Promoting effects of xylazine on development of thyroid tumors in rats initiated with N-bis(2-hydroxypropyl)nitrosamine and the mechanism of action

Kazuo Yasuhara1,5, Takatoshi Koujiti1, Kiyoshi Takegawa2, Masahiro Nasu3, Hiroshi Onodera1, Hisayoshi Takagi4, Masao Hirose1 and Kunitoshi Mitsumori1,4

1Division of Pathology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan, 2Safety Evaluation, Pharmaceutical Research Division, WelFide Corporation, 214-1 Yamasaki, Fukusaki-cho, Kanzaki-gun, Hyogo 679-2296, Japan, 3Panapharm Laboratories Co., Ltd, Kurisaki-machi, Uto, Kumamoto 869-0425, Japan and 4Laboratory of Veterinary Pathology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

To cast light on whether xylazine hydrochloride (XZ), a veterinary medicine commonly used as a sedative agent for food-producing animals, has any promoting potential for thyroid carcinogenesis, the following studies were performed. In Experiment I, male F344 rats received a diet containing 1000 or 0 p.p.m. XZ for 52 weeks with or without initiation with 2400 mg/kg N-bis(2-hydroxypropyl)nitrosamine (DHPN). Focal follicular cell hyperplasias, adenomas and/or carcinomas were induced in the DHPN alone, XZ alone and DHPN + XZ groups, and the incidences and multiplicities of these lesions in the DHPN + XZ group were significantly increased as compared with the DHPN alone case. In Experiment II, male F344 rats received a diet containing 1000 or 0 p.p.m. XZ and were examined for serum levels of triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH) at weeks 1, 2 and 4. In the XZ group, significant increase in thyroid weight and decrease in serum T3 levels were observed at all time points. Serum T3 and TSH levels were significantly decreased and increased, respectively, at week 1, but returned to within the control range thereafter. In Experiment III, male F344 rats received a diet containing 1000 or 0 p.p.m. XZ, they were examined for thyroid iodine uptake and organification of XZ after 1 and 2 weeks. The thyroidal iodine uptake per milligram of thyroid and the amount of iodine bound to 1 mg protein showed a tendency for decrease at week 1 and significant decrease at week 2. These results indicate that XZ has tumor-promoting effects on thyroid follicular cells, and suggest an involvement of alterations in thyroid-related hormone levels due to inhibition of thyroid iodine uptake and organification, resulting, provably, in serum TSH stimulation depending on continuous reduction of serum T4 level through the feedback system in the pituitary–thyroid axis.

Introduction

Xylazine hydrochloride (XZ) is an α2-adrenergic agonist with sedative, muscle relaxant and analgesic properties and is commonly used in veterinary medicine (1,2), not only therapeutically but also to reduce aggressiveness associated with livestock bleeding. One particular application is for treatment of food-producing animals during transport to slaughterhouses to avoid death and loss of meat quality caused by stress reactions (3). XZ itself has been reported to be weakly mutagenic in Ames tests, but no XZ-related pathological change was found in Wistar rats given a diet containing 500 p.p.m. XZ for 32 weeks (4,5). Carcinogenicity studies have not yet been performed but 2,6-dimethylaniline (DMA), one of the major metabolites of XZ, has been shown to cause a significant increase in the incidence of nasal carcinomas in both male and female rats fed a diet containing 3000 p.p.m. DMA for 2 years (6). In addition, DMA proved to induce sister chromatid exchange and to be mutagenic in a gene mutation test using mouse lymphoma cells, although not in the Ames test (7–10). Based on these data, there is some concern about carcinogenic effects of DMA on consumers via ingestion of edible tissues in food-producing animals treated with xylazine (11). The 47th Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECCFA) was unable to establish an acceptable daily intake (ADI) for XZ, because it was concluded that DMA, its metabolite, was carcinogenic (5).

In our previous two-stage nasal carcinogenesis study of DMA and XZ in rats using N-bis(2-hydroxypropyl)nitrosamine (DHPN) as an initiator, it was demonstrated that DMA has tumor-promoting activity in the rat nasal cavity (12), but that this is not the case for XZ, even at a dose of 1000 p.p.m. (13). However diffuse follicular cell hypertrophy of thyroid glands was observed in rats administered 1000 p.p.m. XZ for 28 days (14). Since it is well known that thyroid follicular cell hypertrophy is produced by continuous serum stimulation of thyroid-stimulating hormone (TSH) (15–17), the possibility arises that XZ has tumor-promoting potential in the rat thyroid.

The present studies were performed to examine this question using a two-stage carcinogenesis model in rats initiated with DHPN. As a result, thyroid tumors were induced in XZ-treated rats. Therefore, further studies were conducted to clarify the mechanism of action of XZ.

Materials and methods

Chemicals

XZ, 2-(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine hydrochloride, was purchased from Sigma Chemical Co. (St Louis, MO) (Figure 1). It was a 99% pure, white crystalline powder, freely soluble in water. DHPN was purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Animals and housing conditions

Male F344 rats, 4 or 5 weeks old, were purchased from Charles River Japan Inc. (Kanagawa, Japan). They were housed, three to five animals per cage, in polycarbonate cages with wood chips for bedding, in an air-conditioned barrier system animal room (room temperature 23 ± 2°C; relative humidity 55 ± 5%; air changes, 18 times/h; a 12 h/12 h light/dark cycle). The rats were first quarantined for 7 days in the animal room assigned for the study and only those without any abnormal findings were selected for the experiment. Basal
Based on the results of Experiment I, 42 6-week-old male rats were divided into two groups. The initial mean body weights of each group were approximately equal. Twenty-four rats (XZ group) were fed a pulverized diet containing 1000 p.p.m. of XZ, as described in Experiment I, for 4 weeks. Eighteen rats were fed a pulverized basal diet alone as the control group. At weeks 1, 2 and 4 after the start of experiment, blood was collected from the abdominal aorta of eight rats each from the XZ group and six rats each from the control group under ether anesthesia for hormone assays. Serum triiodothyronine (T3), thyroxine (T4) and TSH were measured by radioimmunoassay kit, using a Coat-A-Count Canine T3 Kit (Diagnostic Products Co., CA), DPC Total T4 Kit (Diagnostic Products Co., CA) and Rat Thyroid Stimulating Hormone (rTSH) [125I] assay system (Amersham Pharmacia Biotech, UK), respectively. After extraction, thyroid glands, pituitary glands and livers were removed from all animals of each group and weighed.

Experiment III: measurement of thyroidal iodine uptake and organification
Forty 6-week-old male rats were divided into four groups, each consisting of 10 rats, to determine iodine uptake and iodination of tyrosine residues in thyroglobulin in the thyroid gland. The initial mean body weights of each group were approximately equal. Two groups received a pulverized diet containing 1000 p.p.m. of XZ, as described in Experiment I, and the remaining two groups received a pulverized basal diet alone as the control group for 8 cell adenomas were characterized by expansive growth of follicular cells with basophilic cytoplasm and bizarre hyperchromatic nuclei with frequent mitotic

Experiment I: two-stage thyroid carcinogenesis study
A total of 60 5-week-old rats were randomly divided into a DHPN-initiated group of 40 and a non-initiated group of 20 animals. These were given a single subcutaneous injection of 2400 mg/kg of DHPN in physiological saline as vehicle and the vehicle alone, respectively. One week after this initiation treatment, each group of animals was subdivided into two equal groups, receiving no treatment (DHPN alone and control groups) or a pulverized diet containing 1000 p.p.m. XZ for 52 weeks (DHPN+XZ, XZ alone groups). The dose of XZ in this experiment was selected based on the results of our previous short-term study, where an increased incidence of follicular cell hypertrophy was seen in rats given a diet containing 1000 p.p.m. XZ for 28 days (14). The XZ containing diet was prepared either weekly or fortnightly and stored in a refrigerator (temperature, 4°C) before use; the adventur is stable at room temperature for only 2 weeks (14). Body weights and food consumption for each group were measured every week during the first 4 weeks and once every 2–6 weeks thereafter. The mean actual intake of XZ was calculated from the mean body weights and mean food consumption. At week 53 after the start, all surviving animals were killed under ether anesthesia for hormone assays. Serum thyroid hormone concentrations were determined by radioimmunoassay using 125I-labeled hormones. Values were calculated as percentages of the total radioactivity of the injected Na125I.

Statistical analysis
Data for body weights and organ weights, multiplicity of thyroid proliferative lesions, serum thyroid-related hormones and thyroid iodine uptake and protein binding iodine are given as mean and standard deviation (SD), and intergroup differences were analyzed using the Student’s t-test. In addition, data for incidences of thyroid proliferative lesions were analyzed using the Fisher’s exact test.

Results

Experiment I
There were no significant differences in body weight and food consumption between the DHPN-alone and DHPN+XZ groups, and between the untreated control and XZ-alone groups (data not shown). Mean actual intakes of XZ in the DHPN+XZ and XZ-alone groups were 56.2 and 53.5 mg/kg/day, respectively.

Histopathologically, focal follicular cell hyperplasias, adenomas and/or carcinomas in the thyroid gland were observed in the DHPN-alone, XZ-alone and DHPN+XZ groups. Focal follicular cell hyperplasias were characterized by one or several cystic or dilated follicles with increased amounts of colloid, lined by low cuboidal or flattened epithelia with hyperchromatic nuclei. Epithelial cells occasionally formed papillary structures with projection into the follicular lumen (Figure 2). Follicular cell adenomas were characterized by expansive growth of follicular cells with basophilic cytoplasm and hyperchromatic nuclei with slight atypia, easily distinguishable from the adjacent surrounding tissue (Figure 3). Carcinomas were characterized by follicular, papillary and/or solid growth of pleomorphic tumor cells with basophilic cytoplasm and bizarre and/or hyperchromatic nuclei with frequent mitotic figures and invasive growth into the surrounding capsule or adjacent tissues (Figure 4). Furthermore, diffuse follicular cell hypertrophy was observed as a non-focal lesion in the XZ alone and DHPN+XZ groups.

Data for incidence and multiplicity of follicular cell proliferative lesions in each group are summarized in Table I. The incidence and multiplicity of focal follicular cell hyperplasias

![Chemical structure of xylazine hydrochloride.](image)

![Follicular cell hyperplasia in the thyroid gland of a rat treated with 1000 p.p.m. XZ for 52 weeks after DHPN initiation, showing cystic growth of follicles with increased amount of colloid. H-E staining (×150 magnification).](image)
were 40% and 0.60 ± 0.88 in the DHPN-alone group, 20% and 0.50 ± 1.08 in the XZ-alone group, and 100% and 5.95 ± 1.67 in the DHPN+XZ group, respectively. Those for adenomas were 10% and 0.10 ± 0.32 in the XZ-alone group, and 60% and 0.80 ± 0.77 in the DHPN+XZ group, respectively. Carcinomas were only observed in the DHPN-alone group. There were no significant inter-group differences in the pituitary weights at any time point.

Serum T₃, T₄ and TSH concentrations at weeks 1, 2 and 4 are also shown in Table III. Significant decreases in serum T₃ and T₄ and increases in serum TSH levels were observed at week 1 in the XZ group as compared with the control group. The serum T₃ and TSH levels in the XZ group had recovered by week 2, but the serum T₄ level demonstrated a persistent decrease at weeks 2 and 4.

**Experiment III**

Thyroid weight and values for iodine uptake and protein binding iodine are shown in Table IV. Thyroid weights in the XZ group were significantly increased as compared with the corresponding control value at all time points. The thyroidal iodine uptake per milligram of thyroid and the amount of iodine bound to 1 mg protein in the XZ group showed a tendency to decrease at week 1 and a significant reduction at week 2 as compared with the corresponding control values.

**Discussion**

It is well known that DHPN has carcinogenic potential in various organs, including the thyroid gland of rats (19–22). It has therefore been used as an initiator for two-stage thyroid carcinogenesis studies, with high incidences of thyroid follicular cell tumors developing in goitrogen-treated rats (23–26). In Experiment I in this study, follicular cell hyperplasias were observed in the DHPN-alone, XZ-alone and DHPN+XZ groups, and adenomas were observed in the XZ-alone and DHPN+XZ groups. Carcinomas were only observed in the DHPN+XZ group. These proliferative lesions were most marked in the DHPN+XZ group. The results strongly suggest that XZ has tumor promotion effects on the rat thyroid gland. Only weak positive results were obtained in *S. typhimurium*.

<table>
<thead>
<tr>
<th>Group¹</th>
<th>Control (10)</th>
<th>DHPN (20)</th>
<th>XZ (10)</th>
<th>DHPN + XZ (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular cell hyperplasia</td>
<td>0</td>
<td>40b</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Follicular cell adenoma</td>
<td>0</td>
<td>0.60 ± 0.88d</td>
<td>0.50 ± 1.08</td>
<td>5.95 ± 1.67c</td>
</tr>
<tr>
<td>Follicular cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0.10 ± 0.32</td>
<td>0.80 ± 0.77</td>
</tr>
<tr>
<td>Follicular cell adenoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25t</td>
</tr>
<tr>
<td>Follicular cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.40 ± 0.82</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0</td>
<td>40b</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>0</td>
<td>0</td>
<td>0.10 ± 0.32</td>
<td>1.20 ± 1.36</td>
</tr>
</tbody>
</table>

¹Number of animals examined is given in parentheses.
²Incidence of proliferative lesions (%).
³Significantly different from the DHPN group using Fisher’s exact test at P < 0.01.
⁴Mean number of proliferative lesions ± SD/rat.
⁵Significantly different from the DHPN and with the Student’s t-test at P < 0.01.
⁶Significantly different from the DHPN group using Fisher’s exact test at P < 0.05.
⁷Follicular cell adenomas and carcinomas combined.
Table II. Body and organ weights for rats fed a diet containing 1000 p.p.m. XZ for 4 weeks.

<table>
<thead>
<tr>
<th>Exp. week</th>
<th>Group</th>
<th>No. animals</th>
<th>Body weight</th>
<th>Thyroid</th>
<th>Pituitary</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>absolute (mg)</td>
<td>relative (mg%)</td>
<td>absolute (mg)</td>
<td>relative (mg%)</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>6</td>
<td>152.2 ± 10.7b</td>
<td>10.0 ± 1.4</td>
<td>6.6 ± 1.1</td>
<td>7.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>8</td>
<td>141.8 ± 11.5</td>
<td>16.6 ± 4.6c</td>
<td>11.6 ± 2.5c</td>
<td>6.1 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>6</td>
<td>178.4 ± 13.7</td>
<td>12.3 ± 2.0</td>
<td>6.9 ± 0.8</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>8</td>
<td>168.5 ± 7.9</td>
<td>17.5 ± 2.9d</td>
<td>10.4 ± 1.6e</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>6</td>
<td>225.0 ± 15.9</td>
<td>14.5 ± 1.2</td>
<td>6.5 ± 0.6</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>8</td>
<td>215.6 ± 9.2</td>
<td>20.5 ± 2.7c</td>
<td>9.5 ± 1.1c</td>
<td>7.5 ± 0.5</td>
</tr>
</tbody>
</table>

aThe ratio of organ weight to body weight.

bSignificantly different from the control group at P < 0.01.
cSignificantly different from the control group at P < 0.05.

Table III. Serum levels of T3, T4 and TSH for rats fed a diet containing 1000 p.p.m. XZ for 4 weeks

<table>
<thead>
<tr>
<th>Exp. Week</th>
<th>Group</th>
<th>No. animals</th>
<th>Serum levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T3 (ng/ml)</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>6</td>
<td>1.54 ± 0.15a</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>8</td>
<td>1.24 ± 0.15b</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>6</td>
<td>1.43 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>8</td>
<td>1.37 ± 0.20</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>6</td>
<td>1.44 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>8</td>
<td>1.41 ± 0.22</td>
</tr>
</tbody>
</table>

Table IV. Thyroidal iodine uptake and organification in rats fed a diet containing 1000 p.p.m. XZ at weeks 1 and 2

<table>
<thead>
<tr>
<th>Exp. week</th>
<th>Group</th>
<th>No. animals</th>
<th>Thyroid (mg)</th>
<th>Uptake/tissue (%125I/mg thyroid)</th>
<th>PBI/protein (%125I/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>10</td>
<td>8.8 ± 1.2c</td>
<td>0.64 ± 0.13</td>
<td>3.37 ± 0.75</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>10</td>
<td>22.4 ± 5.8c</td>
<td>0.48 ± 0.20</td>
<td>2.67 ± 0.91</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>10</td>
<td>10.2 ± 0.8</td>
<td>0.57 ± 0.09</td>
<td>2.91 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>10</td>
<td>19.5 ± 2.8c</td>
<td>0.18 ± 0.06c</td>
<td>0.98 ± 0.33c</td>
</tr>
</tbody>
</table>

Protein binding iodine.

bMean ± SD.

cSignificantly different from the control group at P < 0.01.

K.Yasuhara et al.

Table II. Body and organ weights for rats fed a diet containing 1000 p.p.m. XZ for 4 weeks.

Factors can contribute to decrease of serum thyroid hormone levels. For example, inhibition of iodine transport into the thyroid, thyroid oxidation, iodine organification or coupling catalyzed by thyroid peroxidase, or hormone inactivation by hepatic microsomal enzymes such as T3-uridine diphosphate glucuronosyltransferase (T4-UDP-GT) can all cause reduction of serum T3 or T4 levels (16,17). When XZ was labeled with 35S and 14C on the thiazine ring and administered to rats orally, the compound was found to be completely absorbed and ~70 and 30% of the administered dose was eliminated in the urine and faeces, respectively (4). Furthermore, after intravenous administration of labeled XZ, it was reported to be distributed within a few minutes to almost all organs, especially the kidneys and central nervous system, with a relatively high concentration in the thyroid gland (4). XZ is metabolized to approximately 20 metabolites, and ~8% of the dose was eliminated in the urine as an unchanged compound 24 h after intravenous administration (4). If XZ were to induce microsomal enzymes in the liver, a relatively high radioactivity concentration of XZ would be expected in the liver. The fact that this was not the case argues against UDP-GT participation in the reduction of serum T4 levels in rats treated with XZ.

When XZ was incubated with rat liver microsomes in vitro, four metabolites were identified, the most major being N-(2,6-dimethylphenyl)-thiourea (27). Chemicals exhibiting goitrogenic activity can be divided into three major groups in terms of structure: thionamides, aromatic amines and polyhydric phenols. Thionamides include derivatives of thiourea and heterocyclic compounds, which exert at least part of their activity by directly interfering with the synthesis of thyroid hormones in the thyroid gland (17). Thiourea has been reported to cause thyroid follicular cell tumors by the inhibition of thyroidal iodine uptake and organification followed by decrease of serum thyroid hormone levels and the increased stimulation of follicular cells by the ensuing elevation of serum TSH (17). Therefore, the inhibition of thyroidal iodine uptake and organification observed in the rats treated with XZ is considered to be due to the thiourea derivatives that are produced during the metabolism of XZ.

In Experiment II in the present study, in spite of the fact that the serum T4 levels in the XZ group were decreased significantly as compared with the control group at all time points, serum T3 and TSH levels were within the control range at weeks 2 and 4. On the other hand, thyroidal iodine uptake per milligram of thyroid and the amount of iodine bound to 1 mg protein in the XZ group showed significant decreases at

strains TA1535 and TA1538 for XZ, and the compound was negative for mutagenicity in a forward mutation assay with cultured mammalian cells and in a mouse bone marrow micronucleus test (4). Based on these data, it can be concluded that XZ, per se does not have genotoxic potential in vitro. Therefore, the incidence of thyroid follicular cell hyperplasia in two rats and an adenoma in one rat might be attributable to spontaneous mutations in follicular cells by continuous TSH stimulation during long-term treatment of XZ.

It is generally understood that exposure of the rat thyroid gland to sustained, elevated levels of serum TSH can lead to progression through follicular cell hypertrophy and hyperplasia, eventually to neoplasia (15). Increases in serum TSH levels can result from a reduction in levels of circulating thyroid hormones (T3, T4) though the feedback control of the hypothalamus and pituitary gland (16,17). A number of important
week 2. The question thus arises as to why the serum TSH level was not increased at weeks 2 and 4. Shimo et al. (25) reported time course changes in serum levels of T3, T4, and TSH in male F344 rats administered DHPN followed by 0.1% thiourea at weeks 1, 2, 4, 8, 12 and 16. The serum T4 level was markedly decreased at week 1 and remained significantly lowered throughout the experiment. Serum TSH levels were elevated up to a peak at around week 4 with a return to the normal range at week 12. However, thyroid hypertrophy was present during the treatment period. Okuno et al., (28) examined the serum hormone levels of T3, T4 and TSH, and thyroid iodine uptake and organification in rats administered 0.1% thiourea in drinking water for 4 weeks. Despite the significant decreases in serum T4 levels and decreased thyroidal iodine uptake and organification, the serum TSH levels were only slightly increased without statistical significance. In addition, similar phenomena, such as marked increase in serum TSH level during the early period and reduction to toward normal level thereafter, have also been reported in some of goitrogens such as 2,4-diaminoanisole sulfate (29) and T4-UDP-GT-inducer phenobarbital (30). This variation in serum thyroid-related hormone levels in rats treated with these goitrogens resembles that in our present studies, although thyroid weights of the same group were continuously increased and thyroid follicular cells showed diffuse hypertrophy at week 4 (data not shown). These data suggest that serum TSH levels are not always significantly increased throughout periods of treatment with goitrogens and thyroid follicular cells exposed to such goitrogens can proliferate in response to very small amounts of serum TSH even after the time when serum TSH levels returned to the normal range.

Based on the results of the present studies, 1000 p.p.m. XZ exerts tumor-promoting effects on thyroid follicular cells, involving alteration of thyroid-related hormone levels due to inhibition of thyroid iodine uptake and organification, resulting in provable serum TSH stimulation depending on continuous reduction of serum T4 levels through the negative feedback system in the pituitary–thyroid axis. Mean actual intake of XZ in the groups treated with 1000 p.p.m. XZ in the present study, in which the thyroid tumor-promoting effect was observed, was 53.5–56.2 mg/kg/day, while it has been reported that no XZ-related pathological change was observed in the thyroid of Wistar rats treated with 500 p.p.m. XZ for 32 weeks (4). On the other hand, the actual dose of XZ for application as a veterinary medicine is 0.03–0.02 mg/kg in cattle and 2–3 mg/kg in swine that were administered intramuscularly or intravenously in combination with another anesthetic agents (2). The data from residue-depletion studies in cattle using unlabeled XZ showed that residues were below the limit of detection (0.01 mg/kg) of the analytical method in muscle, kidney, liver or fat (4). Therefore, the possibility of thyroid tumor-promoting effects of XZ to consumers via ingestion of edible tissues from food-producing animals treated with XZ is extremely low, since consumers seldom ingest such a high concentration of XZ or its metabolites from edible tissues. Additional studies of XZ using the DHPN initiated two-stage thyroid carcinogenesis model in rats are now in progress to clarify the threshold dose of thyroid tumor-promoting effect on this chemical.

Acknowledgement

This study was supported in part by a Grant-in-Aid for Food Hygiene from the Ministry of Health and Welfare of Japan.

References


Received August 30, 2000; revised December 18, 2000; accepted December 19, 2000