries Ltd.; Eosin Y, Muto Pure Chemicals Co. Ltd., Bunkyo, Tokyo, Japan). Histopathological alterations following 2EH exposure were observed for each mouse liver at a magnification of 200× and scored in nine adjacent different microscopic fields (0.15 mm^2/field). No lipid droplets observed in the field were scored as 0 (normal), and fields presenting lipid

droplets as vacuolar degeneration were scored as 1 (slight), 2 (mild), 3 (moderate), or 4 (severe), according to their densities in the white area. The average score was used for statistical analysis as a representative value for each mouse.

Next, to histopathologically examine hepatic triglycerides, trimmed livers were dipped in 10%, 20%, and 30% sucrose sequentially, flash-frozen in frozen carbon dioxide/hexane soaked with OCT Compound (Sakura Finetek Japan Co., Ltd, Chuo, Tokyo, Japan), and then cut into 8-μm thick frozen sections using a freezing microtome (Leica, Nussloch, Germany). The sections were placed on slides and washed with distilled water and 60% isopropyl alcohol (IPA). The slides were then stained with Oil Red-O (Muto Pure Chemicals Co. Ltd.) solution at 37°C for 15 min, rinsed with 60% IPA and distilled water, counterstained with Mayer's hematoxylin for 3 min, and observed under an optical microscope. As a positive control, livers of rats exhibiting nonalcoholic steatosis provided by Prof. Naito (Kinjo Gakuin University, Japan) were subjected to the same procedure.

Measurement of hepatic and plasma lipid concentrations and plasma alanine transaminase (ALT)

Livers stored at −80°C were homogenized with 3-fold (v/w) 10 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose, and then, the lipids were extracted using Folch's method[21]. The chloroform layer was transferred, evaporated, and the residue was resuspended in IPA and recomposed at 37°C for 10 min. Lipids present in plasma or the liver extract suspension were measured using LabAssay™ Triglyceride, HDL-Cholesterol E test Wako, and LabAssay™ Phospholipid kits (Wako Pure Chemical Industries Ltd.). Plasma ALT was measured using the Transaminase C2-test Wako (Wako Pure Chemical Industries Ltd.).

Western blot analysis

Total protein concentrations of the homogenate obtained above were measured using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, Waltham, Massachusetts, USA). Briefly, 8 μg of total protein was separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto cellulose nitrate membranes. Then, the membranes were blocked in Tris-buffered saline with 0.1% Tween 20 (TBST) containing 3% bovine serum albumin for 1 h at room temperature, followed by incubation at room temperature with primary rabbit antibodies for 1 h. After washing with TBST, the membranes were incubated with alkaline phosphatase-conjugated AffiniPure goat anti-rabbit IgG (H+L) (Jackson Immuno Research Inc., West Grove, Pennsylvania, USA); then, the complex was detected by 1-Step™ NBT/BCIP (Thermo scientific). The bands were quantified using the UN-SCAN IT gel software (Silk Scientific, Inc., Orem, Utah, USA). The primary antibodies used for western blot analyses were as follows: microsomal triglyceride transfer protein (MTTP) (Aviva System Biology Corporation, San Diego, California, USA), catalase (Abcam plc., Cambridge, Cambridgeshire, UK), peroxisomal bifunctional protein (BP)[22], very-long-chain acyl-CoA dehydrogenase (VLCAD)[23], and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology, Inc., Danvers, Massachusetts, USA).

Data analysis

In the present study, all statistical analyses were performed using the EZR software package (version 1.41; Saitama Medical Center, Jichi Medical University, Saitama, Saitama, Japan)[24]. Data were analyzed using the Jonckheere-Terpstra trend test or one-way analysis of variance, followed by Dunnett's multiple test. Differences were considered statistically significant when the p-value was <0.05.

Results

Body and liver weights

Changes in body weight were monitored to investigate the toxicological effects of 2EH. During the experimental period, no significant difference in the average body weight was observed when compared with the control group in the subchronic experiment (Fig. 1A). In the chronic experiment, the average body weights of mice exposed to 10 (only after 1, 5, and 6 months) and 100 ppm 2EH were significantly lower than those of control mice (Fig. 1B). At the end of the 6-month exposure, the body weight gain was suppressed by 14% and 19% following exposure to 10 ppm and 100 ppm, respectively, when compared with that observed in the control groups.

After 2EH exposure, the absolute liver weight of mice was significantly increased in all exposure groups after 6 months, whereas no significant difference was observed in the liver weight following the 3-month of exposure (Table 1). The relative liver weights increased in a dose-dependent manner, with statistically significant values observed in mice exposed to 150 ppm for 3 months and 100 ppm for 6 months when compared with the respective controls. These findings were in accordance with the notion that hepatic enlargement was attributed to activated PPARα, as the 2EH metabolite, 2EHA, is a weak activator of the receptor[25]. No significant changes were observed in the weights of the kid-

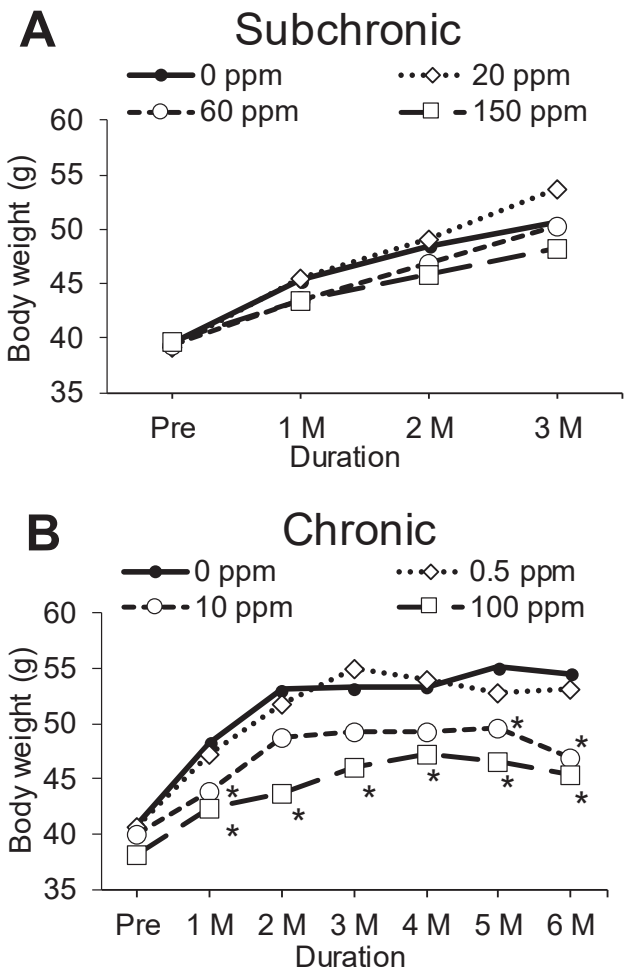

Fig. 1. Time-course changes in body weight of mice exposed to 2EH at different concentrations.
(A) Average body weight of mice exposed to 0 (fresh air), 20, 60, or 150 ppm 2EH for 3 months (3 M) (n=13 for 0 and 60 ppm, and n=14 for 20 and 150 ppm). During the 3 M exposure, a significant difference is not observed. (B) Average body weight of mice exposed to 0 (fresh air), 0.5, 10, or 100 ppm 2EH for 6 months (6 M) (n=7 for 0, 0.5, and 10 ppm, and n=6 for 100 ppm). During the 6 M exposure, the body weights of the 100 ppm group are found to be lower than those in 0 ppm group. Additionally, the 10 ppm group reveals lower body weights than the 0 ppm group during the 1st, 5th, and 6th month. An *asterisk* indicates statistical significance (*p<0.05). 2EH, 2-Ethyl-1-hexanol.

neys, testes, and lungs (data not shown).

Histopathological alteration in mouse liver after 2EH inhalation exposure

We then examined the histopathological effects of 2EH inhalation exposure. Accordingly, mice livers exposed to 2EH for 3 months were stained with H&E (Figs. 2A–D). Zone-specific hepatocellular hypertrophy, such as typical centrilobular hypertrophy, as seen after treatment with a PPARα agonist[26], was not observed, although hepatic enlargement demonstrated as an increase in the relative liver weight was documented. The Jonckheere-Terpstra trend test revealed that 2EH exposure increased the presence of lipid droplets (Fig. 2E) in a dose-dependent manner after the 3-month of exposure, with no significant difference observed between groups (Fig. 2F). Next, Oil Red-O staining was performed to determine whether these lipid droplets were triglycerides. All samples were negative for Oil Red staining, although a positive control (fatty rat liver) was stained (data not shown). No significant difference was observed in mice exposed to 2EH for 6 months.

Table 1. Body weight (B.W.) and absolute and relative liver weight of the mice exposed to 2EH

	ppm	B.W.	Liver	Liver/B.W.x100
3 M	0	51.1 ± 4.3	2.20 ± 0.19	4.32 ± 0.22
	20	53.4 ± 5.0	2.41 ± 0.28	4.51 ± 0.26
	60	51.3 ± 2.7	2.35 ± 0.21	4.58 ± 0.44
	150	50.1 ± 4.4	2.37 ± 0.24	4.74 ± 0.28*
6 M	0	48.4 ± 5.9	2.31 ± 0.28	4.82 ± 0.83
	0.5	53.0 ± 2.8	2.82 ± 0.36*	5.32 ± 0.59
	10	50.3 ± 2.1	2.71 ± 0.15*	5.39 ± 0.36
	100	47.2 ± 3.9	2.70 ± 0.16*	5.73 ± 0.30*

Exposure concentrations were 0, 20, 60, and 150 ppm for 3 months (3 M) (n=7/group), and 0, 0.5, 10, and 100 ppm for 6 months (6 M) (n=7 for 0, 0.5, and 10 ppm, and n=6 for 100 ppm), respectively. Liver weight is significantly higher in mice exposed for 6 months. The relative liver weight is significantly higher in mice exposed to 150 ppm for 3 months and 100 ppm for 6 months. An *asterisk* indicates statistical significance (*p<0.05) when compared with the respective controls. Data are presented as mean ± SD.

Hepatic and plasma lipid levels and plasma ALT

To examine the biochemical effects of 2EH inhalation exposure in mice, three types of lipids (triglycerides, cholesterol, and phospholipids) were measured in the liver and plasma (Fig. 3). 2EH exposure increased hepatic triglyceride (Fig. 3A, 2.8-fold at 20 and 60 ppm and 2.1-fold at 150 ppm) and phospholipid (Fig. 3B, 1.5-fold at 20 and 60 ppm, and 2.0-fold at 150 ppm) levels. Furthermore, 2EH exposure increased the levels of hepatic phospholipids in a dose-dependent manner (Fig. 3B). In contrast, 2EH did not alter the hepatic cholesterol levels (data not shown). Additionally, plasma lipid levels were measured. All lipids presented the same trend, with lipid levels increasing in the 20 ppm and 60 ppm exposure groups and decreasing in the 150 ppm group when compared with the control group; the tendency of plasma phospholipids differed from that of hepatic phospholipids. Plasma ALT levels were unaltered in mice exposed to 2EH for 3 months. No significant differ-

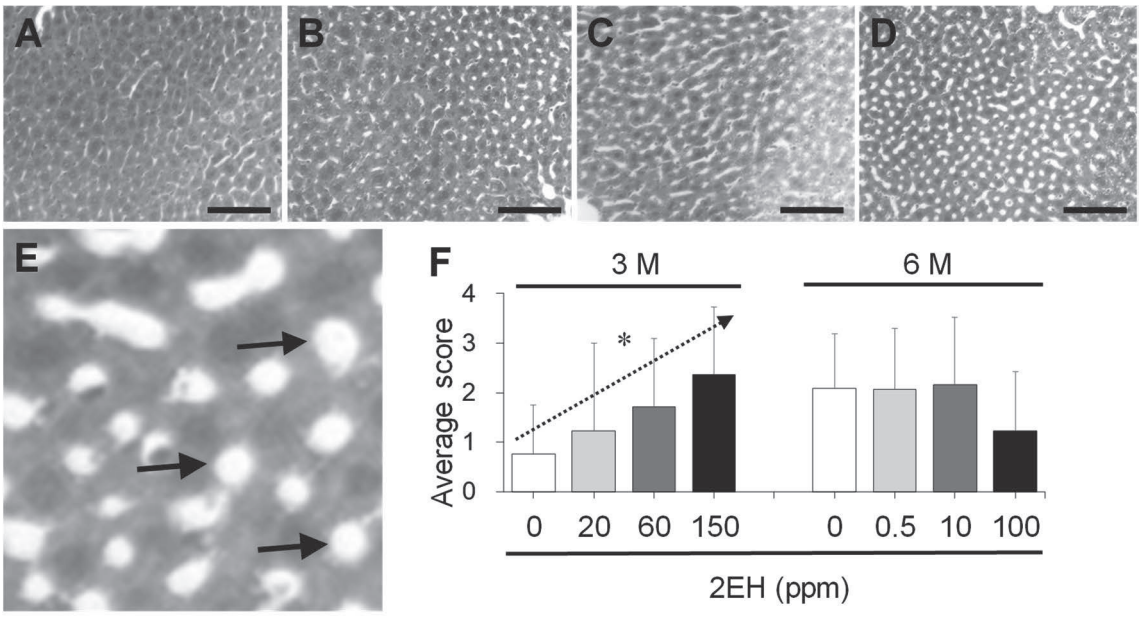

Fig. 2. Liver histopathological findings after 2EH inhalation exposure for 3 months (3 M) or 6 months (6 M). Typical photomicrographs at a magnification of 200× of liver sections obtained from (A) 0, (B) 20, (C) 60, and (D) 150 ppm exposure for 3 M. (E) represents the magnified photograph of (D) and the arrows indicate the presence of lipid droplets. (F) Average score rating of histopathological alterations in each group. The Jonckheere-Terpstra trend test reveals that 2EH exposure for 3 M increases lipid droplets in a dose-dependent manner. An *asterisk* indicates statistical significance (*p<0.05) in the Jonckheere-Terpstra test. Data are presented as mean ± SD for 6–7 mice per group. Scale bar indicates 100 μm.

ences in lipid concentrations were observed in mice exposed to 2EH for 6 months.

Western blot analyses

Western blotting was performed to clarify the role of PPARα-related signaling in 2EH inhalation exposure-induced lipidosis (Fig. 4, Supplemental Fig. 1). Fig. 4A presents typical representative results stained with MTTP and GAPDH. Catalase, a peroxisome marker enzyme, was downregulated only in the livers of mice exposed to 10 ppm 2EH for 6 months (Fig. 4B). VLCAD and BP are hepatic PPARα-target proteins, which demonstrated no differences in protein expression between groups after 2EH exposure (Figs. 4C, D). MTTP, known to transfer triglycerides from the liver, was upregulated in the liver of mice exposed to 60 and 150 ppm of 2EH for 3 months (Fig. 4E), presumably resulting in a dose-independent increase in hepatic triglyceride levels.

Discussion

Herein, we evaluated the hepatotoxicity after inhalation exposure to the indoor air pollutant, 2EH, to which occupants of concrete buildings may be exposed for long-term periods in daily life. In the present study, the relative liver weights were significantly increased in the highest exposure group in the subchronic and chronic experiments. In the subchronic test, histopathological and biochemical lipid accumulation was observed. However, these effects were independent of the mechanism mediated by PPARα, to which 2EHA, a known 2EH metabolite, reportedly binds, based on the induction of PPARα target proteins involved in lipid metabolism. These changes disappeared after the chronic exposure period.

To date, the toxicity of 2EH has mainly been investigated following the oral administration of a metabolite of plasticizer DEHP and di(2-ethylhexyl) adipate[14]; for example, the hepatic toxicity of DEHP, 2EH, and 2EA, particularly focusing on lipid metabolism, has been examined in Fischer 344 (F344) male rats[16, 27]. In contrast to several reports regarding oral administration[8, 9, 13–15, 17, 28, 29], only three studies regarding inhalation exposure are currently available: one acute toxicity study investigating local irritation[30] and two subchronic studies focusing on the effects of 2EH exposure for 3 months on the olfactory epithelium and bulb[20] and general toxicity[31]. Klimisch *et al*.[31] have examined liver histopathological findings, ALT levels, and peroxisome induction by assessing cyanide-insensitive palmitoyl-CoA oxidation, with no adverse effects observed up to 120 ppm (highest concentration) of 2EH exposure in rats.

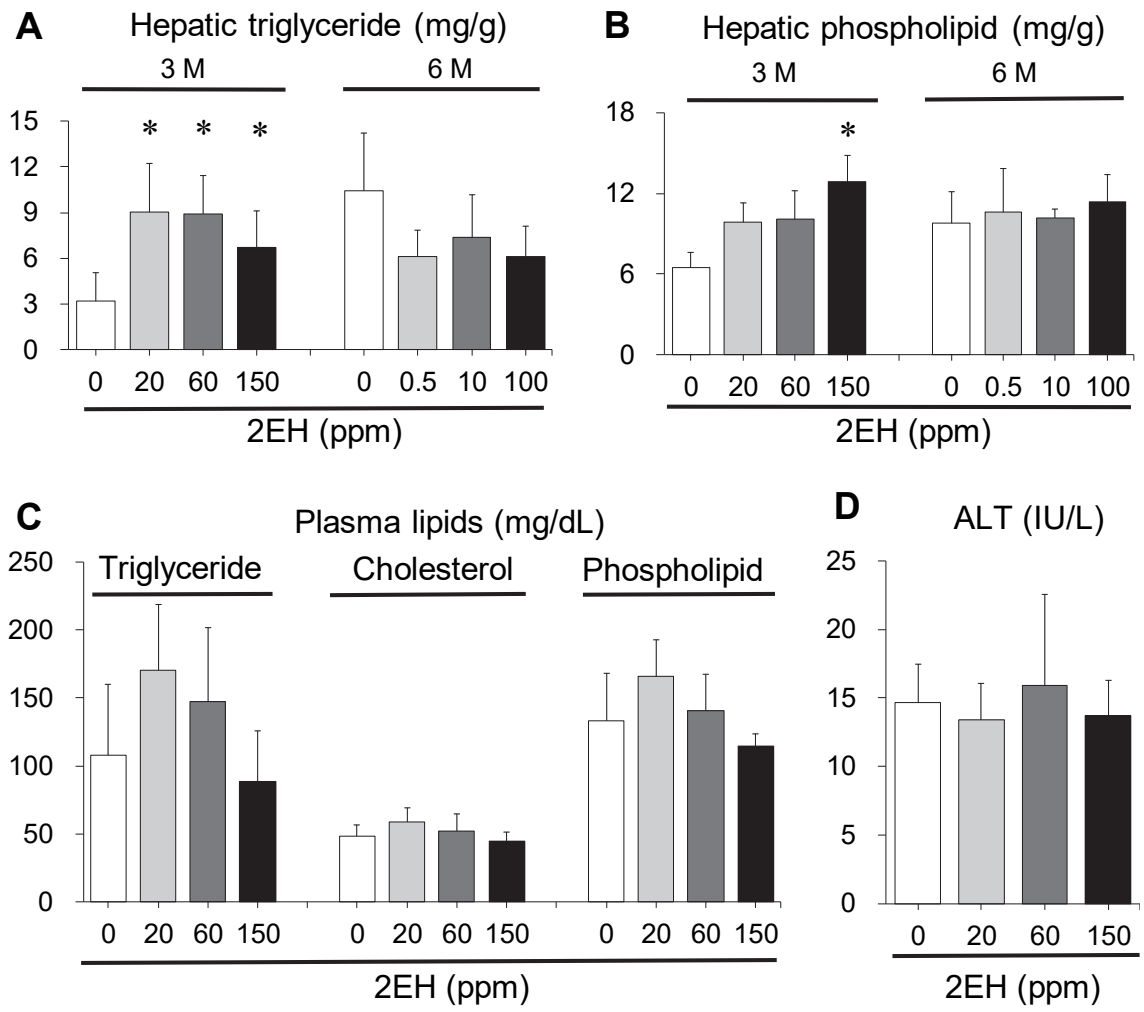

Fig. 3. Hepatic (mg/g) and plasma (mg/dL) lipid concentrations in mice exposed to 2EH.
(A) Hepatic triglycerides and (B) hepatic phospholipids in mice exposed to 2EH for 3 months (3 M) or 6 months (6 M), respectively. (C) Plasma triglycerides, cholesterol, and phospholipids in mice exposed to 2EH for 3 M. (D) Plasma ALT in mice exposed to 2EH for 3 M. The *asterisk* indicates statistical significance (*p<0.05). Data are presented as mean ± SD (n=6 for 100 ppm and n=7 each for the other groups). 2EH, 2-Ethyl-1-hexanol; ALT, alanine aminotransferase.

In the present study, a significant increase in relative liver weights was observed only in the 150 ppm group following a 3-month exposure, although plasma ALT and peroxisome proliferation from the standpoint of BP induction were not altered. This increase in relative liver weight without changes in body weight gain was similarly observed in male B6C3F1 mice orally administered 2EH at a dose of 500 mg/kg/day for 13 weeks[8]. Moreover, increased liver weights have been recorded in mice and rats after oral 2EH administration of 520[17] and 1,335 mg/kg[15] for 7 days, 700 mg/kg for 2 weeks[14], and 833[29], 950[28] and 1,000 mg/kg[13] for 3 weeks.

Hepatomegaly, including centrilobular hepatocellular hypertrophy, is often observed after treatment with PPARα agonists[26] via activation of PPARα, which is highly expressed in the liver[32]. PPARα is involved in lipid metabolism[33], reduces lipid accumulation[34], and lowers plasma triglyceride levels[35]. 2EH is metabolized to 2EHA[36], which acts as a PPARα agonist[12]. In rats, orally administered 2EH (950 mg/kg/day) and a 2% 2EH-containing diet (1,450 mg/kg/day) reportedly decreased triglyceride levels, quantified as Oil-Red-stained tissue areas[28], as well as plasma triglyceride levels[16, 27], respectively. Contrary to these previous reports, 2EH inhalation exposure at a maximum concentration of 150 ppm in mice increased hepatic lipid droplets in a dose-dependent manner and elevated hepatic triglyceride levels following a 3-month of exposure. The enzymatic degradation of 2EH (i.e., conversion from 2EH to 2EHA)[37] and hepatic PPARα-mRNA expression[38] were comparable between rats and mice. Therefore, it can be

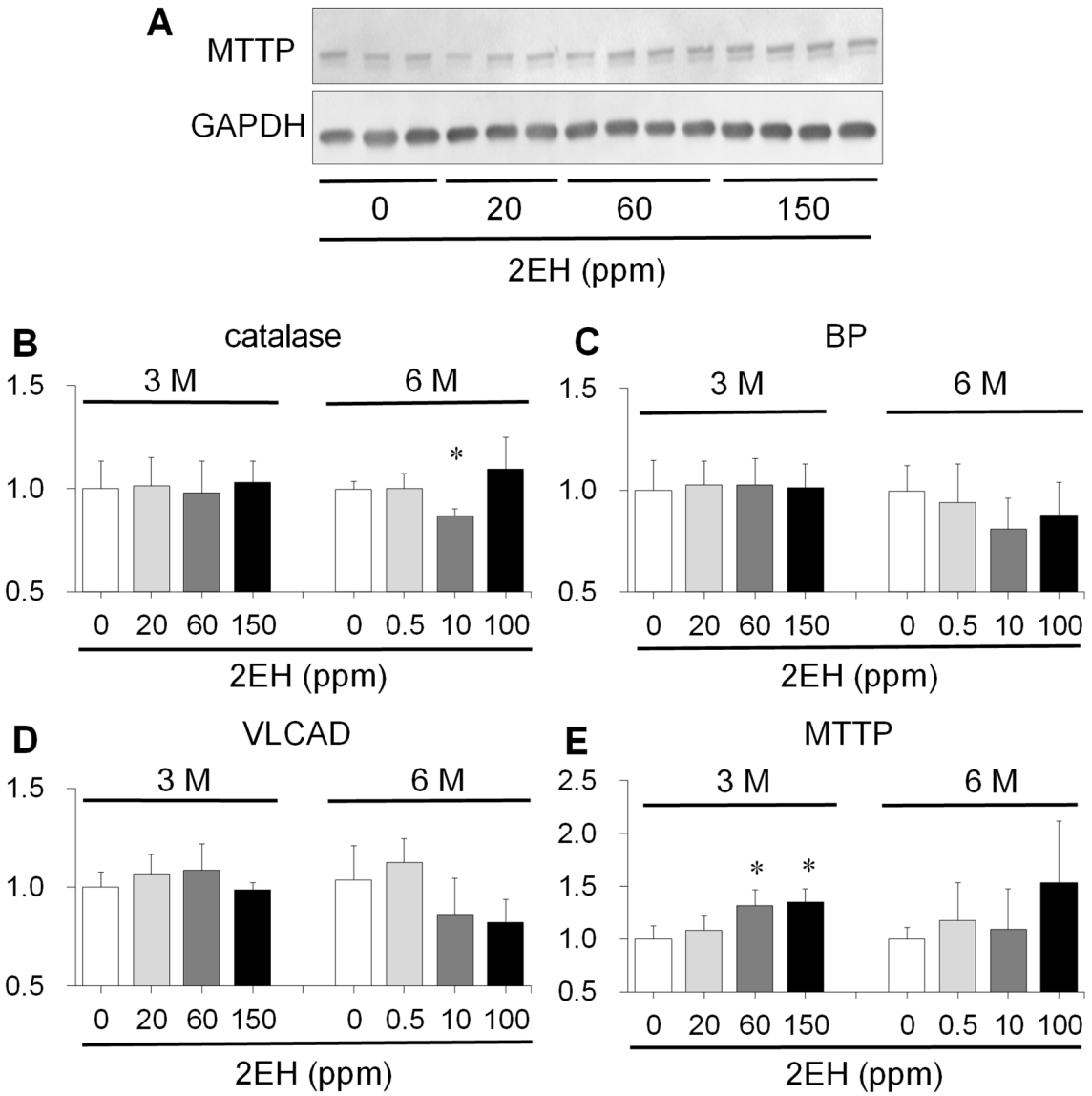

Fig. 4. Relative protein expression levels in the liver of mice exposed to 2EH for 3 months (3 M) or 6 months (6 M). (A) Representative bands of MTTP and GAPDH after western blot analysis in mice exposed to 2EH for 3 M. GAPDH was used as an internal staining standard. For each band, (B) catalase, (C) BP, (D) VLCAD, and (E) MTTP were quantified by densitometric analysis. The *asterisk* indicates statistical significance (*p<0.05) when compared with the respective control. Data are presented as mean ± SD (n=6 for 100 ppm and n=7 each for the other groups). 2EH, 2-Ethyl-1-hexanol; MTTP, microsomal triglyceride transfer protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; VLCAD, very long-chain acyl-CoA dehydrogenase; BP, peroxisomal bifunctional protein.

postulated that the contradictory effects of 2EH exposure can be attributed to the differences in administration routes and exposure levels rather than the species differences. In addition to increased hepatic triglyceride levels, 2EH up-regulated MTTP expression following a 3-month of exposure. Reportedly, activation of PPARα increases MTTP activity in mouse primary hepatocytes[39]. Based on these results, we conclude that 2EH exposure at the concentration range investigated in the present study has a limited effect on PPARα-related signaling. Indeed, the increased hepatic phospholipid levels observed in the present study corroborated with results obtained from a relatively lower dose of a PPARα agonist (dietary 0.5% DEHP for 10 days in rats)[40].

As the first stage of liver disease is characterized by lipid accumulation[41], more severe phenotypes, such as inflammation or fibrosis, may appear if exposure continues for prolonged periods. However, no changes were observed after 6 months. Lipid droplets observed in the subchronic test were transient, suggesting that inhalation exposure to

2EH did not induce histopathological damage to hepatocytes. To corroborate hepatic hypertrophy as toxicity, it is necessary to not only observe an increase in absolute and relative liver weights, but also record changes in hepatic inflammatory markers such as ALT or lipid metabolism[42]. In the present study, no changes in ALT and lipid metabolism markers were noted, although absolute and relative liver weights were increased in mice exposed to 100 ppm of 2EH for 6 months. Therefore, this appears to be an adaptive change to maintain homeostasis rather than an adverse effect. In recent years, it has been highlighted that subtle changes in the liver, rather than apparent liver damage, caused by VOC exposure are crucial for risk assessment from the perspective that a toxicant may initiate or exacerbate liver comorbidities such as nonalcoholic liver injury, which has not been emphasized until recently[41]. Thus, the toxicological significance of hepatic effects observed in the present study needs to be further investigated.

If route-specific toxicity data are unavailable for inhalation exposure, the concentration-related risk may be assessed by route-to-route extrapolation based on oral toxicity data. An adequate strategy for route extrapolation requires at least some basic toxicokinetic data. However, 2EH fails to meet the criteria for reliable route-to-route extrapolation[43]. Therefore, inhalation exposure experiments are indispensable for evaluating the risk of 2EH for establishing an indoor air standard value of 2EH. Inhalation exposure to 2EH for 6 months in the concentration range investigated in this study presented no adverse effects in the liver. However, negative data from long-term 2EH exposure to examine hepatic damage are crucial to distinguish the absence of observable adverse effects for overall toxicological endpoints.

In conclusion, inhalation of 2EH in mice induced histological changes and elevated lipid levels in the liver during the subchronic test, but these changes were not observed in the chronic test. Hepatomegaly was observed in both experiments; however, these effects were presumably independent of a PPARα-mediated mechanism. Hepatocyte hypertrophy observed in the 2EH concentration range investigated in the present study did not meet the criteria for considering liver hypertrophy as toxicity, as no accompanying changes were identified as adverse effects. Therefore, an indoor air standard value for 2EH should be set based on other toxicological endpoints rather than hepatotoxicity.

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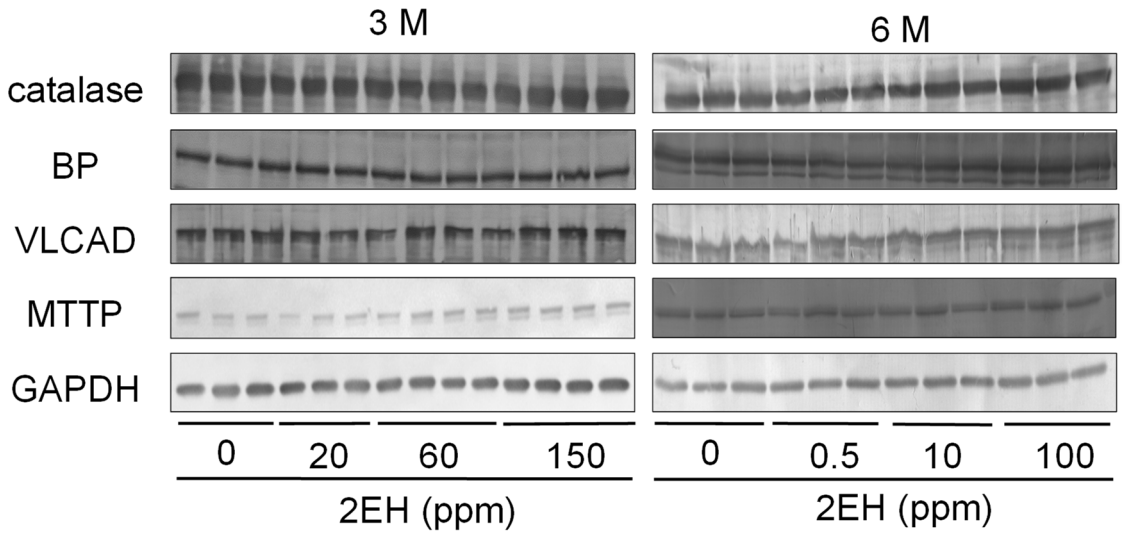

Supplemental Fig. 1. Protein expression in the liver of mice exposed to 2EH for 3 months (3 M) or 6 months (6 M). Representative bands of catalase, BP, VLCAD, MTTP, and GAPDH after western blot analysis in mice exposed to 2EH for 3 M and 6 M. 2EH, 2-Ethyl-1-hexanol; MTTP, microsomal triglyceride transfer protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; VLCAD, very long-chain acyl-CoA dehydrogenase; BP, peroxisomal bifunctional protein.