PYRIDOSTIGMINE BROMIDE

CAS NO. 101-26-8

Prepared for NCI by Technical Resources International, Inc

under Contract No. NO1-CP-56919 (11/93; rev.4/94)

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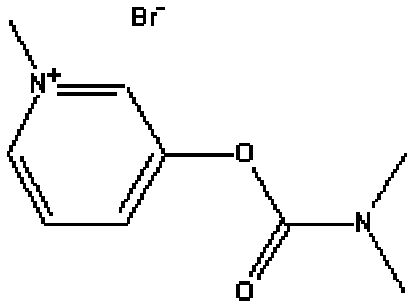


$C_9H_{13}N_2O_2^{\cdot}Br$ Mol. wt.: 261.14

BASIS OF NOMINATION TO THE CSWG

The nomination of pyridostigmine bromide (PB) is based on widespread human exposure during the Persian Gulf War. PB is an anticholinesterase agent which inhibits the hydrolysis of acetylcholine. During the Gulf War it was administered to as many as 400,000 soldiers as protection against nerve agents. It is the drug of choice for symptomatic treatment of myasthenia gravis and is used for reversal of nondepolarizing neuromuscular blocking agents after surgery.

Concern about the possible link of PB to Gulf War veterans' unexplained illnesses has led an Institute of Medicine (IOM) panel to recommend animal studies on the synergistic effects of PB, N,N'-diethyl-m-toluamide (DEET) and permethrin. In addition, testimony to the Veterans Affairs Committee focused on the lack of information on PB.

PB was not clastogenic in the rat micronucleus assay. No drug-related lesions were noted in subchronic studies with rats or dogs. 3-Hydroxy-N-methylpyridinium is the main metabolite of PB in man; however, several minor metabolites have not been fully characterized. PB is structurally related to neostigmine bromide and neostigmine methylsulfate which have demonstrated significant inhibition of chemically-induced liver, stomach, or colon tumors in rats, presumably through a parasympathomimetic mediated mechanism.

SELECTION STATUS

ACTION BY THE CSWG: 6/13/95

Studies Requested:

- Toxicity studies of pyridostigmine bromide (PB) alone, and in combination with other chemicals, such as pesticides, other organophosphorus compounds, carbamates or other relevant chemicals

Priority: High

Rationale/Remarks:

- Significant human exposure

- Used by 400,000 soldiers in the Gulf War as a pretreatment for exposure to nerve agents

- Concern about possible link to unexplained illness of Gulf War veterans

- Army is interested in testing PB alone and in combination with other chemicals to investigate potential synergistic effects, and has issued RFP for such studies

- Recommend that NTP contact Army to discuss the feasibility of doing collaborative studies of PB

- Studies of synergistic effects in animal would provide guidelines as to kinds of adverse effects that might be observed in humans exposed to PB in conjunction with other chemicals

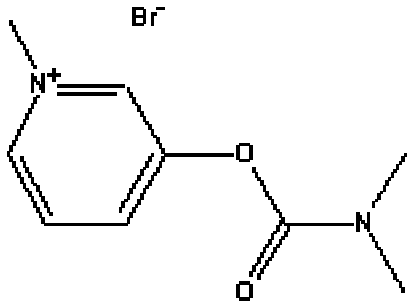
- Contact Army and Veterans Administration to ascertain whether any followup study of Gulf War veterans is in progress

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

A spokesperson for ICN Pharmaceuticals, Inc., verified that they had no chronic animal studies for PB. However, he noted that follow-up safety evaluations of the drug have uncovered no indications of serious long-term effects in patients on PB for life (Granger, 1995).

The FDA furnished copies of the New Drug Application (NDA) for PB for the Summary Basis for Approval and for the toxicology data on PB (FDA, 1995).

CHEMICAL IDENTIFICATION



CAS Registry No.: 101-26-8

Chemical Abstracts Name: 3-[[(Dimethylamino)carbonyl]oxy]-1-methylpyridinium bromide

Synonyms and Trade Names: 3-(Dimethylcarbamyloxy)-1-methylpyridinium bromide; 3-hydroxy-1-methylpyridinium bromide dimethylcarbamate; 1-methyl-3-hydroxypyridinium bromide dimethylcarbamate; Kalymin; Mestinon; Mestinon Bromide; Regonol; Ro 1-5130, [Pyridostigmine[a]]

[a] In the published literature, pyridostigmine is often used interchangeably with pyridostigmine bromide and pyridostigmine iodide.

Chemical and Physical Properties:

Description: Shiny, hygroscopic crystals from absolute ethanol (Budavari, 1989). Hygroscopic, white or practically white crystalline powder; characteristic agreeable odor and bitter taste (McEvoy, 1992)

Melting Point: 152-154°C (Budavari, 1989)

Solubility: Freely soluble in ethanol and water. Practically insoluble in diethyl ether, acetone and benzene (Budavari, 1989). Freely soluble in chloroform. Slightly soluble in hexane (Gennaro, 1990)

Reactivity: Unstable in alkaline solutions (McEvoy, 1992).

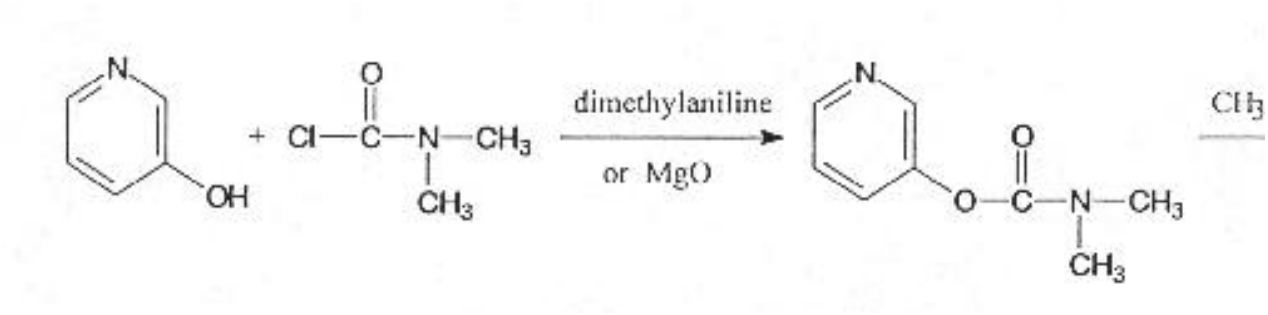
Technical Products and Impurities: The USP grade of pyridostigmine bromide contains not less than 98.5% and not more than 100.5% of $C_9H_{13}N_2O_2 \cdot Br$, calculated on the dried basis. The USP grade of pyridostigmine bromide injection is a sterile solution of pyridostigmine bromide in a suitable medium. It contains not less than 90.0% and not more than 110.0% of the labeled amount of $C_9H_{13}N_2O_2 \cdot Br$. The USP grade of

pyridostigmine bromide syrup contains, in each 100 ml, not less than 1.08 g and not more than 1.32 g of $C_9H_{13}N_2O_2 \cdot Br$. The USP grade of pyridostigmine bromide tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of $C_9H_{13}N_2O_2 \cdot Br$ (US Pharmacopeia, 1995).

Dosage forms include the following injectable solutions, tablets and syrups: Mestinon (ICN Pharmaceuticals, Inc.) 60 mg/tablet; Mestinon Injectable (ICN) 5 mg/ml with parabens 0.2%; Mestinon Syrup (ICN) 60 mg/5 ml with alcohol 5%; Mestinon Timespan (ICN) 180 mg/extended release tablet (McEvoy, 1992); Regonol injection (Organon, Inc.), 5 mg/ml with benzyl alcohol (PDR, 1995).

EXPOSURE INFORMATION

Production and Producers: A US patent for the synthesis pyridostigmine bromide (PB) was issued to Hoffmann-La Roche in 1951 (Budavari, 1989). PB is prepared by condensing 3-pyridinol with dimethylcarbamoyl chloride in the presence of a suitable basic catalyst such as dimethylaniline or magnesium oxide. The resulting ester, 3-pyridyl dimethylcarbamate, is isolated, dissolved in a suitable organic solvent, and quaternized with methyl bromide (Gennaro, 1990).



The United States Tariff Commission and the United States International Trade Commission list PB in their annual reports, but with no specific production levels or sales value. Based on its production category, PB must have a minimal production level of 450 kg [1,000 lbs] and/or a sales value of at least $1,000 (USTC 1969, 1974; USITC 1977-1979, 1981-1991, 1993, 1994).

Annual prescription data for Mestinon Bromide and Mestinon Timespan for 1973-1979 are presented in Table 1 (National Prescription Audit, 1980). The data reflect prescriptions for one supplier only. Although more recent information is not available, the data would seem to indicate a fairly steady demand level.

Mestinon is manufactured by Hoffmann-La Roche, Inc. and is licensed to ICN Pharmaceuticals, Inc. The Regonol brand of PB is manufactured by Organon, Inc. (PDR, 1995).

Use Pattern: PB is a reversible acetylcholinesterase (AChE) inhibitor which competes with acetylcholine for binding to AChE and, like acetylcholine, is hydrolyzed by the enzyme. However, hydrolysis of PB proceeds much more slowly than that of

acetylcholine, resulting in effective inhibition of acetylcholine hydrolysis and enhancement of muscarinic stimulation (Taylor, 1980 as cited in Kluwe *et al*., 1990). For this reason, PB is the drug of choice for the

Table 1. Number of new and refilled prescriptions (US) (thousands)

Year	Mestinon Bromide	Mestinon Timespan
1973	29	6
1974	43	14
1975	60	16
1976	43	16
1977	44	10
1978	66	16
1979	61	16

treatment of myasthenia gravis and has been used for this purpose for nearly 40 years. Many patients with this rare disorder of neuromuscular function can be adequately controlled on oral PB although the dosages needed to improve muscle strength may vary from 30 to 2,000 mg/day (Greene, 1969 as cited in Calvey & Chan, 1977; Calvey & Chan, 1977; Morgan *et al*., 1990a).

PB is a suitable anticholinesterase drug for the reversal of surgically induced non-depolarizing neuromuscular blockade induced by curariform drugs (McNall *et al*., 1969). The prescribed intravenous (iv) doses of PB for this purpose are 10-20 mg (McEvoy, 1992).

PB has been accepted by the US military as pretreatment for exposure to organophosphate chemical warfare agents since 1986. The prescribed prophylactic dose is 30 mg, 3 times a day (Gouge *et al.*, 1994).

Additional uses include doses of 60 to 240 mg in the treatment of paralytic ileus and postoperative urinary retention, and 60 mg up to 3 times a day to relieve severe constipation in patients with Parkinson's disease (Reynolds, 1993).

Human Exposure: The greatest potential for human exposure to PB is through its use in prescription drugs. The number of patients being treated with Mestinon from ICN was reported to be about 6000 (F-D-C Reports, 1989). An estimated 400,000 soldiers were given PB to self-administer as pretreatment for nerve gas exposure during the Persian Gulf War (F-D-C Reports, 1994).

Data from the National Occupational Exposure Survey (NOES) conducted by the

National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983 indicate that 1,619 US workers, including 791 female employees, were potentially exposed to PB in the workplace (NIOSH, 1994). The NOES database does not contain information on the frequency, level or duration of exposure to workers of any chemicals listed therein.

Environmental Occurrence: PB has not been reported to occur naturally. No information was found in the available literature on occurrences of PB in environmental media.

Regulatory Status: PB is approved by the FDA for use in treating myasthenia gravis and for reversing the effects of nondepolarizing neuromuscular blocking agents (PDR, 1995).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposures to pyridostigmine bromide (PB) and cancer risk in humans were identified in the published literature. Adverse effects of PB are chiefly those of exaggerated response to parasympathetic stimulation and include adverse muscarinic effects such as nausea, vomiting, diarrhea, increased peristalsis, miosis, excessive salivation and sweating, increased bronchial secretions, abdominal cramps, bradycardia, and bronchospasm. Nicotinic side effects include weakness, muscle cramps, and fasciculation. Extremely high doses may produce central nervous system symptoms of agitation, restlessness, confusion, visual hallucination, and paranoid delusions. Overdosage can cause cholinergic crisis and death. As with other drugs containing bromide, skin rash may occasionally occur during therapy (McEvoy, 1992; PDR, 1995).

Several studies have examined the adverse effects of PB use during the Persian Gulf War. In one such study, Keeler and coworkers (1991) reported that about half the study population of 41,650 soldiers instructed to take the drug at the onset of hostilities noted gastrointestinal changes that included increased flatus, abdominal cramps, soft stools, and nausea. While under the threat of nerve-agent attack, the drug was self-administered by the troops (30 mg orally every 8 hours for 1 to 7 days). Other reported effects were urinary urgency, headaches, rhinorrhea, diaphoresis, and tingling of the extremities. Fewer than 0.1% of the soldiers had effects sufficient to discontinue the drug. Seventy-five percent of 213 Israeli soldiers surveyed by Sharabi and coworkers (1991) reported at least one symptom following PB use. The most frequent complaints were nonspecific and included dry mouth, general malaise, fatigue, and weakness. Typical effects, such as nausea, abdominal pain, frequent urination and rhinorrhea, were infrequent. The severity of symptoms was generally mild and no correlation was found between levels of cholinesterase and type or severity of complaints. Both of these studies found that the pyridostigmine regimen followed by soldiers under wartime conditions caused a higher incidence of adverse physiologic events than had been reported in earlier peacetime evaluations. It was felt that the combined stresses of anticipated combat, sleep deprivation, and life in the field may have affected or modified many of these responses.

Gouge and coworkers (1994) observed exacerbation of asthma symptoms in 7 of 10 asthmatic soldiers given a single 30 mg PB dose. The authors postulated that the increased irritant effect of desert dust might have predisposed these asthmatics to worsen

after PB treatment, an effect not seen in the laboratory.

The unexplained illnesses experienced by Gulf War veterans, as well as research findings of a 10-fold PB-enhancement of the toxicity of DEET in cockroaches, has led to concern regarding the synergistic effects of PB and insecticides used by the soldiers in the field (Anon., 1994; Ember, 1995). Recent news reports cite a Duke University study in which these synergistic effects resulted in neuropathies in chickens (Washington Post, 1995).

Few data are available regarding the effects of cholinesterase inhibitors, including PB, on the fetus because of the rarity of maternal conditions requiring the use of these drugs during pregnancy. Transient muscular weakness has occurred in 10-20% of neonates whose mothers received anticholinesterase drugs for the treatment of myasthenia gravis, although similar symptoms have also been reported in infants whose mothers were not treated with these drugs (McEvoy, 1992). Anticholinesterase drugs may cause uterine irritability and induce premature labor when given iv to pregnant women near term. Although PB is not known to cause fetal injury or malformation, there are no adequate studies to support its safety during pregnancy (Flagg, 1991; McEvoy, 1992; PDR, 1995).

Animal Data: No 2-year carcinogenicity studies of PB in animals were identified in the published literature. Toxicity information identified was limited to acute and subchronic studies.

The 180-day subchronic oral toxicity of PB was evaluated in 69 male Sprague-Dawley rats. PB was administered in the diet at doses of 0, 1, and 10 mg/kg/day every day, and 10 mg/kg/day 5 days a week for 180 days. Following the 180-day dosing period, subgroups of animals from the control and both 10 mg/kg groups were subjected to a 30-day recovery period during which the test compound was not administered. No morphologic evidence of PB-induced toxicity was observed. All gross lesions were considered to be incidental findings commonly observed in Sprague-Dawley rats. Microscopic lesions with significantly increased incidence in pyridostigmine-treated groups compared to controls included chronic, multifocal hepatic inflammation found in the 10 mg/kg/daily group necropsied at 180 days ($P < 0.05$) and brown pigment, probably hemosiderin, within splenic macrophages found in the 10 mg/kg 5 days a week group necropsied at 210 days ($P \leq 0.05$). These microscopic lesions were also considered to be incidental findings unrelated to treatment. After 180 days, doses of PB that produced up to 63% cholinesterase inhibition in plasma and 49% acetylcholinesterase inhibition in erythrocytes did not have toxic effects other than increased startle reflex associated with the decrease in cholinesterase activity. Increases in aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase were observed at 210 days but the changes could not be attributed to compound administration/ withdrawal (Morgan *et al.*, 1990a).

The same researchers studied the 90-day subchronic oral toxicity of PB in 104 male and 104 female Sprague-Dawley rats. Administration in the diet at 0, 1, 10, 30, 60, and 90 mg/kg a day for 90 days resulted in dose-related decreases in plasma cholinesterase and erythrocyte acetylcholinesterase activity ranging from 5% to 76% and from 18% to 95%, respectively. Toxic signs associated with the decrease in cholinesterase activity included muscarinic (perianal, perioral, and periocular stains or material, diarrhea, and increased salivation) and nicotinic (hypertonia and tremors) effects. No compound-related gross or

microscopic lesions were observed. Blood samples taken at necropsy for hematological and serum chemistry analyses exhibited no significant abnormalities (Morgan *et al.*, 1990b).

Three short-term oral dosing studies were conducted with male and female beagle dogs in order to evaluate the preclinical safety of repeated PB administration. The drug was administered by capsule gavage once a day at 5, 10, or 20 mg/kg for 14 days to 10 dogs of each sex; every 8 hours at 2 or 5 mg/kg for 28 days to 6 dogs of each sex; or every 8 hours at 0.05, 0.5, or 2 mg/kg for 3 months to 37 dogs of each sex. A small portion of the dogs receiving PB for 3 months were allowed an untreated recovery period of an additional 3 months. In the 14-day study, signs of acute anticholinesterase intoxication occurred in all three dose groups. These included lacrimation, hypersalivation, diarrhea or soft stools, occasional emesis, muscle fasciculation, tremors, and occasional convulsions. No lesions were observed at necropsy except for the dogs that died or were euthanized during the study. These four animals exhibited a reddened mucosa in the large and small intestines, occasional ulcerations in the small intestine or colon and ileal intussusception. No morphological abnormalities were observed upon microscopic examination of the diaphragm muscle, a potential target organ. Signs of toxicity in the 28-day and 3-month studies were generally limited to the gastrointestinal tract and included diarrhea or soft stools and reddened or mucoid-containing stools. A single dog given 2 mg/kg every 8 hours developed an apparent intussusception. There were no pathological changes in clinical chemistry, hematology, or urinalysis parameters associated with PB administration for up to 3 months, nor were any drug-related lesions observed upon gross necropsy and microscopic evaluation of the major tissues and organs. These studies suggest that prolonged oral administration of PB at doses sufficient to cause as high as 70% inhibition of red blood cell acetylcholinesterase activity cause mainly local, gastrointestinal distress related to altered intestinal motility (Kluwe *et al.*, 1990).

Gebbers and coworkers (1986) assessed the morphological changes in 26 male and female Tif:RAI f rats following single, sub-lethal gavage doses of PB. Within 24 hours of 20 or 40 mg/kg doses, acute focal necroses, leukocytic infiltrates, and marked changes in the motor endplates appeared in the skeletal muscle. Changes were more evident in the diaphragm than in the quadriceps muscle. Bowman and coworkers (1989) also reported myopathic changes in the diaphragm of 18 male Sprague-Dawley rats following administration of 90 mg/kg pyridostigmine in the diet for 15 days. Within the first day of dosing, 1% of the myofibers in the diaphragm were damaged. By 7 days, although myofibers were damaged as evidenced by centralized nuclei, dilated sarcoplasmic reticulum and disruption of Z-bands, they appeared less severely damaged than those examined earlier, indicative of some mechanism of accommodation that minimizes continued muscle injury.

Pyridostigmine bromide was a nonirritant in a modified Draize dermal irritation assay in New Zealand white rabbits (Magnuson *et al.*, 1990). In guinea pig skin sensitization studies, PB was found to be a potential contact sensitizer that showed a potentiated response in the presence of surfactants. The formulations tested included 50% pyridostigmine bromide, 30% pyridostigmine bromide with 0.198% sodium lauryl sulfate, and 30% pyridostigmine bromide with 0.21% of a proprietary surfactant (Harris

& Maibach, 1989).

The reproductive and developmental toxicity of PB was evaluated through the following gavage studies in Sprague-Dawley rats: fertility study, a) male rats received doses of 0, 5, 15, or 45 mg/kg a day for at least 70 days prior to mating with untreated females or b) female rats received doses of 0, 5, 15, or 45 mg/kg a day for at least 14 days prior to co-housing with untreated males; perinatal/postnatal study, sperm-positive female rats received doses of 0, 3, 10, or 30 mg/kg a day from gestation day 15 until lactation day 21; teratology study, sperm-positive female rats received 0, 3, 10 or 30 mg/kg a day on gestation days 6-15 and were killed on gestation day 20. Dose levels in each study were sufficient to result in overt cholinergic tremors at the high dose. PB administration did not affect fertility or reproductive performance in male or female rats. In the perinatal/postnatal studies, treatment did not alter reproduction indices and did not result in abnormal treatment-related effects in the offspring. Pups born to treated-dams did show slight, transient decreases in body weight gain, apparently secondary to the nursing behavior of dams demonstrating overt tremors. In the teratology study, a significantly increased rate of early resorptions, (approximately 2-fold over the control group, $P \leq 0.05$), was seen at the high-dose level. PB did not result in significant increases in either visceral or skeletal malformations. Skeletal variations indicative of delayed ossification such as hypoplastic supraoccipital, poor ossification of the cervical vertebrae, and missing vertebrae were slightly but significantly increased at the high-dose level ($P \leq 0.05$). These effects, however, were considered secondary to maternal toxicity (Levine & Parker, 1991).

Inhibition of chicken embryo kynurenine formamidase (KFase) results in a decreased concentration of NAD in the embryo and abnormal feathering and micromelia. Many potent avian teratogens produce prolonged inhibition of this enzyme in mice (Moscioni *et al*., 1977). PB was tested for *in vitro* and *in vivo* mouse liver KFase inhibition at doses of 10 μM and 1 mg/kg intraperitoneal (ip), respectively. In addition, teratogenic potency was assessed following injection of 1 mg into white Leghorn eggs at day 4 of incubation. PB was found to be without effect on KFase and it was not teratogenic. Further details of the results with this compound were not provided (Eto *et al.*, 1980). PB at 10 mg or more per egg injected into the yolk sac of chicken eggs at 96 hours of incubation resulted in short and crooked necks as well as muscular hypoplasia of the legs. The experiment was not reported in detail (Landauer, 1975). At 15 mg/egg injected at 96 hours of incubation, the incidences of short/crooked neck and muscular hypoplasia were 95% and 61%, respectively (Landauer, 1976).

Short-Term Tests: The *in vivo* clastogenic potential of PB was evaluated with the rat micronucleus assay. Male and female Sprague-Dawley rats were administered 1, 10, or 30 mg/kg in the diet for 180 days. No differences were found between the treated and vehicle control groups in the numbers of micronuclei or in the percentages of polychromatic erythrocytes. The selected doses produced a dose-dependent inhibition of cholinesterase activity and toxic signs associated with the decreased activity were noted, indicating that pyridostigmine is not a clastogen at doses that produce significant pharmacological activity and/or toxicity in the rat (Orner & Korte, 1990). No further information on the genotoxicity or mutagenicity of PB was found in the available

literature.

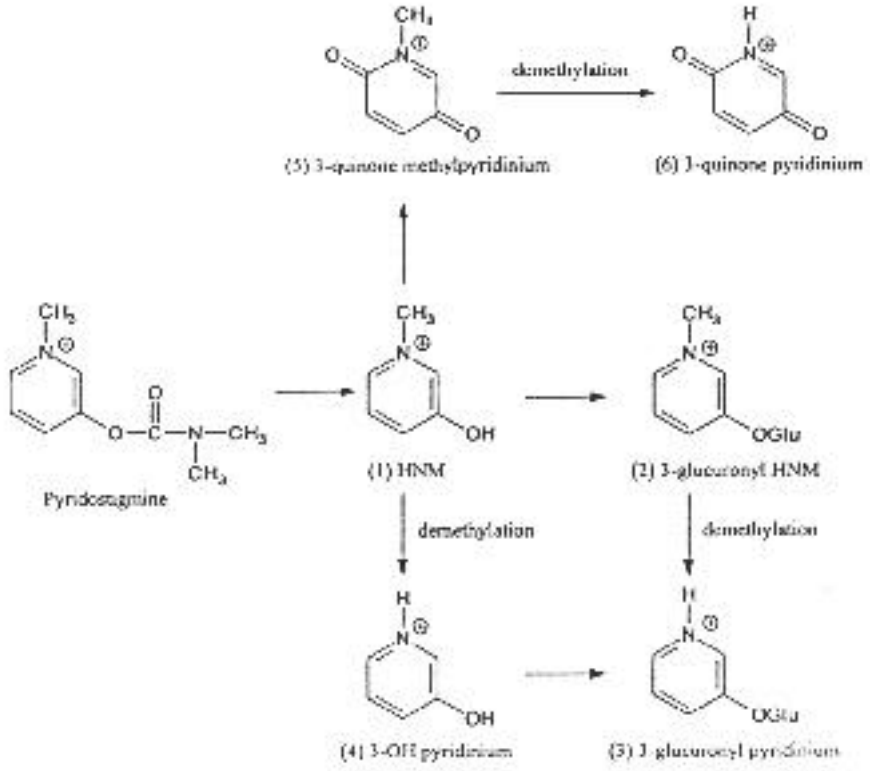
Pyridostigmine bromide (PB) has been reported to be negative in the Ames/*Salmonella* mutagenicity assay conducted for the Short-Term Test Program (STTP) of the National Cancer Institute's Division of Cancer Etiology (NCI/DCE). PB was negative at doses up to 10,000 µg/plate in strains TA98, TA100, TA1535, TA1537, and TA1538, both with and without S9 activation. PB has been selected for the mouse lymphoma assay conducted for the STTP of NCI/DCE (NCI/DCE, 1995).

Metabolism: Metabolism of PB has been studied in both humans and animals.

Human Data: PB is poorly absorbed from the GI tract. After oral administration, onset of action is 30-45 minutes and the duration of action is 3-6 hours (McEvoy, 1992).

Penetration of pyridostigmine into the central nervous system is poor. The drug crosses the placenta and small amounts are excreted in breast milk (Reynolds, 1993). Maternal doses of 180-300 mg/kg PB a day resulted in maternal plasma and breast milk concentration ranges of 6-100 ng/ml and 2-25 ng/ml, respectively. The drug was not identified in infant plasma (Hardell *et al.,* 1982).

3-Hydroxy-N-methylpyridinium (HNM) has been identified as the main metabolite of pyridostigmine in man. Using bidirectional radiochromatography (BDRC), Kornfeld and coworkers (1970) detected as many as eight metabolites in the urine of eight myasthenic patients and five control subjects following iv injection of 2 mg radiolabeled PB. The investigators suggested that the following pyridostigmine (P) biotransformations could account for six of these (unidentified except for HNM) metabolites.



Neither pyridostigmine, its chief metabolite (HNM) nor the other metabolites found in plasma were protein bound. Somani and coworkers (1972) confirmed that pyridostigmine and HNM are the two main compounds in the urine of patients taking oral pyridostigmine iodide. Two additional urinary metabolites were found following intramuscular (im) administration of the radiolabeled drug to a myasthenic patient. The authors proposed that the first metabolite was formed from HNM and could be the 3,4- or 3,6-dihydroxy-N-methylpyridinium compound. Either could be present as the tertiary amine in its resonance form. Methoxy-N-methylpyridinium or acetoxy-N-methylpyridinium were suggested as the other metabolite.

Pyridostigmine undergoes hydrolysis by cholinesterases. It is also metabolized by microsomal enzymes in the liver (McEvoy, 1992).

PB is fairly quickly metabolized and excreted. When the radiolabeled drug was injected iv into patients and volunteers, levels of radioactivity in the urine varied widely among myasthenics and controls. Forty-seven to seventy-seven percent of the injected radioactivity appeared in the first-hour urines and an average of 88% of the radioactivity was excreted in the urine within 24 hours (Kornfeld *et al.*, 1970). The mode of excretion is apparently primarily via renal tubules (Eiermann *et al.*, 1993).

Animal Data: *In vitro* studies of pyridostigmine iodide (PI) with rat liver homogenates demonstrated that hydrolysis predominantly occurs in the soluble fraction of the liver cell, and is independent of the cofactor NADPH. In addition to the major hydrolysis compound, HNM, an additional metabolite was detected but its structure was not

identified. However, it was suggested that it probably contained a carbamate group, since it was not formed when 3-hydroxy-N-^{14}C-methylpyridinium was used as a substrate (Burdfield *et al.,* 1973; Burdfield & Calvey, 1974).

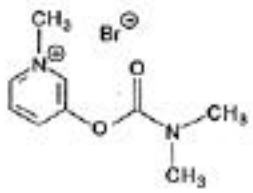
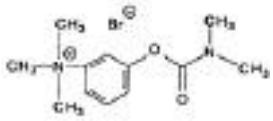
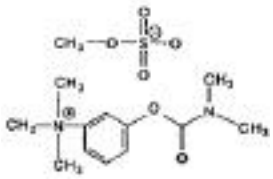
Metabolism and urinary excretion proceeded more slowly than noted for human subjects after administration of 500 μg of ^{14}C-labeled PI to rats (strain not reported) by gavage. After 24 hours 42% of the dose was absorbed and excreted in the urine. About 75% of the radioactivity in the urine was present as unchanged pyridostigmine, the remainder was a metabolite (Husain *et al.,* 1968).

In rats (strain not reported), after im administration of ^{14}C-labeled PI, radioactivity was rapidly excreted in the urine, mostly as pyridostigmine. About 45% of the dose was excreted in the first hour. The excretion of metabolite, HNM, steadily increased and after 3 hours was greater than that of pyridostigmine. The concentration of radioactivity in the liver reached its peak of 70% 20 minutes after injection and rapidly decreased during the next 40 minutes. The peak concentration of HNM occurred after 30 minutes and from 45 minutes onward its concentration exceeded that of pyridostigmine. The authors postulated that the liver is probably the main site of pyridostigmine metabolism and the source of HNM in urine. A second metabolite was detected but was not identified. Radioactivity was detected in most tissues except the brain, intestinal wall, fatty tissue, and the thymus gland (Birtley *et al.,* 1966).

Other Biological Effects: Twenty-two drugs, whose active agents contain dimethylamino groups, were tested for their ability to form N-nitrosodimethylamine under simulated human gastric conditions. No measurable amounts of this carcinogen were formed *in vitro* by incubation of 60 mg PB and nitrite with human gastric juice (Ziebarth & Schramm, 1984).

Structure/Activity Relationships: Nine compounds structurally related to PB, as well as the hydrolysis product HNM, were screened for data relevant to the possible association, either positively or negatively, of mutagenicity or carcinogenicity with compounds of this structural type. Pertinent information was identified for only two of these compounds, neostigmine bromide and neostigmine methylsulfate. These synthetic quaternary ammonium compounds, which behave pharmacologically similarly to pyridostigmine bromide, have demonstrated significant inhibition of chemically-induced liver, stomach, or colon tumors in rats, presumably through a parasympathomimetic mediated mechanism. A summary of the carcinogenicity and mutagenicity information on PB, neostigmine bromide and neostigmine methylsulfate is shown in Table 2. No information on carcinogenicity or mutagenicity for the following structurally related compounds was found in the available literature: pyridostigmine chloride [7681-22-3]; pyridostigmine iodide [4685-03-4]; 3-[[(diethylamino)carbonyl]oxy]-1-methylpyridinium bromide [67465-54-7]; 2-bromo-3-[[(dimethylamino)carbonyl]oxy]-1-methylpyridinium bromide [51581-39-6]; 3-[[(dimethylamino)carbonyl]oxy]-1-(1-methylethyl)pyridinium bromide [69440-43-3]; 3-hydroxy-N-methylpyridinium bromide [31034-86-3]; edrophonium bromide [302-83-0]; and edrophonium chloride [116-38-1]. Structures of these compounds are shown in Table 3.

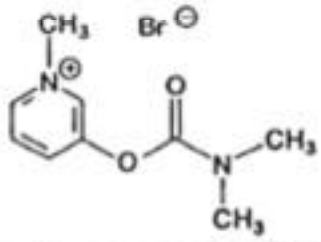
Table 2. Summary of carcinogenicity and mutagenicity information on pyridostigmine bromide and structurally related compounds

Chemical Name [CAS RN]	Carcinogenicity Data	Mutagenicity Data
Pyridostigmine bromide [101-26-8] 	NDF	negative in a rat micronucleus assay (Orner & Korte, 1990)
Neostigmine bromide [114-80-7] 	significant inhibition of chemically-induced liver tumors in rats (Gurkalo & Zabezhinski, 1982)	negative in a DNA-cell binding assay, *E. coli* Q 13 cells (Kubinski *et al.*, 1981)
Neostigmine methylsulfate [51-60-5] 	significant inhibition of chemically-induced stomach and colon tumors in rats (Tatsuta *et al.*, 1988, 1989, 1992)	NDF

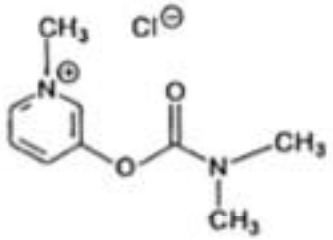
NDF:No data found.

Table 3. Pyridostigmine bromide and structurally related compounds for which no information was found

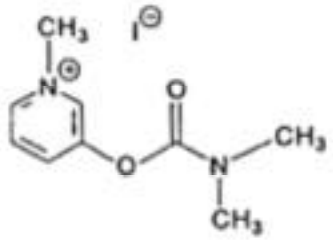
[101-26-8]: Pyridostigmine bromide



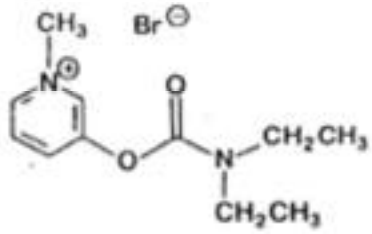
(A) [7681-22-3]: Pyridostigmine chloride



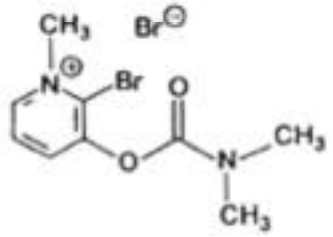
(B) [4685-03-4]: Pyridostigmine iodide



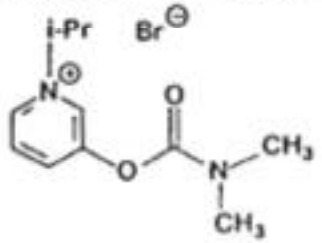
(C) [67465-54-7]: 3-[[(Diethylamino)carbonyl] oxy]-1-methylpyridinium bromide



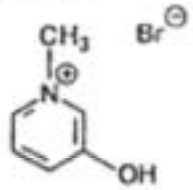
(D) [51581-39-6]: 2-Bromo-3-[[(dimethylamino) carbonyl]oxy]-1-methylpyridinium bromide



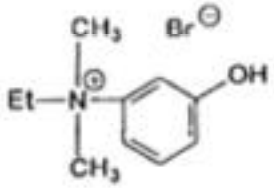
(E) [69440-43-3]: 3-[[(Dimethylamino)carbonyl] oxy]-1-(1-methylethyl)pyridinium bromide



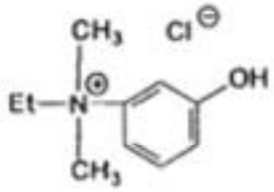
(F) [31034-86-3]: 3-Hydroxy-N-methylpyridinium bromide



(G) [302-83-0]: Edrophonium bromide



(H) [116-38-1]: Edrophonium chloride



Carcinogenic Effects

The following studies examined the role of the autonomic nervous system in the mechanisms of chemical carcinogenesis and the ability of pharmacological neurotropic compounds to modify the carcinogenic process. Gurkalo and Zabezhinski (1982) suggested that compounds that enhance the activity of the sympathetic nerves stimulate carcinogenesis while those that enhance cholinergic functions inhibit carcinogenesis. Neostigmine, an acetylcholinesterase inhibitor, demonstrated significant inhibition of carcinogenesis in these studies.

Neostigmine bromide administered subcutaneously (sc) at 50 µg/kg 3 times a week significantly decreased ($P < 0.05$) both the incidence and size of liver tumors in noninbred male rats treated with 0.7 mmol/l N-nitrosodiethylamine (NDEA) in drinking water for 4 months. At 6 months, 11 of 15 NDEA-treated rats had liver tumors while the incidence was 2 of 11 rats in the neostigmine group (Gurkalo & Zabezhinski, 1982).

The incidence of gastric cancers induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in Wistar rats was significantly ($P < 0.02$) decreased by administration of neostigmine (salt not specified). MNNG in drinking water (50 µg/ml) for 25 weeks followed by neostigmine (0.1 mg/kg/day) for 27 weeks after MNNG treatment resulted in a gastric cancer incidence of 7/19 (37%) versus 15/18 (83%) for the control group (olive oil only from week 25 on). The route of neostigmine administration was not stated (Tatsuta *et al*., 1989). In a second study of gastric cancers in MNNG-treated Wistar rats (75 µg/ml drinking water for 25 weeks), the sc administration of neostigmine methylsulfate (0.075 mg/kg every other day for 27 weeks after MNNG) significantly ($P < 0.05$) inhibited both the incidence (39% vs 80% for the control group) and multiplicity (0.4 vs 1.1 for the control group) of gastric cancers (Tatsuta *et al*., 1992).

Azoxymethane (AOM) induced colon tumors in 18 of 20 Wistar rats following sc administration of 7.4 mg/kg per week for 10 weeks. The tumor incidence was significantly decreased ($P < 0.02$) to 9 of 19 rats by sc administration of 0.1 mg/kg neostigmine methylsulfate every other day beginning 2 weeks before AOM. Colon tumor multiplicities were also reduced to 0.7 in the neostigmine group versus 1.9 in the control group ($P < 0.001$) (Tatsuta *et al*., 1988).

Mutagenic Effects

Neostigmine bromide, tested at 100 or 1,000 µM, was negative in the DNA-cell binding assay with metabolically activated *E. coli* Q13 cells (Kubinski *et al*., 1981).

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Mutagenic Effects

Neostigmine bromide, tested at 100 or 1,000 μM, was negative in the DNA-cell binding assay with metabolically activated *E. coli* Q13 cells (Kubinski *et al*., 1981).