Tissue Distribution of the Tobacco-specific Carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone and Its Metabolites in F344 Rats 1, 2

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ABSTRACT

The tissue distribution of the tobacco-specific N-nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in the F344 rat was studied by whole-body autoradiography and high-performance liquid chromatography. The results of the whole-body autoradiography experiments indicate that the substance is able to freely cross biological membranes and reach all tissues of the body. A high level of tissue-bound metabolites occurred in the mucosa of the ethmoturbinates, in the lung, and the liver, which are the targets for the carcinogenicity of NNK in F344 rats. However, tissue-bound radioactivity was also present in non-target tissues such as the lateral nasal gland (Steno’s gland), the tracheal mucosa, and the mucosa of the nasopharyngeal duct. A high level of unbound radioactivity occurred in the preputial gland, submaxillary and adrenal glands, and the urinary and gastrointestinal systems.

Localisation of unbound radioactivity was observed in the stomach, only not only after p.o. but also after i.v. administration of NNK. Analysis of extracts of the stomach contents by high-performance liquid chromatography indicated that, due to their basicity, NNK and its metabolites were trapped in the gastric juice and later reabsorbed from the intestinal tract. Analysis of unbound metabolites in various tissues and in the urine after i.v. or p.o. administration of [carbonyl-14C]NNK indicated metabolism and excretion of products resulting from α-carbon hydroxylation, carbonyl reduction, and pyridine N-oxidation of NNK. After p.o. administration of [14CH3]NNK, 47% of the dose was recovered as CO2. [carbonyl-14C]NNK, however, was not metabolized to 14CO2. Levels of in vitro metabolism of [14CH3]-NNK to 14CO2 or incorporation of radioactivity into the acid-insoluble material after incubation with [carbonyl-14C]NNK were the highest in the nasal mucosa. Thus, the high activity of NNK-activating enzymes present in the nasal cavity is apparently an important factor in the etiology of NNK-induced neuroepitheliomas. In vitro autoradiography experiments showed that NNK is metabolized in the mucosa of the ethmoturbinates, the lung, and the liver, suggesting that the tumors are induced by metabolites formed locally in the target tissues. In the lung, the labeling was higher in the bronchial tree than in the lung parenchyma.

INTRODUCTION

The tobacco-specific nitrosamine NNK 4 has been identified in tobacco smoke (0.1 μg/cigarette) and in the saliva of snuff dippers (10 ng/g) (14, 15). Its carcinogenic properties have been demonstrated in A/J mice, F344 rats, and Syrian golden hamsters (10, 11, 16). Thus, NNK is a putative human carcinogen contributing to the high incidence of respiratory tract cancer among smokers and oral cavity neoplastic and neoplastic lesions among snuff dippers and tobacco chewers. After s.c. injections of a total dose of 3.4 mmol of NNK, 83% of F344 rats developed neuroepitheliomas of the nasal cavity, 83% developed hepatocarcinomas and hemangiosarcomas of the liver, and 67% developed adenomas and carcinomas of the lung (11). These tumors also developed after administration of NNK at a dose of 1.0 mmol/kg. 5 The potency of NNK has also been observed in A/J mice. A total dose of 0.1 mmol induced 37.6 lung adenomas/animal. The reduction of the carbonyl group of NNK occurred in vivo as well as in animal tissues cultured in vitro (13). The resulting metabolite, NNAI, is also a strong carcinogen in A/J mice and induced 26.3 lung adenomas/animal. 6

In a previous study, we determined the structures of rat urinary and hepatic microsomal metabolites of NNK (13). The purpose of the present study was to investigate the tissue distribution and binding of [carbonyl-14C]NNK using whole-body autoradiography and HPLC. The excretion and tissue-specific metabolism of [carbonyl-14C]NNK and [14CH3]NNK were also compared.

MATERIALS AND METHODS

Chemicals. [carbonyl-14C]NNK (specific activity, 4.2 mCi/mmol) was prepared from [carbonyl-14C]nicotinic acid (California Bionuclear Corporation, Sun Valley, Calif.). [14CH3]NNK (specific activity, 48.1 mCi/mmol) was synthesized from [14CH3]methyamine hydrochloride (New England Nuclear, Boston, Mass.). Both radiochemicals were refined to >99% purity by thin-layer chromatography on silica gel. 7 The syntheses of NNK and its metabolites used as reference compounds in the HPLC analyses have been reported (12, 13).

Animals. Adult male and female F344 rats, originally obtained from Bantin and Kingman, Ltd., Hull, England, were used. They were fed a standard pellet diet (Ewos AB, Södertälje, Sweden) and were given tap water ad libitum.

Whole-Body Autoradiography. A series of 9 male rats (body weight =70 g) were given i.v. or p.o. [carbonyl-14C]NNK (5 μCi, 3.5 mg/kg) dissolved in 0.9% NaCl solution (50 μCi/ml). For the p.o. administration, the animals were maintained in vertical upward position, and the solution was instilled in the oral pharynx with a 200-μl syringe and a blunt needle. The animals were sacrificed by CO2 asphyxiation after 1 min (i.v.), 5 min (i.v.), 30 min (i.v., p.o.), 4 hr (i.v., p.o.), 24 hr (i.v., p.o.), and 7 days (i.v., p.o.).

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAI, 4-(methylnitrosamino)-1-(3-pyridyl)-1-ol; HPLC, high-performance liquid chromatography; NNN, N'-nitrosonornicotine.
5 S. S. Hecht, A. Rivenson, and D. Hoffmann, manuscript in preparation.
7 A. Castonguay and S. S. Hecht, manuscript in preparation.
p.o.), and 4 days (i.v.). They were immediately embedded in carboxy-
methylcellulose, frozen, and sectioned sagittally (20-μm-thick sections) on tape according to published procedures (23). Twenty-five duplicate sections were taken from each block. The sections were freeze-dried and exposed to X-ray film. To localize tissue-bound metabolites, adjacent freeze-dried sections were washed successively with 5% trichlo-
roacetic acid, water, methanol, and heptane for 0.5 min each. The sections were dried and exposed to X-ray film together with the adjacent nonextracted freeze-dried sections. The time of exposure was from 2 to 6 months.

In Vitro Autoradiography. Two 70-g male rats were decapitated, and the trachea, esophagus, pieces of the nasal mucosa, and lungs were removed. The tissues were incubated at 37°C for 1 hr under an O₂ atmosphere in Dulbecco’s solution (pH 7.6) containing 10 mm glucose. To the solutions was added either [carbonyl-14C]NNK (specific activity, 4.2 mCi/mmol) or [14CH₃]NNK (specific activity, 48.1 mCi/mmol; 0.05 μCi/ml). After the incubations, the tissues were rinsed with 0.9% NaCl solution and used for autoradiography. Tissue-bound metabolites were localized by washing some sections with trichloroacetic acid, water, and organic solvents as described above. The time of exposure was 2 months.

Metabolism in Vitro. Male 70-g rats were decapitated, and selected organs were excised and sliced. The tissues were then incubated with [carbonyl-14C]NNK (specific activity, 4.2 mCi/mmol) or [14CH₃]NNK (specific activity, 48.1 mCi/mmol; 0.05 μCi/ml) at 37°C and under an O₂ atmosphere for 1 hr in Krebs-Ringer phosphate buffer (pH 7.0) containing 10 mm glucose. The 14CO₂ formed from the [14CH₃]NNK in the incubation was determined by trapping on filter papers moistened with KOH as described previously (19). For [carbonyl-14C]NNK, the binding of radioactive metabolites to trichloroacetic acid-insoluble macromolecules was determined according to published procedures (5).

Metabolism in Vivo. For determination of the amounts of radioac-
tivity excreted in the urine, feces, and expired air, male rats (250 to 300 g) were given p.o. [carbonyl-14C]NNK (0.036 μCi; 7.1 μg/kg) or [14CH₃]NNK (0.36 μCi; 5.2 μg/kg) dissolved in triacetin (1 ml). Exhaled CO₂ was collected in 1 N NaOH (100 ml), and 14C activity was determined by scintillation counting of 200-μl aliquots in 2 ml of Maxifluor (New England Nuclear). Feces were ground in ethanol and air dried, and 14C activity was determined by combustion analyses. Urine was collected at −76°C and lyophilized. Methanol extracts of the residues were analyzed by HPLC as described previously (13). Radioac-
tivity present in exhaled air was monitored every 24 hr, and feces were monitored every 24 hr.

Unbound metabolites extracted from tissues of rats treated with [carbonyl-14C]NNK were quantitated by HPLC and liquid scintillation counting. Four female rats (140 g) received i.v. (in 0.2 ml 0.9% NaCl solution) or p.o. (in 1 ml triacetin) [carbonyl-14C]NNK (10 μCi; 3.5 mg/kg). The animals were sacrificed 1 hr later, and various tissues were removed and homogenized in cold 0.1 N HCl (1 ml). After centrifugation, the supernatant was decanted, and the pellet was washed with cold 0.1 N HCl (1 ml). The combined supernatants were carefully neutralized to pH 7.0 with NaOH solution, and metabolites were separated by HPLC according to published procedures (16). To determine the recovery during extraction, pieces of liver from untreated rats were supplemented with known amounts of NNK metabolites. The amounts present in the extracts were measured by HPLC and UV absorption at 254 nm. The recoveries varied from 70 to 90% for each metabolite.

RESULTS

Whole-Body Autoradiography. One min after i.v. administration of [carbonyl-14C]NNK, the radioactivity was homogeneously distributed in most tissues of the body at a level similar to that in the blood (Fig. 1a). A few tissues, however, showed labeling which exceeded the background radioactivity. Thus, in the nose, strong labeling was present in the mucosa of the ethmoturbine and in the lateral nasal gland (Steno’s gland) occupying the submucosa anterior, lateral, and inferior to the maxillary sinus. High radioactivity was also present in the liver, the bronchial mucosa, the adrenal cortex, and the preputial gland. In addition, considerable labeling occurred in the sub-
mucosal salivary gland and the contents of the stomach. Similar distribution pictures were observed 5 min after i.v. injection of [carbonyl-14C]NNK. At this interval, radioactivity was in addition observed in the kidney and the urinary bladder. The distribution pictures observed 30 min after i.v. injection were in most aspects similar to the ones at the preceding interval. However, in the respiratory tract, a marked radioactivity was present in the mucosa of the trachea and the nasopharyngeal duct in addition to that in the previously described sites. The radioactivity in the lung parenchyma, in the mucosa of the median and anterior regions of the nasal cavity, and in the esophageal mucosa also slightly exceeded the background labeling. The radioactivity in the stomach lumen was very high, and the contents of the duodenum and the first part of the jejunum were also labeled. Four hr after i.v. administration of [carbonyl-14C]NNK, there was a considerable decrease in the labeling of most tissues. However, there was still a marked radioactivity in the mucosa of the ethmoturbine, the bronchi, the trachea, and the nasopharyngeal duct (Fig. 1b). The lateral nasal gland, the liver, and the preputial gland also showed high radioactivity. The lung parenchyma, the mucosa of the median and anterior regions of the nasal cavity, the submaxillary sali-
vary gland, and the adrenal cortex were moderately labeled. Radioactivity was, in addition, present in the kidney and urinary bladder and in the gastrointestinal contents. At 24 hr after i.v. injection, there was a further decrease in the tissue radioactivity. With the exception of the submaxillary salivary gland, the aforementioned tissues that were labeled at 4 hr showed radioactivity also at 24 hr. In the liver, there was an irregular distribution of the radioactivity; the highest labeling was prob-
ably present in the central parts of the lobuli. At this interval, the radioactivity was low in the urinary and gastrointestinal tract. The distribution pictures 4 days after the i.v. injections were similar to those at 24 hr, although there was a marked further general decrease of the tissue labeling.

Extraction of the sections with trichloroacetic acid and or-

ganic solvents removed the homogeneously distributed radio-
activity as well as the labeling in the submaxillary, preputial, and adrenal glands and the urinary and gastrointestinal sys-
tems (Fig. 1c). Nonextractable, tissue-bound radioactivity was present in the mucosa of the ethmoturbines, the nasopharyn-
geal duct, the trachea, and the bronchi, and in the lateral nasal gland and the liver. A low nonextractable radioactivity was also present in the lung parenchyma and the median and anterior nasal cavities. In the tissues retaining bound radioactivity, a higher proportion of the tissue labeling was nonextractable at long survival intervals than at short survival intervals. The tissue-bound labeling which remained in the liver showed an irregular distribution, the highest radioactivity probably being present in the central parts of the lobuli.

In the autoradiograms obtained after p.o. administration of [carbonyl-14C]NNK, there was high labeling of the mucosa of the mouth and the esophagus, which was not observed in the animals given the substance i.v. (Fig. 2a). There was also a
higher labeling of the upper part of the gastrointestinal tract in the former than in the latter animals. The other previously listed tissues that showed labeling after i.v. injection were also labeled after p.o. administration.

Most of the radioactivity in the mouth and the esophagus was removed by the tissue extractions, but a small amount remained bound to the tissues (Fig. 2b). In other respects, the radioactivity distribution in various tissues after extraction was similar to the distribution described in i.v.-injected animals.

In Vitro Autoradiography. Incubations of the nasal region with [carbonyl-14C]NNK or [14CH3]NNK showed an accumulation of nonextractable radioactivity in the mucosa of the ethmoturbinates (Fig. 3, a to d). In the trachea, tissue-bound radioactivity accumulated in the mucosa (Fig. 3, e to h). In the lung, radioactivity was present over both the parenchyma and the bronchi, the latter labeling being stronger than that of the former (Fig. 3, i and k). After the extractions of the lung incubated with [14CH3]NNK, a marked radioactivity was retained in this tissue (Fig. 3j). In contrast, almost all the labeling was lost from the lung which had been incubated with [carbonyl-14C]NNK (Fig. 3f). When incubations were performed with the esophagus, there was a labeling of the mucosa which exceeded that of the submucosal and muscular layers (Fig. 3, m and o). The extractions removed this radioactivity from the esophagus incubated with both [carbonyl-14C]NNK and [14CH3]NNK (Figs. 3, n and p).

Metabolism in Vitro. The mucosa of the ethmoturbinates was found to have a high capacity to form CO2 from [14CH3]-NNK and to incorporate the radioactivity in the acid-insoluble macromolecules from [carbonyl-14C]NNK (Table 1). This CO2 formation and radioactivity incorporation was higher in the lung than in the nasal region. The results indicated a relatively low level of metabolism by the esophagus, submaxillary salivary, and preputial glands.

Metabolism in Vivo. The rate of urinary excretion of radioactive metabolites over a 48-hr period was determined after p.o. administration of [carbonyl-14C]NNK (7.1 μg/kg) and to incorporate the radioactivity in the acid-insoluble macromolecules from [carbonyl-14C]NNK (Table 1). This CO2 formation and radioactivity incorporation was higher in the liver than in the lung. The results indicated a relatively low level of metabolism by the esophagus, submaxillary salivary, and preputial glands.

Table 1

<table>
<thead>
<tr>
<th>Tissues</th>
<th>CO2a</th>
<th>CO2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal mucosa</td>
<td>7332 ± 639</td>
<td>7270 ± 611</td>
</tr>
<tr>
<td>Liver</td>
<td>2879 ± 157</td>
<td>1261 ± 180</td>
</tr>
<tr>
<td>Lung</td>
<td>754 ± 83</td>
<td>781 ± 73</td>
</tr>
<tr>
<td>Esophagus</td>
<td>310 ± 19</td>
<td>352 ± 52</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>243 ± 8</td>
<td>369 ± 96</td>
</tr>
<tr>
<td>Preputal glands</td>
<td>ND</td>
<td>534 ± 88</td>
</tr>
<tr>
<td>Control</td>
<td>183 ± 13</td>
<td>341 ± 19</td>
</tr>
</tbody>
</table>

a Incubation with [14CH3]NNK (specific activity, 48.1 mCi/mmol).

b Incubation with [carbonyl-14C]NNK (specific activity, 4.2 mCi/mmol).

The tissues were incubated in Krebs-Ringer phosphate buffer containing 14C and 12O2 p.o. or i.v. are shown in Table 2. Metabolites resulted from α-carbon hydroxylation, carbonyl reduction, or pyridine N-oxidation of NNK (Chart 2). The highest amount of extractable metabolites was observed in the stomach contents, independent of the routes of administration. The stomach contents weighed between 1.5 and 2.0 g at the time of sacrifice and contained 27% of the dose administered p.o. and 0.8% of the dose administered i.v. The 2 major radioactive components were unmetabolized NNK and NNAI. The ratio of NNAI to NNK was 2.1 after i.v. administration but was only 0.03 after p.o. administration. In all other tissues analyzed, including the nasal mucosa, the levels of NNAI were higher than those of NNK.

DISCUSSION

The autoradiography at short survival intervals indicates that NNK, like many other N-nitrosamines (2, 4–6, 18, 20), is distributed quickly and homogeneously throughout the body and thus has the ability to freely cross cellular membranes and partition evenly in the intra- and extracellular tissue water. The autoradiography showed a rapid localization and a retention of tissue-bound metabolites in target tissues, while the in vitro experiments demonstrated the competence of these tissues to metabolize NNK. These data suggest that the reactive intermediates inducing the tumors are formed locally in the target tissues.

Accumulation of tissue-bound metabolites as studied by whole-body autoradiography does correlate to a certain extent with the susceptibility of some rat tissues to nitrosamine carcinogenicity. For example, the nasal mucosa and esophagus, which are the main target organs of NNN, showed localization of tissue-bound metabolites after i.v. injections of [2-14C]NNN...
Tissue Distribution of NNK

Table 2
Percentage of administered dose excreted after p.o. administration of [14C]NNK to F344 rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>[carbonyl-14C]NNK</th>
<th>[14CH3]NNK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>88</td>
<td>39</td>
</tr>
<tr>
<td>Air</td>
<td>&lt;0.5</td>
<td>47</td>
</tr>
<tr>
<td>Feces</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

*Mean of duplicate values obtained from 2 rats.

(5, 11, 17). However, a low level of nonextractable radioactivity was also present in the liver, but liver tumors are rarely induced by NNN administered s.c. or in drinking water (11, 22).

The results of the present study indicate that the mucosa of the ethmoturbinates is highly active in metabolizing NNK. This tissue has previously been shown to have the ability to metabolize other N-nitrosamines (2, 3, 5, 6, 9). The nasal mucosa is a main target for the carcinogenicity of several N-nitrosamines, and a high level of activating enzymes is probably an important factor in the etiology of the nasal cavity tumors.

After the administration of [carbonyl-14C]NNK, the highest level of tissue-bound radioactivity in the lung was present in cells lining the bronchial tree, but bound radioactivity was also present in the lung parenchyma. The in vivo autoradiography showed a higher labeling of the lung parenchyma than was observed in the in vitro autoradiography. It is possible that in vivo metabolites formed in the parenchyma may be removed to a considerable extent by the blood but will not take place in vitro. The lung tumors induced by NNK in F344 rats are adenomas or carcinomas (11). Our autoradiograms showed localization of tissue-bound metabolites from [carbonyl-14C]-NNK also in the mucosa of the trachea and the nasopharyngeal duct and in the lateral nasal gland. NNK does not induce tumors in these tissues in F344 rats (11). It is obvious that metabolites may bind to certain tissues without inducing tumors. It may be noted, however, that s.c. injection of NNK does induce tracheal papillomas in Syrian golden hamsters (16).

The liver is extensively involved in the metabolism of N-nitrosamines whether it is a target or a non-target organ. Studies with rat hepatic microsomes have shown that N-nitrosamines bind as both substrates and ligands to cytochrome P-450 (1). This enzyme system probably plays an important role in the metabolism of NNK in the liver. A large proportion of the hydroxylated metabolites of NNK which are eliminated via the urine probably originates from α-carbon hydroxylation followed by hydrolysis of the alkylation species.

The autoradiograms in the F344 rats observed in the present study after the i.v. injections of [carbonyl-14C]NNK are in several aspects similar to those observed after i.v. administration of the related nitrosamine [2-[14C]NNN, but there are also certain dissimilarities (5). Thus, with both substances, tissue-bound metabolites were observed in the nasal and tracheobronchial mucosa, in the lateral nasal gland, and in the liver, and non-tissue-bound labeling was observed in the preputial and the submaxillary salivary glands. However, the labeling of the esophageal mucosa was much more pronounced after the administration of [2-[14C]NNN than of [carbonyl-14C]NNK. Even after p.o. administration of [carbonyl-14C]NNK, there was only a low amount of tissue-bound metabolites present in the esophageal mucosa. In vitro formation of bound metabolites by the esophagus was observed from [2-[14C]NNN but not from [carbonyl-14C]NNK. Zymbal’s gland and the tarsal glands of the eyelids were labeled after [2-[14C]NNN administration, but not after the injection of [carbonyl-14C]NNK (5). It is known that
NNK and NNN have metabolic pathways in common. Thus, hydroxylation at the N-methyl group of NNK gives the same oxobutylcarbocation ion that is formed by 2'-carbon hydroxylation of NNN, and some identical metabolites are excreted in the urine of rats treated with [2'-14C]NNN and [carbonyl-14C]NNK (8, 13). The similarities in the autoradiographic distribution patterns could reflect the tissue disposition of the common metabolites.

It was proposed that hydroxylation of NNK at the α-methylene group, which would result in methyl diazohydroxide, a methylyating species, may be responsible for its strong carcinogenicity (11, 13). The liberation of 14CO2 from [14CH3]NNK by the liberation of methanol from 10.1 #Ci; 3.6 mg/kg). The animals were sacrificed 1 hr later, and the radioactive metabolites were extracted with 0.1 N HCl as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Route</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>7</th>
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<th>9</th>
<th>10</th>
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<th>12</th>
<th>13</th>
<th>14</th>
<th>NNAI</th>
<th>NNK</th>
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</thead>
<tbody>
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<td>3.4</td>
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<td>1.8</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NNK</td>
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<td>4.0</td>
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<td>NNK</td>
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<tr>
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<td>ND</td>
<td>ND</td>
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<td>p.o.</td>
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<td>NNK</td>
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<tr>
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<tr>
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<td>0.8</td>
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a Values are not corrected. Recovery of each metabolite varies between 70 and 90%. Mean of values from 2 rats.

b Numbers refer to structures in Chart 2.

c ND, not detected. Limit of detection was 0.2 nmol/g of wet tissue.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Autoradiograms of F344 rats 1 min (a) and 4 hr (b and c) after i.v. injections of [carbonyl-¹⁴C]NNK (5 μCi; 3.5 mg/kg). a and b, autoradiograms of freeze-dried, nonextracted tissue sections; c, autoradiogram of tissue section adjacent to b which was extracted with trichloroacetic acid and organic solvents before the autoradiographic exposure. White areas, radioactivity. Time of exposure: 2 months (a); and 6 months (b and c).
Fig. 2. Autoradiograms of F344 rats 4 hr after p.o. administration of [carbonyl-14C]NNK (5 μCi; 3.5 mg/kg). a, autoradiogram of freeze-dried, nonextracted tissue section. b, autoradiogram of tissue section adjacent to a, which was extracted with trichloroacetic acid and organic solvents before the autoradiographic exposure. Time of exposure, 6 months.
Fig. 3. Autoradiograms of tissues incubated with \([1^{14}C]N\)NK (a and b; e and f; i and j; m and n) or [carbonyl-\(^{14}C\)]NNK (c and d; g and h; k and l; o and p) for 1 hr. a to d, nose; e to h, trachea; i to l, lung; m to p, esophagus. a, e, i, and m, and c, g, k, and o, autoradiograms of freeze-dried, nonextracted tissue sections. b, f, j, and n, and d, h, l, and p, autoradiograms of tissue sections adjacent (in the same order, respectively) to the preceding ones which were extracted with trichloroacetic acid and organic solvents before the autoradiographic exposure. Time of exposure, 2 months.
Tissue Distribution of the Tobacco-specific Carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone and Its Metabolites in F344 Rats

Andre Castonguay, Hans Tjalve and Stephen S. Hecht