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Tracing Tissues with *N*-Nitrosamine-metabolizing Capacity

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INTRODUCTION

N-nitrosamines are a class of carcinogens which show remarkable organ specific carcinogenic activity. These compounds are chemically stable under physiological conditions and it is now generally accepted that the tumourigenesis by these substances results from metabolic activation to reactive electrophilic metabolites which bind to DNA and other cellular components. The mechanism underlying the neoplastic transformation of certain cell types is not yet known in detail, but high capacity for N-nitrosamine metabolism may be one factor of great importance. It is of interest therefore to trace the tissues which have a capacity to metabolize N-nitrosamines and to evaluate whether the susceptibility of the tissues for the tumourigenesis can be correlated with N-nitrosamine metabolism.

In a series of studies at our department, the tissue-disposition of various N-nitrosamines has been examined in experimental animals. A major aim in the studies has been the tracing of N-nitrosamine-metabolizing tissues.

EXPERIMENTAL DESIGN

In the experiments whole-body autoradiography was used to study the disposition of the ^{14}C- or ^{3}H-labelled N-nitrosamines. Some of the studied N-nitrosamines are volatile, and low-temperature autoradiography and autoradiography with heated tape sections were then used to distinguish the tissue localization of the non-metabolized N-nitrosamines from the distribution of the non-volatile metabolites (4,19). Autoradiography with sections extracted with trichloroacetic acid and organic solvents was used to localize metabolites firmly bound to the tissues. In addition to the toxic alkylating species, the metabolism of some N-nitrosamines may also yield aldehydes and alcohols, which can be used in normal metabolic pathways of the tissues. It is necessary to distinguish the part of the radioactivity which is derived from the biosynthetic activities in the body and this was done by comparative autoradiography with substances such as ^{14}C-acetate, ^{14}C-acetaldehyde and ^{14}C-formaldehyde (19,20). For some N-nitrosamines it was possible to perform autoradiography in

vitro with tissue-pieces incubated with the labelled N-nitrosamines (5,7). Microautoradiography, with material fixed in formaldehyde or glutaraldehyde and embedded in paraffin or resin, was used to examine in detail the localization of metabolites in some tissues (24,27,38).

Based on the autoradiographic data, experiments were then performed in vitro in which the capacity of various tissues to form metabolites from the N-nitrosamines was studied.

TISSUE-SPECIFICITY OF N-NITROSAMINE METABOLISM

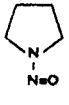
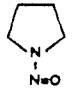
N-nitrosamines studied include N-nitrosodiethylamine (4,24), N-nitrosodimethylamine (19), N-nitrosopyrrolidine (5), N-nitrosodibutylamine (6,25), N-nitrosodiethanolamine (26), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) (8,36) and N'-nitrosonornicotine (NNN) (7) (Table 1). Rats, mice and hamsters were used as experimental animals.

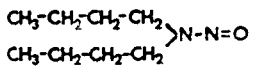
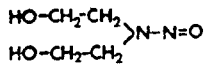
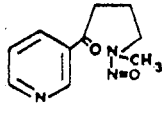
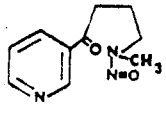
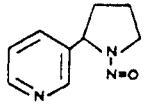
The autoradiographic studies showed that the N-nitrosamines are rapidly distributed throughout the body fluids. The autoradiography further showed an accumulation of N-nitrosamine-metabolites in several tissues in vivo (Table 1). When various tissues were tested for N-nitrosamine-metabolizing capacity in vitro, it was found that the ability of a tissue to metabolize a N-nitrosamine almost invariably correlated to a capacity of the same tissue to accumulate N-nitrosamine-metabolites in vivo. These data provide strong evidence that the localization of metabolites in a tissue in vivo is due to a local metabolism.

Examination of the data in Table 1 shows that there is a spectrum of tissues which accumulates N-nitrosamine-metabolites and which has a capacity to perform the N-nitrosamine-metabolism. Tissues active in this respect include the liver, the nasal mucosa, the tracheal mucosa, the bronchial mucosa, the mucosa of the tongue and the oesophagus and the kidney. However, variations in the tissue-specificity can be observed for different N-nitrosamines and differences may also exist between species (Table 1, Figure 1).

For most N-nitrosamines an initial α-carbon hydroxylation is considered to be the decisive and rate-limiting step in the activation process. The metabolism of the N-nitrosamines is likely to involve the cytochrome P-450-system (2,22), and the localization of N-nitrosamine-metabolites in the various extrahepatic tissues should correlate with the presence of cytochrome P-450. Indeed, cytochrome P-450-activity has been shown in most of the tissues engaged in the metabolism of the N-nitrosamines, such as the nasal epithelia and glands (9,37,39), the oesophagus (21), the epithelia of the mouth (31), the trachea (24), cells in the bronchi and bronchioles (3) and the kidney (10). However, cytochrome P-450 is not a single entity but consists of various forms which may have different substrate specificities (29). The differences seen in

Table 1. Summary of data from our studies of the tissue-disposition of N-nitrosamines.

N-nitrosamine studied and strain/species used	Structural formula	Principal tissues accumulating metabolites *in vivo*	Tissues identified to metabolize the N-nitrosamine *in vitro*	Principal target for the carcinogenicity in the strain/species
N-nitrosodiethylamine in the Syrian golden hamster [Löfberg and Tjälve, 1984 (24)]	$CH_3-CH_2 \rangle N-N=O$ CH_3-CH_2	Nasal mucosa, tracheal mucosa, mucosa of bronchi and bronchioles, lateral nasal gland, liver, kidney	Nasal mucosa, trachea, lung, lateral nasal gland, liver, kidney	Nasal cavity, trachea, lung, liver (36)
N-nitrosodiethylamine in the C57Bl mouse [Brittebo Löfberg and Tjälve, 1981 (4)]	$CH_3-CH_2 \rangle N-N=O$ CH_3-CH_2	Nasal mucosa, liver, salivary glands, mucosa of bronchi, bronchioles and trachea, oesophageal mucosa, mucosa of tongue, lacrimal glands, conjunctival part of eyelid	Nasal mucosa, liver, salivary glands, lung, oesophagus, tongue, lacrimal gland and conjunctival part of the eyelid	Not evaluated in detail in the C57Bl mouse [in other strains of mice: lung, liver, nasal cavity, oesophagus (18)]
N-nitrosodimethylamine in the C57Bl mouse [Johansson and Tjälve, 1978 (19)]	$CH_3 \rangle N-N=O$ CH_3	Liver (dominating), kidney	Not examined in the study	Liver (12,18)
N-nitrosopyrrolidine in the Sprague-Dawley rat [Brittebo, Löfberg and Tjälve, 1981 (5)]		Liver, nasal mucosa, kidney	Liver, nasal mucosa, kidney	Liver (dominating), nasal cavity (23)
N-nitrosopyrrolidine in the C57Bl mouse [Brittebo, Löfberg and Tjälve, 1981 (5)]		Liver, nasal mucosa, bronchial mucosa, tracheal mucosa, kidney	Liver, nasal mucosa, lung, trachea, kidney	Not evaluated in detail in the C57Bl mouse [lung adenomas in Swiss mice (11)]
N-nitrosodibutylamine in the Sprague-Dawley rat [Brittebo and Tjälve, 1982 (6)]	$CH_3-CH_2-CH_2-CH_2 \rangle N-N=O$ $CH_3-CH_2-CH_2-CH_2$	Nasal mucosa, liver, bronchial mucosa, oesophageal mucosa, kidney, urinary bladder	Nasal mucosa, liver, lung, oesophagus	Liver, lung, oesophagus, urinary bladder, kidney (34)

N-nitrosodibutylamine in the Syrian golden hamster [Löfberg and Tjälve, 1986 (25)]		Nasal mucosa, bronchial mucosa, tracheal mucosa, liver, kidney	Nasal mucosa, lung, trachea, liver, kidney	Nasal cavity, lung, trachea, urinary bladder (1,30)
N-nitrosodiethanolamine in the Sprague-Dawley rat [Löfberg and Tjälve, 1985 (26)]		Liver, nasal mucosa	Liver, nasal mucosa	Liver, nasal mucosa (32)
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) in the F344 rat [Castonguay, Tjälve and Hecht, 1983 (8)]		Nasal mucosa, bronchial mucosa, liver	Nasal mucosa, lung, liver	Nasal cavity, lung, liver (13)
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) in the Syrian golden hamster [Tjälve and Castonguay, 1983 (36)]		Nasal mucosa, tracheal mucosa, bronchial mucosa, liver	Nasal mucosa, trachea, lung, liver	Trachea, nasal cavity, lung (14,17)
N'-nitrosonornicotine in the Sprague-Dawley and F344 rats [Brittebo and Tjälve, 1981 (7)]		Nasal mucosa, oesophageal mucosa, mucosa of the tongue, bronchial mucosa, tracheal mucosa, liver	Nasal mucosa, oesophagus, liver	Nasal cavity, oesophagus (13,16,35)

our studies between the metabolism of the N-nitrosamines in the various tissues may reflect variations in the distribution of the cytochrome P-450 isozymes active in the metabolism of these substances.

Our data have shown that the metabolism of N-nitrosamines is very active in the epithelial linings of tissues which are exposed orally or by inhalation to drugs or other xenobiotics. It has been shown that these structures are also active in the metabolism of other compounds, such as carbon tetrachloride (37) and chloroform (28), which like the N-nitrosamines are metabolized by cytochrome P-450-dependent pathways. The role of this enzyme system may be to defend the body from unrestrained uptake of xenobiotics. However, in certain cases, and this applies to the N-nitrosamines, the metabolism may instead result in a bioactivation.

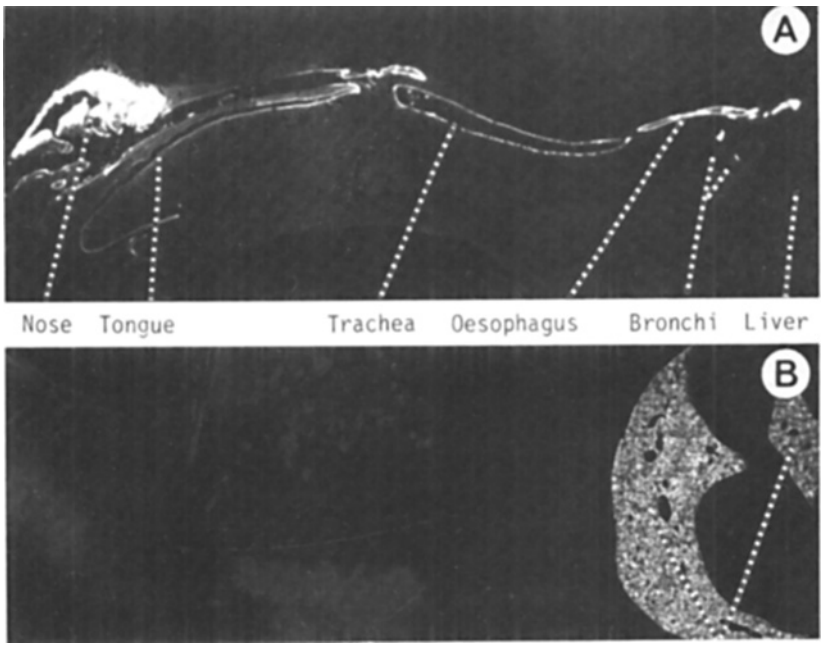


Figure 1. Whole-body autoradiography of ^{14}C-labelled N-nitrosodiethylamine (A) and ^{14}C-labelled N-nitrosodimethylamine (B) in Sprague-Dawley rats killed 1 min. after i.v. injections. The sections were dried and extracted with trichloroacetic acid and organic solvents before the autoradiographic exposure, and the autoradiograms show the distribution of firmly bound metabolites. For N-nitrosodiethylnitrosamine (A) high levels of metabolites are bound to the nasal mucosa, the mucosa of the tongue and the oesophagus and the tracheal and bronchial mucosa, whereas for N-nitrosodimethylamine (B) the localization of metabolites is restricted to the liver.

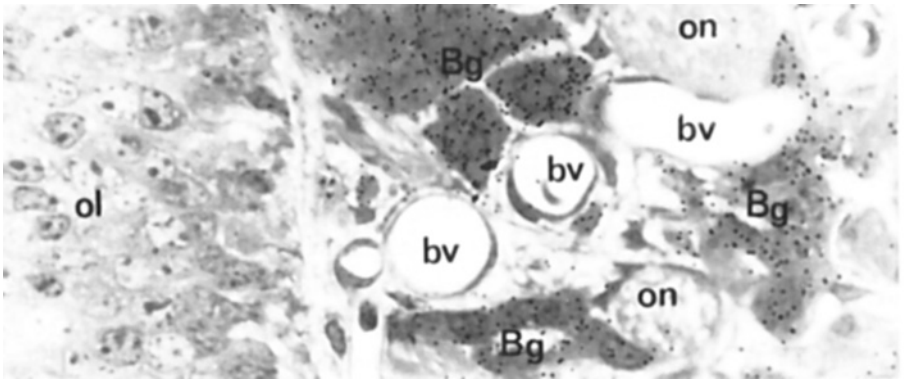


Figure 2. Microautoradiogram of the nasal olfactory mucosa of a Fischer (F344) rat killed 4 hours after a subcutaneous injection of ^{3}H-labelled N'-nitrosonornicotine. The section is from material fixed in glutaraldehyde and embedded in resin and the labelling represents metabolites firmly bound to the tissues. There is a strong labelling of the subepithelial glands (Bowman's glands), whereas other structures, such as the olfactory surface epithelium, blood-vessels and fasciculi of olfactory nerves are virtually devoid of radioactivity. **Bg** = Bowman's glands; **bv** = blood vessels; **on** = fasciculi of olfactory nerves; **ol** = olfactory surface epithelium. (x 600)

The nasal cavity has attracted special attention in our studies. This is a tissue which is one of the most prevalent sites for the N-nitrosamine carcinogenicity. The tumours usually originate from the olfactory part of the nasal cavity. We have examined in detail by microautoradiography the localization of bound metabolites in this tissue for some N-nitrosamines (24,27,38). A consistent finding in these studies has been a strong labelling of the subepithelial glands (Bowman's glands) beneath the olfactory epithelium (Figure 2). Localization of cytochrome P-450 and NADPH-cytochrome P-450 reductase in Bowman's glands has been shown histochemically (39), and the cells of these glands have been reported to be involved in N-nitrosamine carcinogenesis originating from this area of the nasal cavity.

It appears from the information in Table 1 that there is a good correlation between the ability of the tissues to accumulate N-nitrosamine metabolites/metabolize N-nitrosamines and the ability of the N-nitrosamines to induce tumours in the same tissues. These data indicate that the susceptibility of the tissues for the tumourigenesis to a considerable extent is correlated with N-nitrosamine-metabolism. However, in a few cases metabolism of N-nitrosamines was observed in tissues which were reported not to be the targets for the carcinogenicity. The reason for this is not known, but factors which may be of importance are effective repair of DNA lesions or formation in the tissues of metabolites with low miscoding frequency. N-nitrosodibutylamine, which may induce cancers in the respiratory tissues and the liver (Table 1), is unique

among N-nitrosamines in also being a strong inducer of tumours in the urinary bladder. It is generally assumed that the metabolites underlying these neoplasms are formed at other sites, probably mainly the liver (33). Thus, this target is an exception from the general rule that tumours are induced by locally formed metabolites.

CONCLUSIONS

Our data provide evidence that the localization of N-nitrosamine metabolites in the various tissues in vivo almost invariably is due to a local metabolism in the same tissues and that the tumourigenesis by the N-nitrosamines to a considerable extent is correlateed with this metabolism. The epithelial linings of the respiratory pathways and the upper digestive tract are usually very active in the N-nitrosamine-metabolism and these tissues are also prevalent sites for the N-nitrosamine-carcinogenesis. The cytochrome P-450-system which appears to be active in these sites may have the role of defending the body from noxious effects of xenobiotics, but in some instances, as with the N-nitrosamines, injuries may instead be induced.

REFERENCES

1. Althoff, J.: Simultaneous tumours in the respiratory system and urinary bladder of Syrian golden hamsters. Z. Krebforsch. 82: 153-158, 1974.
2. Appel, K.E., Ruf, H.H., Mahr, B., Schwarz, M., Rickart, R. and Kunz, W.: Binding of nitrosamines to cytochrome P-450 of liver microsomes. Chem.-Biol. Interact. 28: 17-33, 1979.
3. Boyd, M.R.: Evidence for the Clara cells as a site of cytochrome P450-dependent mixed-function oxidase activity in the lungs. Nature 269: 713-715, 1977.
4. Brittebo, E.B., Löfberg, B. and Tjälve, H.: Sites of metabolism of N-nitrosodiethylamine in mice. Chem.-Biol. Interact. 34: 209-221, 1981.
5. Brittebo, E.B. Löfberg, B. and Tjälve, H.: Extrahepatic sites of metabolism of N-nitrosopyrrolidine in mice and rats. Xenobiotica 11: 619-625, 1981.
6. Brittebo, E.B. and Tjälve, H.: Tissue-specificity of N-nitrosodibutylamine metabolism in Sprague-Dawley rats. Chem.-Biol. Interact. 38: 231-242, 1982.
7. Brittebo, E.B. and Tjälve, H.: Formation of tissue-bound N'-nitrosonornicotine metabolites by the target tissues of Sprague-Dawley and Fischer rats. Carcinogenesis 2: 959-963, 1981.
8. Castonguay, A., Tjälve, H. and Hecht, S.S.: Tissue distribution of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its metabolites in F344 rats. Cancer Res. 43: 630-638, 1983.
9. Dahl, A.R., Hadley, W.M., Hahn, F.F., Benson, J.M. and McClellan, R.O.: Cytochrome P-450-dependent monooxygenases in the olfactory epithelium of dogs: possible role in tumorigenicity. Science 216: 57-59, 1982.
10. Endou, H.: Cytochrome P-450 monooxygenase system in the rabbit kidney: Its intranephron localization and its induction. Jpn J. Pharmacol. 33: 423-430, 1983.
11. Greenblatt, M. and Lijinsky, W.: Failure to induce tumours in Swiss mice after concurrent administration of amino acids and sodium nitrite. J. Natl Cancer Inst. 48: 1389-1392, 1972.
12. Griciute, L., Castegnaro, M. and Bereziat, J.-C.: Influence of ethyl alcohol on carcinogenesis with N-nitrosodimethylamine. Cancer Lett. 13: 345-352, 1981.

13. Hecht, S.S., Chen, C.B., Ohmori, T. and Hoffmann, D.: Comparative carcinogenicity in F344 rats of the tobacco-specific nitrosamines, N'-nitrosonornicotine and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res. 40: 298-302, 1980.
14. Hecht, S.S., Adams, J.D., Numoto, S. and Hoffmann, D.: Induction of respiratory tract tumours in Syrian golden hamsters by a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and the effect of smoke inhalation. Carcinogenesis 4: 1287-1290, 1983.
15. Herrold, K.M. and Dunham, L.J.: Induction of tumors in the Syrian hamster with diethylnitrosamine (N-nitrosodiethylamine). Cancer Res. 23: 773-777, 1963.
16. Hoffmann, D., Rainieri, R., Hecht, S.S. and Maronpot, R.R.: Effects of N'-nitrosonornicotine and N'-nitrosoanabasine in rats. J. Natl Cancer Inst. 55: 977-981, 1975.
17. Hoffmann, D., Castonguay, A., Rivenson, A. and Hecht, S.S.: Comparative carcinogenicity and metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine in Syrian golden hamsters. Cancer Res. 41: 2386-2393, 1981.
18. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Some N-Nitroso Compounds, Vol. 17, IARC, Lyon, 1980.
19. Johansson, E.B. and Tjälve, H.: The distribution of [^{14}C]dimethylnitrosamine in mice: autoradiographic studies in mice with inhibited and non-inhibited dimethylnitrosamine metabolism and a comparison with the distribution of [^{14}C]formaldehyde. Toxicol. Appl. Pharmacol. 45: 565-575, 1978.
20. Johansson-Brittebo, E. and Tjälve, H.: Studies on the tissue-disposition and fate of N-[^{14}C]ethyl-N-nitrosourea in mice. Toxicology 13: 275-285, 1979.
21. Labuc, G.E. and Archer, M.C.: Esophageal and hepatic microsomal metabolism of N'-nitrosomethylbenzylamine and N-nitrosodimethylamine in the rat. Cancer Res. 42: 3181-3186, 1982.
22. Lai, D.Y., Arcos, J.C. and Argus, M.F.: Factors influencing the microsome- and mitochondria-catalyzed in vitro binding of diethylnitrosamine and N-nitrosopiperidine to deoxyribonucleic acid. Biochem. Pharmacol. 28: 3545-3550, 1979.
23. Lijinsky W. and Taylor H.W.: The effect of substituents on the carcinogenicity of N-nitrosopyrrolidine in Sprague-Dawley rats. Cancer Res. 36: 1988-1990, 1976.
24. Löfberg, B. and Tjälve, H.: The disposition and metabolism of N-nitrosodiethylamine in adult, infant and foetal tissues of the Syrian golden hamster. Acta Pharmacol. Toxicol. 54: 104-114, 1984.
25. Löfberg, B. and Tjälve, H.: Perinatal metabolism of N-nitrosodibutylamine in Syrian golden hamsters. Cancer Lett. 31: 153-161, 1986.
26. Löfberg, B. and Tjälve, H.: Autoradiography of [^{14}C]N-nitrosodiethanolamine in Sprague-Dawley rats. Cancer Lett. 26: 129-137, 1985.
27. Löfberg, B., Brittebo, E.B. and Tjälve, H.: Localization and binding of N'-nitrosonornicotine metabolites in the nasal region and in some other tissues of Sprague-Dawley rats. Cancer Res. 42: 2877-2883, 1982.
28. Löfberg, B. and Tjälve, H.: Tracing tissues with chloroform-metabolizing capacity in rats. Toxicology 39: 13-35, 1986.
29. Lu, A.Y.H. and West, S.B.: Multiplicity of mammalian microsomal cytochromes P-450. Pharmacol. Rev. 31: 277-295, 1980.
30. Mohr, U., Althoff, J., Schmähl, D. and Krüger, F.W.: The carcinogenic effect of dibutylnitrosamine in Syrian and Chinese hamsters. Z. Krebsforsch. 74: 112-113, 1970.
31. Rao, G.S.: Drug metabolism in oral soft tissues. Pharm. Int. 4: 103-107, 1983.
32. Preussmann, R., Habs, M., Habs, H. and Schmähl, D.: Carcinogenicity of N-nitrosodiethanolamine in rats at five different dose levels. Cancer Res. 42: 5167-5171, 1982.

33. Okada, M.: Comparative metabolism of N-nitrosamines in relation to their organ and species specificity. In: N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer (eds I.K. O'Neill, R.C. von Borstel, C.T. Miller, J. Long and H. Bartsch) pp. 401-409, IARC Scientific Publications No. 57, IARC, Lyon, 1984.
34. Rogers, A.E., Sanchez, O., Feinsod, F.M. and Newberne, P.M.: Dietary enhancement of nitrosamine carcinogenesis. Cancer Res. 34: 96-99, 1974.
35. Singer, G.M. and Taylor, H.W.: Carcinogenicity of N'-nitrosonornicotine in Sprague-Dawley rats. J. Natl Cancer Inst. 57: 1275-1276, 1976.
36. Tjälve, H. and Castonguay, A.: The in vivo tissue disposition and in vitro target-tissue metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in Syrian golden hamsters. Carcinogenesis 4: 1259-1265, 1983.
37. Tjälve, H. and Löfberg, B.: Extrahepatic sites of metabolism of carbon tetrachloride in rats. Chem.-Biol. Interact. 46: 299-316, 1983.
38. Tjälve, H., Castonguay, A. and Rivenson, A.: Microautoradiographic localization of bound metabolites in the nasal cavities of F344 rats treated with the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. J. Natl Cancer Inst. 74: 185-189, 1985.
39. Voigt, J.M., Guengerich, F.P. and Baron, J.: Localization of a cytochrome P-450 isozyme (cytochrome P-450 PB-B) and NADPH-cytochrome P-450 reductase in rat nasal mucosa. Cancer Lett. 27: 241-247, 1985.

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