

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Tobacco-specific *N*-nitrosamines, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N'*-nitrosonornicotine (NNN), *N'*-nitrosoanabasine (NAB) and *N'*-nitrosoanatabine (NAT), occur widely in tobacco and tobacco smoke. They are formed by the nitrosation of nicotine and other tobacco alkaloids and have been detected in green tobacco leaves from *Nicotiana tabacum* and *N. rustica* species; however, the largest quantities of tobacco-specific *N*-nitrosamines are formed during tobacco curing and processing and additional amounts are formed during smoking. Tobacco-specific *N*-nitrosamines occur in all

commercially and non-commercially prepared tobacco products including cigarettes, cigars, *bidis*, pipe tobacco and smokeless tobacco products. *N*-Nitrosamines occur in a wide variety of both food and non-food products, but the amounts of tobacco-specific *N*-nitrosamines in all tobacco products exceed the levels of other *N*-nitrosamines in other commercial products by several orders of magnitude. The highest levels of tobacco-specific *N*-nitrosamines are measured in smokeless tobacco products. For example, levels of NNK up to 17.8 µg/g have been measured in North American and European smokeless tobacco products; up to 245 µg/g have been measured in products used in India; and up to 7870 µg/g have been measured in Sudanese *toombak*. Levels of NNN up to 135 µg/g have been measured in North American and European smokeless tobacco products; up to 1356 µg/g have been measured in products used in India; and up to 3085 µg/g have been measured in Sudanese *toombak*. These compounds are also present in secondhand tobacco smoke. The degree of exposure to tobacco-specific *N*-nitrosamines depends not only on the levels of these compounds in tobacco products or smoke, but also on the manner in which the products are used.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

NNK

In numerous studies in mice, NNK induced lung adenomas independent of the route of administration.

In studies by subcutaneous injection, benign and malignant tumours of the lung, nasal cavity and liver were induced in rats. In two of four experiments in hamsters, lung adenomas and adenocarcinomas or adenosquamous carcinomas were induced in males and females. In the two other experiments, adenomas were observed. Nasal cavity tumours involving the forebrain were observed in a limited study in mink.

In a study by administration in the drinking-water and another by oral swabbing, combined benign and malignant lung tumours (adenoma, adenosquamous carcinoma and carcinoma) were induced in male rats. In the drinking-water study, NNK produced benign and malignant pancreatic tumours. In the oral swabbing study, combined benign and malignant tumours of the liver and nasal cavity were observed. A significant increase in the incidence of liver and lung tumours was reported in female rats when NNK was instilled into the urinary bladder.

In two studies, the offspring of mice were exposed transplacentally by intraperitoneal injection of the dams. Liver tumours were observed in male offspring in both studies and in female offspring in one study. In one of these studies, lung tumours were also observed in male offspring.

In studies of the offspring of hamsters given NNK during pregnancy, intratracheal instillation of the dams resulted in adenocarcinomas of the nasal cavity in male offspring and adrenal pheochromocytomas in male and female offspring in one study. In a second study, subcutaneous injection of NNK into dams induced respiratory tract (nasal cavity, larynx and trachea) tumours in male and female offspring. When dams were injected subcutaneously or treated by intratracheal instillation, nasal cavity and adrenal gland tumours developed in male and female offspring in a third study.

Intraperitoneal administration of NNK-*N*-oxide induced lung adenomas in female mice.

In an oral swabbing study, NNK in combination with NNN increased the incidence of oral tumours in rats.

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)

In one study in male rats in which NNAL, a principal metabolite of NNK, was administered in the drinking-water, adenomas, adenocarcinomas and adenosquamous carcinomas of the lung and benign and malignant pancreatic tumours were induced.

In three studies in female mice, intraperitoneal injection of NNAL induced lung adenomas. In one of these studies, adenocarcinomas were also observed.

NNN

In four studies in female mice, intraperitoneal injection of NNN produced lung adenomas. In another study in mice, lung adenomas were induced by intraperitoneal injection of NNN in males and females.

In two studies in rats in which NNN was given in the drinking-water and one study in which it was added to a liquid diet, benign and malignant oesophageal tumours were observed in males and females. Benign nasal cavity tumours were also observed in rats treated through the liquid diet.

In rats, subcutaneous injection of NNN induced malignant or benign (combined) and malignant nasal cavity tumours in males and females in two studies. In one study in hamsters, subcutaneous injection of NNN induced tracheal tumours in males and females and benign and malignant tumours of the nasal cavity in males. In two limited studies in mink treated with NNN by subcutaneous injection, nasal cavity tumours that invaded the forebrain were observed in females.

Skin application of NNN in female mice induced a non-significant increase in the incidence of skin papillomas and carcinomas.

Metabolites of NNN (3'-hydroxy-NNN, 4'-hydroxy-NNN or NNN-1-*N*-oxide) were tested by intraperitoneal injection into female mice and resulted in the induction of lung adenomas in mice exposed to 4'-hydroxy-NNN. Administration of NNN-1-*N*-oxide in the drinking-water increased the incidence of benign and malignant oesophageal tumours in male and female rats in one study and that of colon tumours in female hamsters in another study.

In one oral swabbing study, NNN in combination with NNK increased the incidence of oral tumours in rats.

NAB

In one study in female mice, intraperitoneal injection of NAB induced lung adenomas.

In one study in rats in which NAB was administered in the drinking-water, oesophageal carcinomas and/or papillomas were induced in males and females. Another study in male rats gave negative results.

Subcutaneous injection of NAB into hamsters gave negative results in one study.

NAT

Subcutaneous injection of NAT into male and female rats did not induce tumours at any site.

5.4 Other relevant data

NNK and its metabolite NNAL

Extensive studies have examined the metabolism of NNK and the formation of DNA adducts by NNK and its metabolite NNAL in humans and laboratory animals; the metabolic pathways and structures of DNA adducts have been characterized comprehensively. NNK and NNAL have been detected in the saliva of smokeless tobacco users, and NNAL and another metabolite of NNK, NNAL-glucuronide, have been quantified in human urine. The presence of these metabolites, which are specific to exposure to tobacco products (e.g. in smokers, users of smokeless tobacco and nonsmokers exposed to secondhand tobacco smoke), signals human uptake and metabolism of NNK, and their quantification allows an estimation of the dose of NNK absorbed. Dose calculations show that the total amounts of NNK taken up by people who used tobacco products for a period of 30 years or more approximate the total amounts that induce tumours in rats.

The metabolic activation of NNK and NNAL to DNA adducts is critical for the expression of their carcinogenic activities. The metabolic activation process has been documented extensively in laboratory animals. Cytochrome P450 enzymes are the principal catalysts of this process, and those in the 2A family appear to be the most efficient in both humans and laboratory animals. Macromolecular adducts formed after the metabolic activation of NNK and/or NNN have been detected in smokers, in smokeless tobacco users and in laboratory animals treated with these carcinogens. In laboratory animals, persistence of these adducts is associated with tumour formation.

NNK is a genotoxic compound. It was shown to be mutagenic in bacteria, in rodent fibroblasts and in human lymphoblastoid cells *in vitro*. It caused cytogenic effects in a variety of mammalian cells *in vitro* and induced transformation of the pancreatic duct cells of hamsters. *In vivo*, NNK induced micronucleus formation in the bone marrow of

mice and DNA strand breaks in the hepatocytes of rats and hamsters. NNAL was reported to be mutagenic in *Salmonella* in a single study.

In addition to the classical mechanisms of carcinogenesis that proceed through the formation of DNA adducts, NNK also binds to nicotinic and other receptors, which leads to downstream effects that contribute to the development of cancer. These effects have been observed in experimental systems including pancreatic and lung cells from humans and laboratory animals.

NNN

The major route of metabolic activation to DNA adducts is α -hydroxylation adjacent to the nitroso group, which is mediated principally by cytochrome P450 enzymes and, in particular, by those of the 2A family. The human oesophagus catalyses α -hydroxylation of NNN, and this process is especially efficient in the rat oesophagus and nasal mucosa, which are target tissues for the carcinogenicity of NNN. NNN has been detected in the saliva of smokeless tobacco users. The uptake and metabolism of NNN by smokers and smokeless tobacco users has been demonstrated by its quantitation and that of its glucuronide in human urine. The metabolic pathways of NNN have been characterized extensively in laboratory animals and there are distinct parallels can be seen between the metabolism of NNN in humans and laboratory animals.

NNN is a genotoxic compound. It was shown to be mutagenic in bacteria, but not in mammalian cells *in vitro*. NNN induced DNA strand breaks in human fetal lung cells and in primary rat hepatocytes *in vitro*. It did not show cytogenetic activity *in vitro*, but induced micronuclei in the bone marrow of mice *in vivo* in a single study.

NAB

NAB has been detected in the saliva of *toombak* users and has been quantified together with its glucuronide in the urine of smokers and smokeless tobacco users. The metabolism of NAB by α -hydroxylation and other pathways has been characterized in rats.

The genotoxicity of NAB has not been tested extensively. It was shown to be mutagenic in various strains of *Salmonella typhimurium*, each of which co-expressed a different form of human cytochrome P450 enzyme.

NAT

NAT has been detected in the saliva of smokeless tobacco users, and has been quantified together with its glucuronide in the urine of smokers and smokeless tobacco users.

The genotoxicity of NAT has not been tested extensively. It was shown to be mutagenic in a strain of *S. typhimurium* that expressed human cytochrome P450 2A6.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of tobacco-specific N-nitrosamines.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).

There is *sufficient evidence* in experimental animals for the carcinogenicity of N'-nitrosonornicotine (NNN).

There is *limited evidence* in experimental animals for the carcinogenicity of N'-nitrosoanabasine (NAB).

There is *inadequate evidence* in experimental animals for the carcinogenicity of N'-nitrosoanatabine (NAT).

Overall evaluation

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are *carcinogenic to humans (Group 1)*.

N'-Nitrosoanabasine (NAB) is *not classifiable as to its carcinogenicity to humans (Group 3)*.

N'-Nitrosoanatabine (NAT) is *not classifiable as to its carcinogenicity to humans (Group 3)*.

In making the overall evaluation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine, the Working Group took into consideration the following mechanistic evidence (detailed in Section 5.4).

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine are the most abundant strong carcinogens in smokeless tobacco; uptake and metabolic activation in smokeless tobacco users have been clearly observed. In rats, combined application of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine induced oral tumours consistent with their induction by smokeless tobacco. One of the mechanisms of carcinogenicity is cytochrome P450-mediated α -hydroxylation, which leads to the formation of DNA and haemoglobin adducts that are commonly detected in users of tobacco.