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# Absorption, Tissue Distribution, Excretion, and Metabolism of Clothianidin in Rats

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Absorption, distribution, excretion, and metabolism of clothianidin [(*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] were investigated after a single oral administration of [*nitroimino*-$^{14}$C]- or [*thiazolyl*-2-$^{14}$C]clothianidin to male and female rats at a dose of 5 mg/kg of body weight (bw) (low dose) or 250 mg/kg of bw (high dose). The maximum concentration of carbon-14 in blood occurred 2 h after administration of the low oral dose for both labeled clothianidins, and then the concentration of carbon-14 in blood decreased with a half-life of 2.9−4.0 h. The orally administered carbon-14 was rapidly and extensively distributed to all tissues and organs within 2 h after administration, especially to the kidney and liver, but was rapidly and almost completely eliminated from all tissues and organs with no evidence of accumulation. The orally administered carbon-14 was almost completely excreted into urine and feces within 2 days after administration, and ~90% of the administered dose was excreted via urine. The major compound in excreta was clothianidin, accounting for >60% of the administered dose. The major metabolic reactions of clothianidin in rats were oxidative demethylation to form *N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine and the cleavage of the carbon−nitrogen bond between the thiazolylmethyl moiety and the nitroguanidine moiety. The part of the molecule containing the nitroguanidine moiety was transformed mainly to *N*-methyl-*N'*-nitroguanidine, whereas the thiazol moiety was further metabolized to 2-(methylthio)thiazole-5-carboxylic acid. With the exception of the transiently delayed excretion of carbon-14 at the high-dose level, the rates of biokinetics, excretion, distribution, and metabolism of clothianidin were not markedly influenced by dose level and sex.

**KEYWORDS: Clothianidin; absorption; excretion; distribution; metabolism; neonicotinoid; rat**

## INTRODUCTION

Clothianidin [(*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] (commercial names, Dantop and Poncho) (**Figure 1**) is a novel, highly effective systemic and contact insecticide exhibiting low mammalian toxicity (*1*−*3*). Clothianidin is active on hemipteran pest species in particular, such as aphids, leafhoppers, and planthoppers, and also on many coleopteran and some lepidopteran pest species with a low application rate and has been commercialized to control these pests (*4*, *5*). From the physicochemical properties and excellent root systemic properties, clothianidin can be used for a wide range of application techniques, including foliar, seed treatment, soil drench, and soil application. Clothianidin is one of the latest members of the neonicotinoid insecticides (*6*, *7*). As do all other neonicotinoid insecticides, clothianidin also acts as an agonist at the nicotinic acetylcholine receptors (nAChR) located in the central nervous system at much lower concentration in insects than in mammals (*4*, *8*, *9*). This target site selectivity is a major factor in the favorable toxicological properties of the neonicotinoids.

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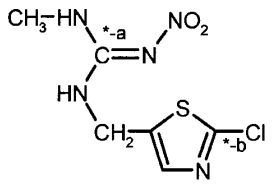


**Figure 1.** Chemical structure of clothianidin. Asterisks indicate the position of carbon-14 label: a, [*nitroimino*-$^{14}$C]clothianidin; b, [*thiazolyl*-2-$^{14}$C]-clothianidin.

The present paper deals with the absorption, tissue distribution, excretion, and metabolism of clothianidin in rats carried out using clothianidin labeled with carbon-14 at the nitroimino moiety ([*nitroimino*-$^{14}$C]clothianidin) or the thiazolyl ring ([*thiazolyl*-2-$^{14}$C]clothianidin).

## MATERIALS AND METHODS

**Chemicals.** [*nitroimino*-$^{14}$C]Clothianidin and [*thiazolyl*-2-$^{14}$C]-clothianidin were synthesized by Nemoto Science Co., Ltd. [*nitroimino*-$^{14}$C]Clothianidin was labeled with carbon-14 at the nitroimino moiety with a specific radioactivity of 2.06 GBq/mmol. [*thiazolyl*-2-$^{14}$C]-

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**Table 1.** TLC $R_f$ Values and Retention Times on HPLC of Authentic Standards

| | TLC | | | | HPLC | |
|---|---|---|---|---|---|---|
| | $R_f$ value with solvent system[a] | | | | | |
| compound | A | B | C | D | mobile phase[b] | retention time (min) |
| clothianidin | 0.56 | 0.59 | –[c] | – | I | 28.1 |
| TZMU | 0.54 | 0.50 | – | – | I | 19.0 |
| TZNG | 0.50 | 0.56 | – | – | I | 24.4 |
| MNG | 0.31 | 0.39 | – | – | II | 5.2 |
| TZU | 0.31 | 0.33 | – | – | | – |
| NTG | 0.15 | 0.29 | – | – | | – |
| MTCA | 0.00 | 0.00 | 0.22 | 0.72 | I | 6.5 |
| TMG | 0.00 | 0.00 | 0.20 | 0.40 | I | 10.8 |
| MG | 0.00 | 0.00 | 0.10 | 0.29 | | – |

[a] Solvent system: (A) chloroform/methanol (8:1, v/v); (B) chloroform/acetone (1:1, v/v); (C) chloroform/methanol (7:3, v/v); (D) chloroform/acetone/methanol/formic acid (2:2:1:1, v/v/v/v). [b] Mobile phase: (I) 10 min isocratic with 5% MeCN/95% 0.05 M $NH_4Cl$ followed by a 10 min linear gradient to 15% MeCN/85% 0.05 M $NH_4Cl$ (final mobile phase composition maintained for 20 min); (II) isocratic with $H_2O$ (20 min). [c] –, not determined.

Clothianidin was labeled at the 2-position of the thiazolyl ring with a specific radioactivity of 2.21 GBq/mmol (**Figure 1**). These chemicals had a radiochemical purity of >99.5% as determined by HPLC analysis.

The following authentic standards (unlabeled compound) were prepared in our laboratories: clothianidin, 2-chlorothiazole-5-carboxylic acid (CTCA), *N*-methyl-*N*′-nitroguanidine (MNG), 2-(methylthio)-thiazole-5-carboxylic acid (MTCA), *N*-(2-chlorothiazol-5-ylmethyl)-*N*′-methylguanidine (TMG), *N*-(2-chlorothiazol-5-ylmethyl)-*N*′-methylurea (TZMU), *N*-(2-chlorothiazol-5-ylmethyl)-*N*′-nitroguanidine (TZNG), and 2-chlorothiazol-5-ylmethylurea (TZU). Methylguanidine (MG) and nitroguanidine (NTG) were purchased from Tokyo Kasei Kogyo Co., Ltd. All other chemicals were of reagent grade unless otherwise noted in this paper.

**Radioanalysis.** Radioactivity in urine, plasma, trapping solution for expired air, solvent extracts, and silica gel regions scraped off from TLC plates was quantified by liquid scintillation counting (LSC) with an LS-6000TA (Beckman). Radioactivity in fecal homogenates, whole blood, unextractable residue, tissues, and organs was quantified by combustion in an oxidizer (307 sample oxidizer, Packard). The liberated $CO_2$ was absorbed in a cocktail (Carbo-sorb E, Packard BioScience) and measured by LSC. The recovery of carbon-14 was ≥95% for combustion.

**Thin-Layer Chromatography (TLC).** TLC analysis was carried out with precoated silica gel 60 $F_{254}$ chromatoplates (20 × 20 cm, 0.25-mm layer thickness, Merck, Darmstadt, Germany). The $R_f$ values for authentic standards and solvent systems used are shown in **Table 1**. The developed TLC plates were exposed to imaging plates, which were scanned by a Bio-Imaging Analyzer (BAS2000, Fuji Photo Film Co., Ltd.) to produce the autoradiogram (TLC-ARG). The TLC spots of the reference compounds were visualized either by UV light or by iodine vapor.

**High-Performance Liquid Chromatography (HPLC).** HPLC was performed with a system composed of an SCL-6B system controller (Shimadzu), an LC-6A pump (Shimadzu), an SPD-6A ultraviolet detector (Shimadzu) set at 254 nm, and a model 171 radioisotope detector (Beckman). Analytical conditions were as follows: column, Develosil ODS-HG-5 (5 μm, 4.6 mm i.d. × 150 mm; Nomura Chemical Co., Ltd.); guard column [LiChrospher100 RP-18(e); Merck]; flow rate, 1 mL/min; mobile phase, (I) 10 min isocratic with 5% acetonitrile/95% 0.05 M ammonium chloride followed by a 10 min linear gradient to 15% acetonitrile/85% 0.05 M ammonium chloride (final mobile phase composition maintained for 20 min), (II) 20 min isocratic with $H_2O$. The retention times of authentic standards are listed in **Table 1**.

**Animals and Dosage Form.** Wistar male and female rats were purchased at 7 weeks of age from Clea Japan, Inc., and acclimatized for 1 week before the start of the study. The animals were given pelleted diet (MF, Oriental Yeast Co., Ltd.) and water ad libitum and housed under conditions that were controlled at all times: temperature, 23 ± 2 °C; light, 12 h light/dark cycle. For the oral administration, [*nitroimino*-$^{14}$C] or [*thiazolyl*-2-$^{14}$C]clothianidin ([$^{14}$C]clothianidin) was dissolved or suspended in corn oil at a concentration of 5 (low dose) or 250 (high dose) mg/2.5 mL and administered orally to rats at a dose of 2.5 mL/kg of body weight (bw) using a syringe equipped with a gastric probe (1 mm in diameter and 100 mm in length). For the intravenous administration, [$^{14}$C]clothianidin was dissolved in dimethylformamide at a concentration of 5 mg/mL and intravenously injected in the femoral region using a 27G needle at a dose of 1 mL/kg of bw.

**Blood and Plasma Concentration of Radioactivity.** After oral or intravenous administration of [$^{14}$C]clothianidin to groups of rats each consisting of three males and three females at a low dose, blood samples were taken from the tail vein 0.25, 0.50, 0.75, 1, 2, 4, 6, 10, 24, and 48 h after administration. Each blood sample was collected in a heparinized tube, and the plasma was separated by centrifugation at 10000 rpm for 5 min.

**Tissue and Organ Distribution.** After oral administration of [$^{14}$C]-clothianidin to groups of rats each consisting of three males and three females at a low or high dose, the rats of each sex were anesthetized with diethyl ether 2 h (low dose), 24 h (low dose), and 7 days (low and high doses) after administration, exsanguinated from the abdominal aorta, and sacrificed, and 18−19 major tissues and organs were sampled by dissection.

**Urinary and Fecal Excretion.** After oral administration of [$^{14}$C]-clothianidin to groups of rats each consisting of five males and five females at low or high dose, rats were individually housed in glass Metabolica $CO_2$ cages (MC-$CO_2$, Sugiyamagen Iriki Co., Ltd.), in which their urine and feces were separately collected and expired $CO_2$ was trapped into monoethanolamine/water (7:3, v/v; 250 mL) by sucking the air through the trap. Urine and feces were collected at intervals of every 24 h for 7 days, and expired air was collected every 24 h for 2 days after administration.

**Analysis of Metabolites in Excreta.** Feces collected separately from urine were homogenized in a 3-fold weight of distilled water. An aliquot of the homogenates was mixed with methanol/water (1:1, v/v), shaken, and centrifuged at 3000 rpm for 10 min. The precipitates were further extracted with methanol in the same manner. The collected extracts were combined and concentrated by evaporation.

To quantify the metabolites, the urine and extract of feces were subjected to one- or two-dimensional TLC, and radioactive zones of interest on the TLC plates were scraped off and subjected to LSC to measure radioactivity. The unchanged parent compound and all major metabolites were identified by cochromatography with authentic reference compounds using independently optimized chromatographic methods (**Table 1**).

## RESULTS AND DISCUSSION

**Blood and Plasma Concentration of Carbon-14.** The concentration of carbon-14 in blood and plasma of male and female rats administered [*nitroimino*-$^{14}$C]clothianidin at the low dose is shown in **Figure 2**. The pharmacokinetic parameters, such as maximum time ($t_{max}$, h), maximum concentration ($C_{max}$, μg equiv of clothianidin/mL), elimination half-lives ($t_{1/2}$, h), and $AUC_{0-48}$ (area under the blood concentration−time curve from 0 to 48 h) derived from blood and plasma curve analysis, are shown in **Table 2**. After a single oral administration of [*nitroimino*-$^{14}$C]clothianidin, the concentration of carbon-14 in blood reached maxima of 1.86 μg equiv/mL in male rats and 2.36 μg equiv/mL in female rats at 2 h after administration. Thereafter, the concentration of carbon-14 in blood declined rapidly and approached the limit of quantification 48 h after administration. The elimination half-lives ($t_{1/2}$, h) of carbon-14 in blood for male and female rats were estimated to be 3.8 and 2.9 h, respectively, using first-order kinetics. The depletion curve for blood and plasma of male and female rats were very similar

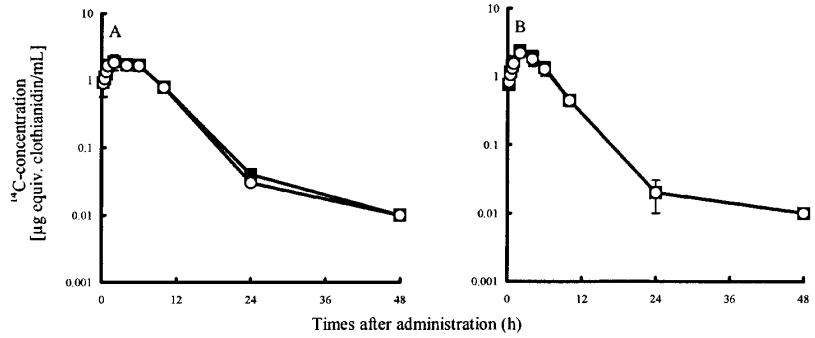


**Figure 2.** Concentration of carbon-14 in blood (■) and plasma (○) of male (**A**) and female (**B**) rats after a single oral administration of [*nitroimino*-$^{14}$C]clothianidin at a dose of 5 mg/kg. Points and bars are the mean values and standard deviations for data from three animals.

**Table 2.** Pharmacokinetic Parameters of Carbon-14 in Blood and Plasma after a Single Oral or Intravenous Administration of [*nitroimino*-$^{14}$C]- or [*thiazolyl*-2-$^{14}$C]Clothianidin

| dosing compound | dosing route | sex | $t_{max}$ (h) | | $C_{max}$ (μg equiv/mL) | | $t_{1/2}$[a] (h) | | $AUC_{0-48}$ (μg equiv·h/mL) |
|---|---|---|---|---|---|---|---|---|---|
| | | | blood | plasma | blood | plasma | blood | plasma | blood |
| [*nitroimino*-$^{14}$C]clothianidin | po | male | 2.0 | 2.0 | 1.86 ± 0.47 | 1.81 ± 0.22 | 3.8 ± 0.1 | 3.6 ± 0.2 | 20.80 ± 0.59 |
| | | female | 2.0 | 2.0 | 2.36 ± 0.27 | 2.16 ± 0.31 | 2.9 ± 0.2 | 3.2 ± 0.2 | 16.38 ± 1.99 |
| | iv | male | | | 5.62 ± 0.29 | 5.38 ± 0.16 | 2.4 ± 0.3 | 2.4 ± 0.3 | 21.50 ± 1.64 |
| | | female | | | 5.19 ± 0.17 | 4.96 ± 0.08 | 1.8 ± 0.1 | 1.8 ± 0.2 | 17.43 ± 0.50 |
| [*thiazolyl*-2-$^{14}$C]clothianidin | po | male | 2.0 | 2.0 | 2.15 ± 0.20 | 2.14 ± 0.20 | 4.0 ± 0.1 | 3.8 ± 0.1 | 19.20 ± 1.11 |
| | | female | 2.0 | 2.0 | 2.08 ± 0.48 | 2.01 ± 0.36 | 3.8 ± 0.3 | 3.6 ± 0.1 | 17.08 ± 1.91 |
| | iv | male | | | 4.90 ± 0.28 | 4.95 ± 0.38 | 2.2 ± 0.2 | 2.3 ± 0.1 | 19.47 ± 1.31 |
| | | female | | | 5.26 ± 0.23 | 4.73 ± 0.08 | 1.9 ± 0.1 | 1.9 ± 0.2 | 17.13 ± 0.53 |

[a] Elimination half-lives for oral administration and intravenous administration were calculated using the concentrations for 2–24 and 0–10 h, respectively. Data are the mean ± standard deviation (SD) of three rats.

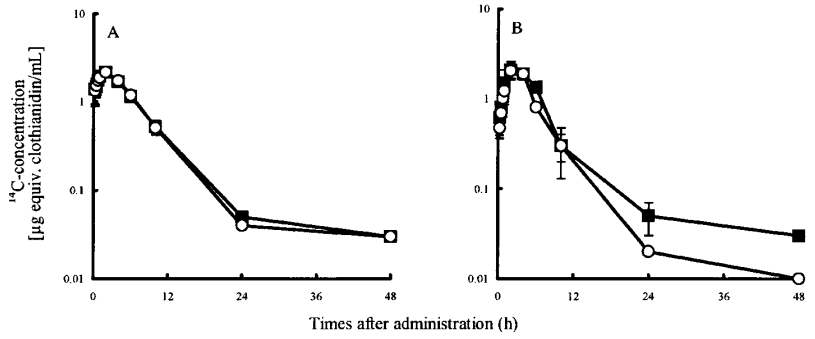


**Figure 3.** Concentration of carbon-14 in blood (■) and plasma (○) of male (**A**) and female (**B**) rats after a single oral administration of [*thiazolyl*-2-$^{14}$C]clothianidin at a dose of 5 mg/kg. Points and bars are the mean values and standard deviations for data from three animals.

for the whole time range of 48 h after administration. From the ratio of AUC po to AUC iv for blood, the absorption rate of carbon-14 in male and female rats was estimated to be >94%. The pharmacokinetic parameters and depletion curve for blood and plasma of the male and female rats administered [*thiazolyl*-2-$^{14}$C]clothianidin were very similar to those administered [*nitroimino*-$^{14}$C]clothianidin (**Figure 3**).

These results indicated that most of the carbon-14 administered was absorbed from the gastrointestinal tract and eliminated rapidly from the body. The depletion curve for the concentration of carbon-14 in plasma was similar to that in blood, suggesting that binding or accumulation of carbon-14 in blood cells is insignificant. The pharmacokinetic parameters were not statistically different either between the two kinds of radiolabeled clothianidin or between the male and female rats, indicating that neither the labeling position nor sex has any influence of the absorption and elimination of carbon-14.

**Tissue and Organ Distribution of Carbon-14.** The distribution of carbon-14 in tissue and organ after a single oral administration of [*nitroimino*-$^{14}$C]clothianidin at the low and high dose is shown in **Table 3**. In the low-dose group, radioactivity was rapidly distributed to all tissues and organs within 2 h after administration. At 2 h after administration, the concentration of carbon-14 in blood was 1.95−2.23 μg equiv/g, and concentrations in the same or lower ranges were measured in most tissues and organs. Remarkably higher concentrations of carbon-14 than in blood were measured in stomach (7.17−7.96 μg equiv/g, administered organ), kidney (5.04−5.69 μg

**Table 3.** Residual Radioactivity in Tissues and Organs of Male and Female Rats after a Single Oral Administration of [*nitroimino*-$^{14}$C]Clothianidin at a Dose of 5 (Low Dose) or 250 (High Dose) mg/kg of Body Weight[a]

| | μg equiv of clothianidin/g of tissue | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | male | | | | female | | | |
| | low dose | | | high dose | low dose | | | high dose |
| tissue/organ | 2 h | 1 day | 7 days | 7 days | 2 h | 1 day | 7 days | 7 days |
| adrenal | 2.80 ± 0.34 | 0.04 ± 0.00 | 0.01 ± 0.01 | 0.23 ± 0.13 | 2.94 ± 0.41 | 0.18 ± 0.04 | <0.01 ± 0.00 | 0.41 ± 0.29 |
| blood | 1.95 ± 0.40 | 0.03 ± 0.00 | 0.01 ± 0.00 | 0.63 ± 0.20 | 2.23 ± 0.48 | 0.08 ± 0.01 | 0.01 ± 0.00 | 0.52 ± 0.10 |
| brain | 0.55 ± 0.11 | 0.01 ± 0.00 | <0.01 ± 0.00 | 0.11 ± 0.07 | 0.54 ± 0.10 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.03 ± 0.03 |
| cecum | 1.55 ± 0.27 | 0.14 ± 0.02 | <0.01 ± 0.00 | 0.16 ± 0.02 | 1.39 ± 0.22 | 0.17 ± 0.07 | <0.01 ± 0.00 | 0.10 ± 0.10 |
| fat | 0.26 ± 0.03 | <0.01 ± 0.00 | <0.01 ± 0.00 | 0.28 ± 0.17 | 0.27 ± 0.03 | 0.02 ± 0.01 | <0.01 ± 0.00 | 0.17 ± 0.06 |
| heart | 2.36 ± 0.28 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.10 ± 0.04 | 2.60 ± 0.36 | 0.10 ± 0.02 | <0.01 ± 0.00 | 0.12 ± 0.02 |
| intestine | 1.15 ± 0.37 | 0.04 ± 0.03 | <0.01 ± 0.00 | 0.45 ± 0.60 | 1.36 ± 0.35 | 0.05 ± 0.01 | <0.01 ± 0.00 | 0.12 ± 0.04 |
| kidney | 5.69 ± 1.54 | 0.09 ± 0.02 | <0.01 ± 0.00 | 0.33 ± 0.05 | 5.04 ± 0.82 | 0.18 ± 0.03 | <0.01 ± 0.00 | 0.35 ± 0.07 |
| liver | 3.92 ± 0.64 | 0.14 ± 0.03 | 0.02 ± 0.00 | 0.86 ± 0.15 | 4.23 ± 0.85 | 0.24 ± 0.03 | 0.01 ± 0.00 | 0.67 ± 0.03 |
| lung | 2.20 ± 0.42 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.11 ± 0.06 | 2.44 ± 0.53 | 0.08 ± 0.02 | <0.01 ± 0.00 | 0.21 ± 0.07 |
| muscle | 1.66 ± 0.23 | 0.02 ± 0.00 | <0.01 ± 0.00 | 0.15 ± 0.03 | 1.82 ± 0.23 | 0.06 ± 0.01 | <0.01 ± 0.00 | 0.17 ± 0.09 |
| ovary | | | | | 1.79 ± 0.32 | 0.05 ± 0.01 | <0.01 ± 0.00 | 0.04 ± 0.05 |
| pancreas | 1.52 ± 0.34 | 0.02 ± 0.00 | <0.01 ± 0.00 | 0.08 ± 0.04 | 1.51 ± 0.35 | 0.06 ± 0.01 | <0.01 ± 0.00 | 0.14 ± 0.03 |
| sciatic nerve | 0.74 ± 0.26 | 0.02 ± 0.01 | <0.01 ± 0.00 | 0.55 ± 0.50 | 0.85 ± 0.24 | 0.06 ± 0.04 | <0.01 ± 0.00 | 0.62 ± 0.48 |
| skin | 2.05 ± 0.33 | 0.05 ± 0.03 | <0.01 ± 0.00 | 0.62 ± 0.46 | 1.50 ± 0.29 | 0.10 ± 0.10 | <0.01 ± 0.00 | 0.13 ± 0.07 |
| spinal cord | 0.64 ± 0.12 | 0.01 ± 0.00 | <0.01 ± 0.00 | 0.10 ± 0.03 | 0.56 ± 0.12 | 0.02 ± 0.00 | <0.01 ± 0.00 | 0.02 ± 0.02 |
| spleen | 1.99 ± 0.32 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.12 ± 0.12 | 2.14 ± 0.41 | 0.07 ± 0.01 | <0.01 ± 0.00 | 0.14 ± 0.03 |
| stomach | 7.17 ± 2.88 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.11 ± 0.09 | 7.96 ± 1.72 | 0.09 ± 0.01 | <0.01 ± 0.00 | 0.09 ± 0.06 |
| testis | 1.54 ± 0.24 | 0.02 ± 0.00 | <0.01 ± 0.00 | 0.13 ± 0.02 | | | | |
| thyroid | 1.64 ± 0.41 | 0.02 ± 0.01 | <0.01 ± 0.00 | 0.33 ± 0.48 | 1.25 ± 0.22 | 0.05 ± 0.01 | 0.01 ± 0.00 | 0.10 ± 0.12 |
| uterus | | | | | 1.36 ± 0.20 | 0.05 ± 0.00 | <0.01 ± 0.00 | 0.07 ± 0.12 |

[a] Data are the mean ± SD of three rats.

**Table 4.** Residual Radioactivity in Tissues and Organs of Male and Female Rats after a Single Oral Administration of [*thiazolyl*-2-$^{14}$C]Clothianidin at a Dose of 5 (Low Dose) or 250 (High Dose) mg/kg of Body Weight[a]

| | μg equiv of clothianidin/g of tissue | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | male | | | | female | | | |
| | low dose | | | high dose | low dose | | | high dose |
| tissue/organ | 2 h | 1 day | 7 days | 7 days | 2 h | 1 day | 7 days | 7 days |
| adrenal | 2.69 ± 0.76 | 0.05 ± 0.01 | 0.01 ± 0.01 | 0.13 ± 0.15 | 1.88 ± 0.62 | 0.06 ± 0.02 | <0.01 ± 0.00 | 0.59 ± 0.48 |
| blood | 1.94 ± 0.59 | 0.07 ± 0.01 | 0.02 ± 0.00 | 0.95 ± 0.25 | 1.81 ± 0.61 | 0.09 ± 0.02 | 0.01 ± 0.00 | 0.79 ± 0.08 |
| brain | 0.58 ± 0.13 | <0.01 ± 0.00 | <0.01 ± 0.00 | 0.06 ± 0.06 | 0.42 ± 0.18 | 0.01 ± 0.00 | <0.01 ± 0.00 | 0.08 ± 0.04 |
| cecum | 1.08 ± 0.14 | 0.09 ± 0.02 | 0.01 ± 0.00 | 0.15 ± 0.06 | 0.81 ± 0.23 | 0.06 ± 0.02 | <0.01 ± 0.00 | 0.06 ± 0.02 |
| fat | 0.43 ± 0.18 | 0.01 ± 0.01 | <0.01 ± 0.00 | 0.07 ± 0.05 | 0.10 ± 0.06 | <0.01 ± 0.00 | <0.01 ± 0.00 | 0.06 ± 0.08 |
| heart | 2.13 ± 0.60 | 0.04 ± 0.00 | <0.01 ± 0.00 | 0.13 ± 0.07 | 1.86 ± 0.52 | 0.05 ± 0.02 | <0.01 ± 0.00 | 0.40 ± 0.25 |
| intestine | 1.37 ± 0.25 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.16 ± 0.10 | 1.01 ± 0.30 | 0.04 ± 0.02 | <0.01 ± 0.00 | 0.17 ± 0.21 |
| kidney | 6.83 ± 1.95 | 0.25 ± 0.10 | 0.02 ± 0.00 | 0.57 ± 0.23 | 5.65 ± 1.50 | 0.31 ± 0.09 | 0.02 ± 0.00 | 0.57 ± 0.06 |
| liver | 3.76 ± 1.37 | 0.18 ± 0.04 | 0.02 ± 0.00 | 1.34 ± 0.32 | 3.21 ± 1.07 | 0.17 ± 0.05 | 0.01 ± 0.00 | 0.59 ± 0.26 |
| lung | 2.10 ± 0.64 | 0.04 ± 0.01 | <0.01 ± 0.00 | 0.18 ± 0.21 | 1.72 ± 0.56 | 0.05 ± 0.02 | <0.01 ± 0.00 | 0.42 ± 0.08 |
| muscle | 1.51 ± 0.50 | 0.02 ± 0.01 | <0.01 ± 0.00 | 0.08 ± 0.08 | 2.33 ± 1.53 | 0.03 ± 0.01 | <0.01 ± 0.00 | 0.08 ± 0.06 |
| ovary | | | | | 1.12 ± 0.39 | 0.03 ± 0.01 | <0.01 ± 0.00 | 0.05 ± 0.09 |
| pancreas | 2.01 ± 0.77 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.11 ± 0.07 | 1.21 ± 0.54 | 0.03 ± 0.01 | <0.01 ± 0.00 | 0.21 ± 0.05 |
| sciatic nerve | 0.45 ± 0.08 | <0.01 ± 0.00 | <0.01 ± 0.00 | 0.53 ± 0.50 | 0.35 ± 0.09 | 0.01 ± 0.01 | <0.01 ± 0.00 | 0.22 ± 0.36 |
| skin | 1.21 ± 0.14 | 0.05 ± 0.01 | 0.01 ± 0.00 | 0.64 ± 0.27 | 1.12 ± 0.33 | 0.04 ± 0.01 | <0.01 ± 0.00 | 0.40 ± 0.29 |
| spinal cord | 0.58 ± 0.13 | <0.01 ± 0.00 | <0.01 ± 0.00 | 0.08 ± 0.09 | 0.31 ± 0.10 | 0.01 ± 0.00 | <0.01 ± 0.00 | 0.05 ± 0.05 |
| spleen | 1.92 ± 0.60 | 0.04 ± 0.01 | <0.01 ± 0.00 | 0.16 ± 0.06 | 1.54 ± 0.54 | 0.04 ± 0.02 | <0.01 ± 0.00 | 0.21 ± 0.05 |
| stomach | 9.98 ± 3.23 | 0.08 ± 0.03 | <0.01 ± 0.00 | 0.48 ± 0.11 | 11.20 ± 1.52 | 0.10 ± 0.03 | <0.01 ± 0.00 | 0.70 ± 0.23 |
| testis | 1.36 ± 0.45 | 0.03 ± 0.00 | <0.01 ± 0.00 | 0.19 ± 0.04 | | | | |
| thyroid | 0.83 ± 0.31 | 0.02 ± 0.00 | <0.01 ± 0.00 | 0.64 ± 0.23 | 0.44 ± 0.14 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.11 ± 0.23 |
| uterus | | | | | 1.44 ± 0.15 | 0.03 ± 0.01 | <0.01 ± 0.00 | 0.13 ± 0.10 |

[a] Data are the mean ± SD of three rats.

equiv/g), and liver (3.92−4.23 μg equiv/g). Thereafter, the concentrations for each tissue and organ rapidly decreased by several orders of magnitude, and the concentrations for each tissue and organ were close to or less than the limit of quantification (0.01 μg equiv/g) at 7 days after administration. In the high-dose group, the concentrations for each tissue and organ 7 days after administration were 0.86 μg equiv/g or less. Comparison of the dose-normalized concentrations between the low- and high-dose groups revealed no significant differences.

The distribution of carbon-14 in tissues and organs after a single oral administration of [*thiazolyl*-2-$^{14}$C]clothianidin at the low and high doses is shown in **Table 4**. In the low-dose group, the radioactivity was rapidly distributed to all tissues and organs 2 h after administration and eliminated rapidly thereafter. The concentration in each tissue and organ 7 days after administration decreased and approached the limit of quantification. In the high-dose group, the concentrations in each tissue and organ 7 days after administration were ≤1.34 μg equiv/g. Comparison

**Table 5.** Amounts of Metabolites in the Urine and Feces within 7 Days after a Single Oral Administration of [*nitroimino*-$^{14}$C]Clothianidin to Rats at a Dose of 5 (Low Dose) or 250 (High Dose) mg/kg of Body Weight

| | composition (% of administered radioactivity) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | low dose | | | | | | high dose | | | | | |
| | male | | | female | | | male | | | female | | |
| metabolite | urine | feces | total | urine | feces | total | urine | feces | total | urine | feces | total |
| extractable $^{14}$C | 92.3 | 4.8 | 97.1 | 95.0 | 3.9 | 98.9 | 90.6 | 6.1 | 96.7 | 92.5 | 3.7 | 96.2 |
| clothianidin | 72.2 | 1.8 | 74.0 | 77.4 | 1.8 | 79.2 | 61.4 | 2.5 | 63.9 | 65.5 | 1.6 | 67.1 |
| TZMU | 1.0 | ND[a] | 1.0 | 0.8 | ND | 0.8 | 0.5 | 0.1 | 0.6 | 0.4 | 0.1 | 0.5 |
| TZNG | 7.1 | 0.1 | 7.2 | 4.9 | <0.1 | 4.9 | 13.5 | 0.5 | 14.0 | 14.4 | 0.2 | 14.6 |
| MNG | 7.5 | 0.1 | 7.6 | 7.8 | 0.1 | 7.9 | 9.6 | 0.1 | 9.7 | 8.4 | 0.1 | 8.5 |
| TZU | 1.5 | ND | 1.5 | 1.6 | ND | 1.6 | 1.2 | 0.1 | 1.3 | 1.1 | 0.1 | 1.2 |
| NTG | 1.5 | ND | 1.5 | 0.8 | ND | 0.8 | 2.3 | ND | 2.3 | 1.7 | ND | 1.7 |
| TMG | 0.3 | 2.5 | 2.8 | 0.4 | 1.8 | 2.2 | 2.1 | 2.6 | 4.7 | 1.0 | 1.5 | 2.5 |
| Ni-UK-1 | 0.5 | ND | 0.5 | 0.4 | ND | 0.4 | ND | ND | ND | ND | ND | ND |
| Ni-UK-2 | 0.5 | ND | 0.5 | 0.6 | ND | 0.6 | ND | ND | ND | ND | ND | ND |
| MG | ND | 0.3 | 0.3 | ND | 0.2 | 0.2 | ND | 0.2 | 0.2 | ND | 0.1 | 0.1 |
| origin | 0.2 | ND | 0.2 | 0.3 | ND | 0.3 | ND | ND | ND | ND | ND | ND |
| unextractable $^{14}$C | ND | 0.9 | 0.9 | ND | 0.5 | 0.5 | ND | 0.8 | 0.8 | ND | 0.9 | 0.9 |
| total | 92.3 | 5.7 | 98.0 | 95.0 | 4.4 | 99.4 | 90.6 | 6.9 | 97.5 | 92.5 | 4.6 | 97.1 |

[a] Not detected on TLC.

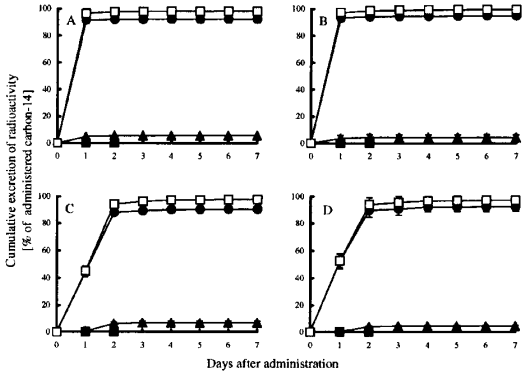


**Figure 4.** Cumulative excretion of carbon-14 in urine (●), feces (▲), and expired air (■) [total (□)] after a single oral administration of [*nitroimino*-$^{14}$C]clothianidin to rats: (**A**) male, at a dose of 5 mg/kg; (B) female, at a dose of 5 mg/kg; (C) male, at a dose of 250 mg/kg; (D) female, at a dose of 250 mg/kg. Points and bars are the mean values and standard deviations for data from five animals.

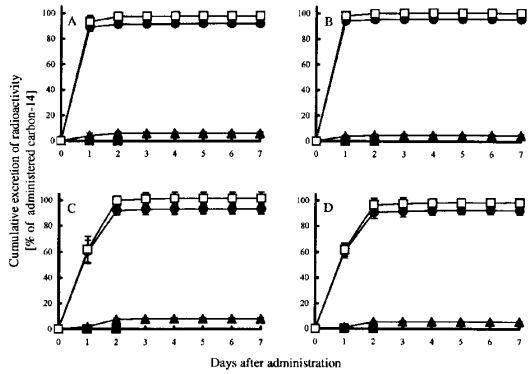


**Figure 5.** Cumulative excretion of carbon-14 in urine (●), feces (▲), and expired air (■) [total (□)] after a single oral administration of [*thiazolyl*-2-$^{14}$C]clothianidin to rats: (**A**) male, at a dose of 5 mg/kg; (**B**) female, at a dose of 5 mg/kg; (**C**) male, at a dose of 250 mg/kg; (**D**) female, at a dose of 250 mg/kg Points and bars are the mean values and standard deviations for data from five animals.

of the dose-normalized concentrations between the low- and high-dose groups revealed no significant differences.

Tissue and organ distribution of carbon-14 indicated that carbon-14 was rapidly distributed into all tissues and organs within 2 h after administration at the low dose, and higher concentrations of carbon-14 were distributed in kidney and liver. These high concentrations in the excretory and metabolizing organs indicate the start of excretion and metabolism immediately after absorption. Thereafter, the concentration in the tissues and organs declined rapidly, and there was no sign of accumulation in any one of the organs or tissues examined. These results were consistent with the rapid and almost complete elimination of carbon-14 from blood, and neither the labeling position nor sex has any influence on the tissue and organ distribution of carbon-14.

**Carbon-14 Excretion.** The cumulative excretion of carbon-14 in urine, feces, and expired air within 7 days after a single oral administration of [*nitroimino*-$^{14}$C]clothianidin at the low or high dose is shown in **Figure 4**. At the low-dose level, >95% of the administered dose was excreted 1 day after administration. At the high-dose level, the cumulative excretion of carbon-14 for 1 and 2 days after administration accounted for 45−53 and >95%, respectively. For both dose levels, the cumulative excretion of radioactivity in urine and feces accounted for 90−95 and 4−7%, respectively. The carbon-14 in the expired air accounted for <0.1% for 2 days after a single oral administration.

The cumulative excretion of carbon-14 in urine, feces, and expired air within 7 days after a single oral administration of [*thiazolyl*-2-$^{14}$C]clothianidin at the low or high dose is shown in **Figure 5**. At the low-dose level, >95% of the administered dose was excreted 1 day after administration. At the high-dose level, the cumulative excretion of carbon-14 for 1 and 2 days after administration accounted for 60−65 and >95%, respectively. For both dose levels, the cumulative excretion of radioactivity in urine and feces accounted for 92−96 and 4−9%, respectively. The carbon-14 in the expired air accounted for <0.1% for 2 days after a single oral administration.

**Table 6.** Amounts of Metabolites in the Urine and Feces within 7 Days after a Single Oral Administration of [*thiazolyl*-2-$^{14}$C]Clothianidin to Rats at a Dose of 5 (Low Dose) or 250 (High Dose) mg/kg of Body Weight

| | composition (% of administered radioactivity) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | low dose | | | | | | high dose | | | | | |
| | male | | | female | | | male | | | female | | |
| metabolite | urine | feces | total | urine | feces | total | urine | feces | total | urine | feces | total |
| extractable $^{14}$C | 92.0 | 5.0 | 97.0 | 95.8 | 3.8 | 99.6 | 93.4 | 5.7 | 99.1 | 92.7 | 4.1 | 96.8 |
| clothianidin | 68.4 | 2.8 | 71.2 | 75.7 | 1.2 | 76.9 | 61.7 | 1.2 | 62.9 | 69.9 | 1.4 | 71.3 |
| TZMU | 0.9 | ND[a] | 0.9 | 0.8 | ND | 0.8 | 0.9 | <0.1 | 0.9 | 0.5 | 0.1 | 0.6 |
| TZNG | 8.2 | 0.2 | 8.4 | 6.0 | 0.1 | 6.1 | 17.5 | 0.2 | 17.7 | 13.0 | 0.2 | 13.2 |
| TZU | 2.1 | ND | 2.1 | 2.3 | ND | 2.3 | 0.3 | 0.2 | 0.5 | 0.3 | 0.1 | 0.4 |
| MTCA | 5.3 | 0.3 | 5.6 | 5.4 | 0.1 | 5.5 | 9.8 | 0.2 | 10.0 | 7.7 | 0.1 | 7.8 |
| TMG | 1.2 | 1.7 | 2.9 | 1.6 | 2.0 | 3.6 | 0.5 | 2.9 | 3.4 | 0.5 | 2.0 | 2.5 |
| Th-UK-1 | ND | ND | ND | ND | ND | ND | 1.8 | 0.3 | 2.1 | 0.4 | 0.1 | 0.5 |
| Th-UK-2 | 2.3 | ND | 2.3 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Th-UK-3 | 1.2 | ND | 1.2 | 1.1 | 0.1 | 1.2 | 0.6 | ND | 0.6 | 0.2 | ND | 0.2 |
| Th-UK-4 | 1.2 | ND | 1.2 | 1.1 | 0.2 | 1.3 | 0.3 | 0.7 | 1.0 | 0.2 | 0.1 | 0.3 |
| Th-UK-5 | ND | ND | ND | ND | 0.1 | 0.1 | ND | ND | ND | ND | ND | ND |
| origin | 1.2 | ND | 1.2 | 1.8 | ND | 1.8 | ND | ND | ND | ND | ND | ND |
| unextractable $^{14}$C | ND | 1.0 | 1.0 | ND | 0.9 | 0.9 | ND | 2.5 | 2.5 | ND | 1.6 | 1.6 |
| total | 92.0 | 6.0 | 98.0 | 95.8 | 4.7 | 100.5 | 93.4 | 8.2 | 101.6 | 92.7 | 5.7 | 98.4 |

[a] Not detected on TLC.

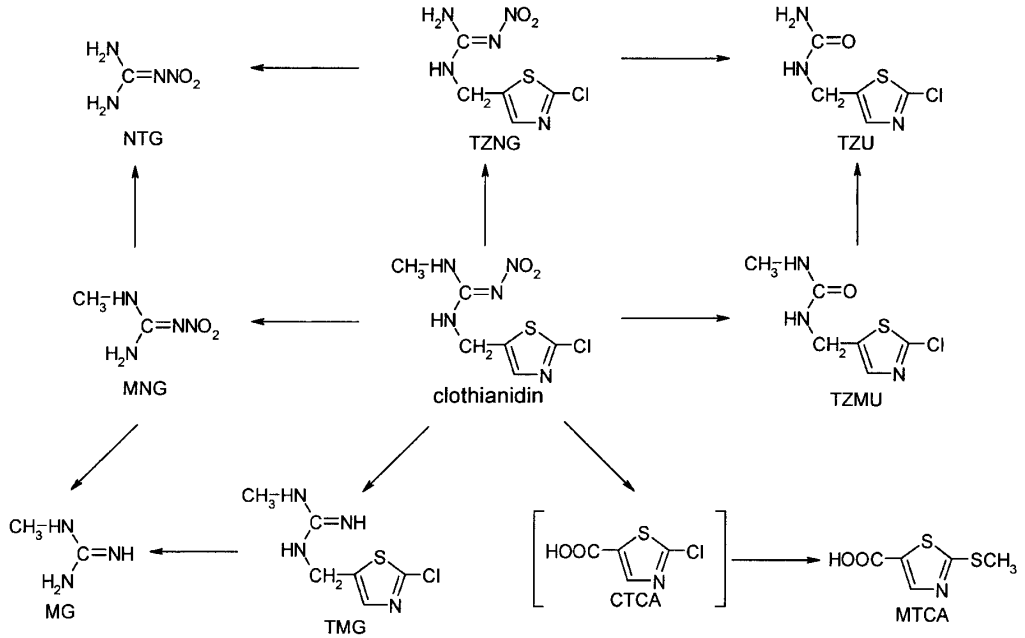


**Figure 6.** Proposed metabolic pathways of clothianidin in rats.

The administered carbon-14 was rapidly and extensively excreted in urine, feces, and expired air and predominantly excreted via the urine compared with fecal excretion. The extent of urinary excretion was not influenced by the dose level, the position of the carbon-14 label, or the sex, because the cumulative excretion of carbon-14 in urine was within the narrow range of 90−96% of the administered dose in all cases. In the high-dose group, the excretion of administered carbon-14 was transiently delayed to some extent, but the renal excretion was still up to 90% of the administered dose. This delay of the excretion in the high-dose group may be caused by the saturation of the absorption and/or excretion of administered radioactivity.

**Metabolites in Excreta.** Amounts (expressed as percentage of the administered carbon-14) of urinary and fecal metabolites are summarized in **Tables 5** and **6**. The metabolic profiles are very similar in all dose groups. The predominant component identified in all cases was the unchanged parent compound, clothianidin, which accounted for 71.2−79.2% of the administered dose at the low dose and 62.9−71.3% at the high dose, and almost all of it was excreted in the urine. The major metabolites identified in excreta were TZNG, MNG, and MTCA. The amounts of TZNG for the low- and high-dose groups were 4.9−8.4 and 13.2−17.7%, respectively. The amounts of MNG for the low- and high-dose groups were 7.6−

7.9 and 8.5−9.7%, respectively. The amounts of MTCA for the low- and high-dose groups were 5.5−5.6 and 7.8−10.0%, respectively. These metabolites were excreted predominantly in urine. The main fecal metabolite was TMG, but the amount of TMG in the total excreta was 4.7% or less of the administered dose. All other metabolites, TZMU, TZU, NTG, and MG, were also detected (0.1−2.3%) together with the unidentified metabolites (0.2−2.3%). In all dose groups, >90% of the administered dose was identified.

There were no significant sex-related effects in the metabolism of clothianidin in rats, and the main component identified in all dose groups was clothianidin itself, accounting for 62.9−79.2% of the administered dose. On the basis of these results, clothianidin should thus be considered moderately metabolizable and easily excretable in urine. This is supported by the relatively high level of carbon-14 in kidney 2 h after administration. The extent of metabolism of the parent compound was slightly larger in the high-dose group than in the low-dose group for both sexes. This dose-related metabolic difference may be explained as follows. The absorption and excretion of administrated clothianidin were saturated in the high-dose group, resulting in a longer residence time of clothianidin in the organs and tissues, and as a result a relatively large amount of clothianidin was metabolized in the liver prior to excretion.

In the present study using the two labeling positions of [$^{14}$C]-clothianidin, eight metabolites were identified and/or characterized by cochromatographic methods. From those identified and/or characterized metabolites, the proposed metabolic pathway of clothianidin in the rats is shown in **Figure 6**. On the basis of the quantified metabolites in the excreta, the major metabolic reactions of clothianidin were concluded to be as follows: (1) oxidative demethylation to form TZNG and (2) cleavage of the carbon−nitrogen bond between the thiazolylmethyl moiety and the nitroguanidine moiety. The part of the molecule traced by the [*nitroimino*-$^{14}$C]clothianidin was transformed mainly to MNG, whereas the molecule traced by [*thiazolyl*-2-$^{14}$C]-clothianidin was transformed into a putative intermediate, CTCA. The putative intermediate, CTCA, was further metabolized to MTCA via glutathione conjugation (*10*). The metabolism of clothianidin by transformation of the nitroimino moiety to the urea compounds (TZMU and TZU) and into the guanidine derivative (TMG) and the formation of NTG and MG were considered to be minor metabolic reactions.

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