PARACETAMOL (ACETAMINOPHEN)

1. Chemical and Physical Data

1.1 Synonyms and trade names

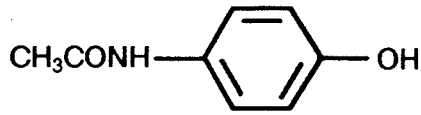
Chem. Abstr. Services Reg. No.: 103-90-2

Chem. Abstr. Name: Acetamide, *N*-(4-hydroxyphenyl)-

Synonyms: 4′-Hydroxy-acetanilide; *para*-acetaminophenol; acetophenum; *para*-acetylamidophenol; *N*-acetyl-*para*-aminophenol; *para*-acetylaminophenol; *para*-hydroxyacetanilide; *N-para*-hydroxyphenylacetamide

A large number of fixed combinations containing paracetamol are available.

1.2 Structural and molecular formula and molecular weight



$C_8H_9NO_2$ Mol. wt: 151.16

1.3 Chemical and physical properties of the pure substance

From Fairbrother (1974) and El-Obeid and Al-Badr (1985)

(*a*) *Description*: White odourless crystalline powder; large monoclinic prisms from water

(*b*) *Melting-point*: 169-170.5°C

(*c*) *Solubility*: Soluble in water (1:70, 1:20 at 100°C), ethanol (1:7), acetone (1:13), chloroform (1:50), glycerol (1:40), methanol (1:10), propylene glycol (1:9) and solutions of alkali hydroxides; insoluble in diethyl ether. A saturated aqueous solution has a pH of ~6.

(*d*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance, fluorescence and mass spectra have been reported.

(*e*) *Stability*: Dry, pure paracetamol is stable to 45°C. Contamination with traces of *para*-aminophenol, and humid conditions that cause hydrolysis to *para*-aminophenol, result in further degradation and discoloration. Slightly light-sensitive in solution, and degradation is catalysed by acids or bases.

(*f*) *Dissociation constant*: pK_a = 9.0-9.5

(*g*) *Partition coefficient*: P_c = 6.237 (octanol: pH 7.2 buffer)

1.4 Technical products and impurities

Paracetamol is available in pure form as numerous trade-name preparations for oral use. It is also found combined in over 200 preparations with other drugs.

Trade names: Abensanil; Acamol; Acephen; Acetalgin; Acetamol; Aferadol; Alba-Temp; Alpiny; Alvedon; Amadil; Anacin-3; Anaflon; Anhiba; Anuphen; Apamide; APAP; Atasol; Ben-u-ron; Bickie-mol; Bramcetamol; Calip; Calpol; Calpon; Campain; Capital; Captin; Ceetamol; Cetadol; Cetamol; Cetapon; Claradol; Claratal; Custodial; Dafalgan; Datril; Dial-a-gesix; Dirox; Disprol Paediatric; Dolamin; Dolanex; Doliprane; Doloral; Dolorol; Dolprone; Dorcol Children's Fever and Pain Reducer; Doregrippin; Dymadon; Efferalgan; Enelfa; Eneril; Ennagesic; Eu-Med; Exdol; Fanalgic; Febrigesic; Febrilix; Fendon; Fevamol; Finimal; Fonafor; Gelocatil; Glenpar; Gynospasmine; Hedex; Homoolan; Kinderfinimal; Kinder-Finiweh; Korum; Liquiprin; Lyteca; Malgis; Melabon; Momentum; Napamol; Naprinol; Nebs; Neuridal; Nevral; Nina 120; Nobedon; Ophinal; Oraphen; Pacemo; Pacemol; Painamol; Painaway; Paldesic; Pamol; Panado; Panadol; Panaleve; Panamax; Panasorb; Panets; Panex; Panodil; Panofen; Pantalgin; Paracet; Paracetamolum; Paraclear; Paralgin; Parapain; Paraprom; Parasin; Paraspen; Paratol; Parmol; Pasolind; Phendex; Pinex; Placemol; Praecimed; Proval; Puernol; Pyragesic; Pyralen; Reliv; Repamol; Resolve; Robigesic; Rounox; Salzone; Schmerzex; Sedapyren; Servigesic; Setamol; SK-APAP; Summadol; Tabalgin; Tachipirina; Tapar; Temlo; Tempra; Tenasfen; Ticelgesic; Tralgon; Treupel; Treuphadol; Tricocetamol; Tylenol; Tymol; Valadol; Zolben

Paracetamol is available as 325-mg or 500-mg tablets, which may include calcium stearate or magnesium stearate, cellulose, docusate sodium and sodium benzoate or sodium lauryl sulfate, starch, hydroxypropyl methylcellulose, propylene glycol, sodium starch glycolate, polyethylene glycol and Red #40.

It is also available as 500-mg gelatin capsules and as a mint-flavoured liquid containing 500 mg/15 ml solution, which can include 7% ethanol, citric acid, glycerine, polyethylene glycol, sodium benzoate, sorbitol, sucrose, Yellow #6, #10 and Blue #1. For children, drops (80 mg/0.8 ml), chewable tablets (80 mg), elixir (160 mg/5 ml) and coated capsules (160 mg/capsule) are available (Barnhart, 1989).

Characteristic impurities may include *para*-nitrophenol, *para*-aminophenol, *para*-chloroaniline, *ortho*-acetyl paracetamol, azobenzene (see IARC, 1975), azoxybenzene, quinone (see IARC, 1977), quinonimine, inorganic chloride, inorganic sulfate, inorganic sulfide and water (Fairbrother, 1974).

2. Production, Occurrence, Use and Analysis

2.1 Production and occurrence

Paracetamol may be made by acetylation of *para*-aminophenol (obtained by reduction of *para*-nitrophenol) with acetic acid or acetic anhydride. A number of other synthetic routes have been described (Fairbrother, 1974).

Paracetamol is synthesized in Argentina, Brazil, China, Colombia, France, the Federal Republic of Germany, India, Japan, Mexico, Poland, Republic of Korea, Romania, Taiwan, Turkey, the UK and the USA (Chemical Information Services Ltd, 1989-90).

In Sweden, paracetamol sales in 1988 were 20.02 defined daily doses per 1000 inhabitants (Apoteksbolaget, 1988, 1989).

Paracetamol is not known to occur naturally, but it is the major metabolite of phenacetin (see IARC, 1980, 1987).

2.2 Use

Paracetamol is used as an analgesic and antipyretic drug. It is the preferred alternative analgesic-antipyretic to aspirin (acetylsalicylic acid), particularly in patients with coagulation disorders, individuals with a history of peptic ulcer or who cannot tolerate aspirin, as well as in children (American Medical Association, 1986). Paracetamol was first used in clinical medicine in 1893. Following initial use as a prescription product in the USA in 1951, it subsequently became available without prescription in 1955 (Ameer & Greenblatt, 1977). In many countries, it is widely available without prescription.

The conventional oral dose for adults is 500-1000 mg. Dosing may be repeated every 4 h as necessary, but the total daily dose should not exceed 4000 mg. For children, the recommended dose is 10-15 mg/kg bw; no more than five doses should be administered over 24 h. Prolonged use (for more than ten days) and use for young children is not recommended (Flower *et al.*, 1985).

The usual dose for rectal administration is equal to that for oral administration (American Medical Association, 1986).

2.3 Analysis

Methods for the analysis of paracetamol have been reviewed (El-Obeid and Al-Badr, 1985).

Paracetamol and its metabolites can be analysed in biological fluids by high-performance liquid chromatography (HPLC; Manno *et al.*, 1981; Kinney & Kelly, 1987; Aguilar *et al.*, 1988; Meatherall & Ford, 1988), HPLC-mass spectrometry (Betowski *et al.*, 1987) and fluorescence polarization immunoassay (Koizumi *et al.*, 1988). It can be analysed in pharmaceutical preparations by HPLC (Biemer, 1987) and spectrophotometric (US Pharmacopeial Convention, Inc., 1989) methods.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Since paracetamol is a metabolite of phenacetin (Reynolds, 1989), carcinogenicity studies of phenacetin result in exposure of animals to paracetamol. For the results of studies on phenacetin, see IARC (1987).

(*a*) *Oral administration*

Mouse: Groups of 60 male and 60 female young adult IF strain mice were fed paracetamol (> 98% pure; dissolved in acetone then evaporated) at 5000 or 10 000 mg/kg of diet for 18 months (approximate daily intake, 250 or 500 mg/kg bw, respectively). A group of 52 males and 52 females fed basal diet served as controls. Shortly after the beginning of treatment, 33 males and seven females in the higher-dose group died from liver necrosis. Subsequent survival in all groups was high. All survivors were killed at 18 months after the beginning of the experiment, and complete necropsy was carried out with histological examination of the liver, lungs, pancreas, kidneys, spleen, bladder and adrenal glands. The effective numbers of animals were 50 male and 48 female controls, 54 males and 57 females in the lower-dose group and 23 males and 47 females in the higher-dose group. The incidences of large, often multiple liver neoplasms (adenomas and carcinomas combined) were 20/23 (87%: 15 adenomas, 5 carcinomas) in higher-dose males, 9/47 (7 adenomas, 2 carcinomas) in higher-dose females, 1/54 (adenoma) in lower-dose males, 0/57 in lower-dose females, 1/50 (adenoma) in control males and 0/48 in control females (Flaks & Flaks, 1983). [The Working Group noted that the high dose produced early lethal hepatotoxicity in half the males.]

Groups of 50 and 55 male or female (C57Bl/6 × C3H/He)F1 (B6C3F1) mice, eight to nine weeks of age, were fed paracetamol (>98% pure) at 3000 and 6000 mg/kg of diet, respectively. The total intake of paracetamol in the high-dose groups was 863 g/kg bw for males and 675 g/kg bw for females. Two groups of 50 males and females were maintained on basal diet. All survivors were killed at 134 weeks. Survival among males was 43/50 (controls), 39/50 (low-dose) and 45/55 (high-dose), and that among females was 49/50 (controls), 46/50 (low-dose) and 50/55 (high-dose). The numbers of mice scored for tumours were 27/43 control males, 32/49 control females, 21/39 low-dose males, 33/46 low-dose females, 23/45 high-dose males and 33/50 high-dose females. No difference was found in the incidence of tumours at any site between treated and control mice (Amo & Matsuyama, 1985).

Groups of 60 and 120 male B6C3F1 mice, six weeks of age, received paracetamol at 5000 or 10 000 mg/kg of diet, respectively, for up to 70 weeks, at which time the remaining animals were killed. A group of 30 mice served as controls. Survival in the high-dose group was less than 50% at 24 weeks and 16% at 72 weeks; in the low-dose group, the survival was greater than 90%. Severe hepatotoxicity was a common finding in mice that died. No increased incidence of neoplasms was observed (Hagiwara & Ward, 1986). [The Working Group noted the poor survival in the high-dose group.]

Rat: Groups of 30 male SPF Sprague-Dawley rats, six weeks of age, were fed paracetamol (99.5-99.7% pure) at 0 or 5350 mg/kg of diet for 117 weeks (total paracetamol intake, 86.5 g per rat). All animals were necropsied, and kidneys, urinary bladder, adrenal glands, liver, stomach, spleen, lungs, heart and any grossly abnormal organs or tissues were examined histologically. No significant difference in survival rates was observed. In the treated group, 4/30 rats developed bladder papillomatosis or tumours *versus* 2/30 controls (Johansson, 1981). [The Working Group noted the relatively small number of animals used in the study.]

Groups of 50 male and 50 female Fischer 344/DuCrj rats, five weeks of age were fed pharmacopoeial-grade paracetamol at 0, 4500 or 9000 mg/kg (males) and 0, 6500 or 13 000 mg/kg (females) of diet for 104 weeks and were then observed for a further 26 weeks (average daily intakes: lower-dose males, 195 mg/kg bw; lower-dose females, 336 mg/kg bw; higher-dose males, 402 mg/kg bw; higher-dose females, 688 mg/kg bw), at which time all survivors were killed. Survival rates at 104 weeks varied between 86 and 90% in males and 80 and 82% in females, with no significant difference between treated and control rats. All rats were necropsied, and major organs, tissues and gross abnormalities were examined histologically. No difference was seen in tumour incidence between the groups (Hiraga & Fujii, 1985).

Groups of 50 male and 50 female young adult Leeds inbred rats were fed paracetamol (>98% pure) at 5000 or 10 000 mg/kg of diet for up to 18 months (mean

daily intake, 300 and 600 mg/kg bw, respectively), at which time all survivors were killed. A group of 40 males and 40 females fed basal diet alone served as controls. Survival was high: male controls, 40/40; female controls, 40/40; lower-dose males, 48/50; lower-dose females, 49/50; higher-dose males, 45/50; and higher-dose females, 49/50. All animals were necropsied, and samples from each liver lobe, lungs, kidneys, pancreas, mammary glands, spleen, adrenal glands and from grossly visible lesions were examined histologically. No tumour was observed among controls. In treated animals, no hepatocellular carcinoma was observed, but hepatocellular neoplastic nodules occurred in 0/40, 1/48 and 9/45 control, lower-dose and higher-dose males and 0/40, 0/49 and 10/49 control, lower-dose and higher-dose females; and 20-25% of rats in each treated group developed hyperplasia of the bladder epithelium. Bladder calculi were present in about 30% of all treated male animals and in 6% of females; no clear association was seen between hyperplasia and the presence of bladder calculi. Bladder papillomas were observed in 5/49 higher-dose males and bladder carcinomas in 1/49 higher-dose males; the total bladder tumour incidence was significantly higher [$p = 0.02$, Fisher's exact test] among high-dose males. In the low-dose group, 4/49 females developed bladder papillomas and 1/49 females developed bladder carcinoma. Total bladder tumour incidence was significantly higher in low-dose female rats [$p = 0.045$, Fisher's exact test] (Flaks *et al.*, 1985). [The Working Group noted that there were increased incidences of calculi, hyperplasia and tumours of the bladder in treated animals but there was no relationship between the presence of calculi and the presence of either hyperplasia or tumours.]

(*b*) *Administration with known carcinogens*

Mouse: Groups of 30 and 60 male B6C3F1 mice, six weeks of age, received paracetamol at 5000 or 10 000 mg/kg of diet, respectively, continuously for up to 70 weeks following a single intraperitoneal injection of 40 mg/kg bw *N*-nitrosodiethylamine at four weeks of age. A group of 30 mice that received *N*-nitrosodiethylamine alone served as controls. Mice were sacrified at either 24 or 72 weeks after injection of the nitrosamine. Survival in the higher-dose group was very poor; severe hepatotoxicity was a common finding in mice that died. No increased incidence of neoplasms was found (Hagiwara & Ward, 1986).

Rat: Two groups of 25 or 30 male Fisher 344 rats weighing 150 g were administered *N*-nitrosoethyl-*N*-hydroxyethylamine (NEHEA) at 0 or 0.1% (v/v) in drinking-water for two weeks and one week later were fed diets containing paracetamol [purity unspecified] at 1.3% for 29 weeks. One group of 25 rats received NEHEA in the drinking-water followed by no further treatment. All animals were killed at the end of week 32, and samples from liver, kidneys and other organs with gross abnormalities were examined histologically.

γ-Glutamyltranspeptidase foci, hyperplastic nodules, hepatocellular carcinomas, renal-cell carcinomas, as well as 'atypical cell foci' and adenomas were measured. Paracetamol inhibited the formation of NEHEA-induced γ-glutamyltranspeptidase foci, hyperplastic nodules and carcinomas in comparison with animals treated with NEHEA only. No liver lesion was found in any animal treated with paracetamol only. In contrast, the incidence and multiplicity of preneoplastic renal lesions and renal-cell adenomas were significantly increased in NEHEA-initiated animals treated with paracetamol in comparison with animals treated with NEHEA only. No such renal lesion was observed in groups treated with paracetamol alone (Tsuda *et al.*, 1984). [The Working Group noted that the progression of the lesions described as preneoplastic to neoplasms was not documented.]

Groups of 25 male Fischer 344 rats, seven weeks old, were administered *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine at 0 or 0.05% (v/v) in the drinking-water for four weeks to initiate bladder carcinogenesis and were then fed paracetamol [purity unspecified] at 13 000 mg/kg of diet for a further 32 weeks, at which time all rats were killed. One group received treatment with the nitrosamine only. Urinary bladders, livers and kidneys were examined histologically. No significant difference in the incidence of bladder tumours was observed between the groups (Kurata *et al.*, 1986).

Groups of male Fischer 344 rats [numbers unspecified], six weeks of age, were subjected to a two-thirds partial hepatectomy and 24 h later received either intragastric intubations of paracetamol (purity, >99%) at 0 or 1000 mg/kg bw in 0.2% tragacanth gum twice a week for five weeks, or a single intragastric instillation of paracetamol at 500 mg/kg bw. Two weeks after the end of paracetamol treatment, the animals were administered phenobarbital (pharmacopoeial grade) at 0 or 1 mg/ml drinking-water for 12 weeks. The experiment was terminated at the end of phenobarbital treatment (weeks 13 and 18). Livers, kidneys, thyroid glands and any gross lesions were examined histologically. The tumour-initiating activity of paracetamol was evaluated by the formation of placental-type glutathione *S*-transferase-positive foci in liver cells; treatment with paracetamol did not result in the induction of such foci (Hasegawa *et al.*, 1988). [The Working Group noted that the rate of absorption of paracetamol from the tragacanth suspension was not measured, and the limited reporting of the experiment.]

To examine possible interference with the activation of 2-acetylaminofluorene, groups of 20 female SPF CD rats were given diets containing acetylaminofluorene at 250 mg/kg alone or with paracetamol at 11 000 mg/kg for 20 weeks and were observed for an additional ten weeks. Mammary tumours were seen in 14/20 females given acetylaminofluorene and in 7/20 ($p = 0.028$, Fisher's exact test) animals given acetylaminofluorene and paracetamol (Weisburger *et al.*, 1973).

Hamster: Groups of 30 male and 30 female Syrian golden hamsters, six weeks old, were given *N*-hydroxyacetylaminofluorene at 430 mg/kg alone or with paracetamol at 11 000 mg/kg of diet for 39 weeks. The experiment was terminated at 47 weeks. The incidences of liver cholangiomas in animals treated with *N*-hydroxyacetylaminofluorene were 13/26 in males and 22/25 in females; in the group treated with *N*-hydroxyacetylaminofluorene and paracetamol, no liver tumour was seen in 24 males but two occurred in 24 females. Similar results were found in groups given acetylaminofluorene at 400 mg/kg alone or with paracetamol at 11 000 mg/kg: with acetylaminofluorene, the incidence of liver cholangiomas was 6/30 males and 28/30 females; in the group treated with acetylaminofluorene and paracetamol, the incidence was 0/29 males ($p = 0.013$, Fisher's exact test) and 4/28 females ($p < 0.001$, Fisher's exact test) (Weisburger *et al.*, 1973).

3.2 Other relevant data

(*a*) *Experimental systems*

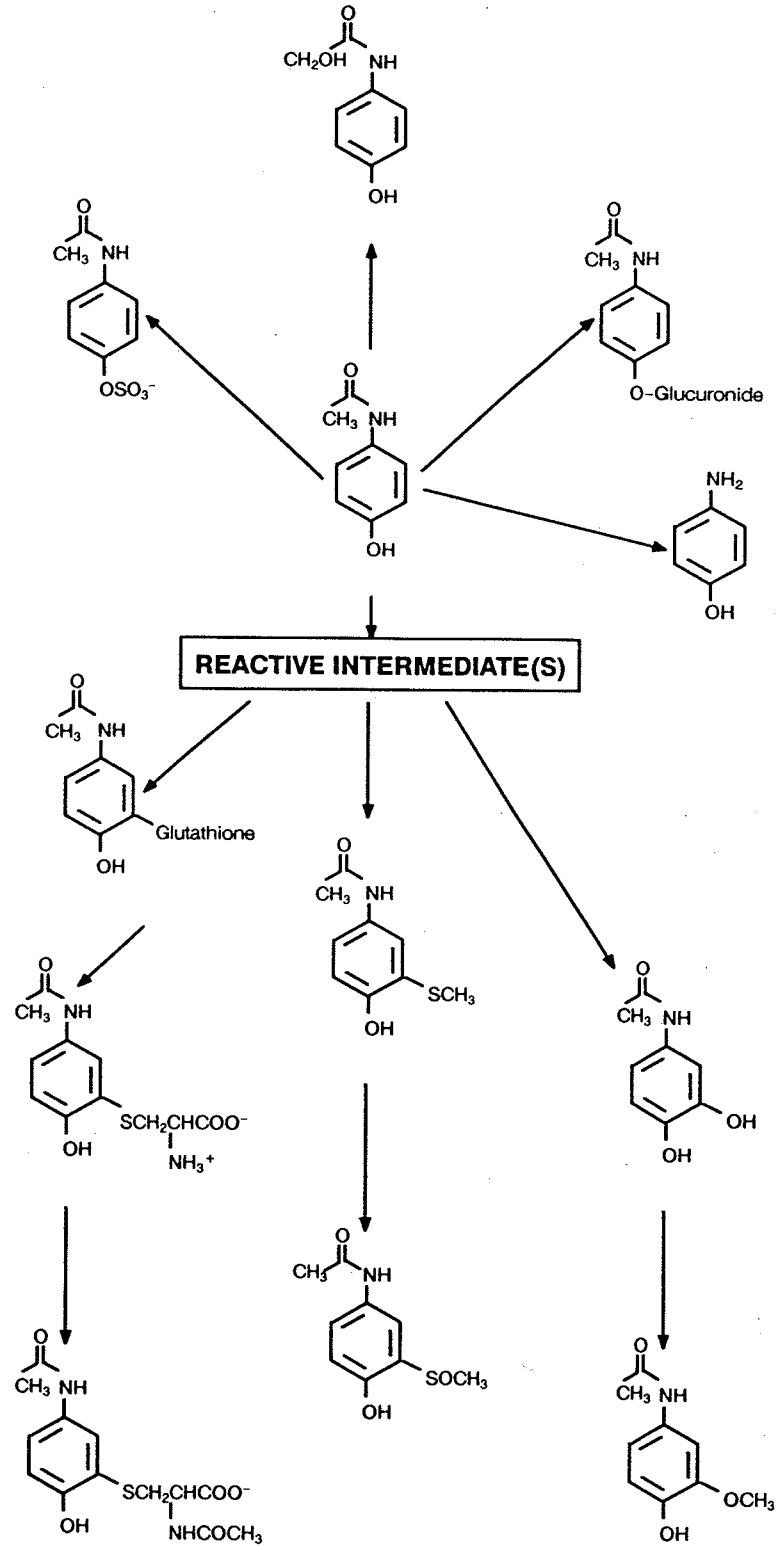
(i) *Absorption, distribution, excretion and metabolism*

Dogs receiving a single oral administration of a wide range of doses of paracetamol excreted about 85% of the administered dose within the first 24 h (Savides *et al.*, 1984).

A summary of the proposed metabolic pathways of paracetamol is shown in Figure 1. The major urinary metabolites (the glucuronide, sulfate and 3-mercapto derivatives) are observed in most species, although there is much species variation regarding the percentages of these conjugates excreted in the urine (Davis *et al.*, 1976). Each of the other metabolites shown in Figure 1 has been identified in one species (see Gemborys & Mudge, 1981, for details). In rats, biliary excretion of the various metabolites of paracetamol increased from 20 to 49% as doses were increased from 37.5 to 600 mg/kg bw. The glucuronide conjugate was the major metabolite recovered in the bile at all doses (Hjelle & Klaassen, 1984). The putative reactive intermediates are not known but are thought to include benzoquinone (Hinson *et al.*, 1977).

A minor but important metabolic pathway involves the conversion of paracetamol to a reactive metabolite by the hepatic cytochrome P450-dependent mixed-function oxidase system (Mitchell *et al.*, 1973; Potter *et al.*, 1973). The reactive metabolite is thought to be either *N*-acetyl-*para*-benzoquinoneimine (Corcoran *et al.*, 1980) or the corresponding semiquinone free radical (De Vries, 1981; Nelson *et al.*, 1981). With low doses of paracetamol, a conjugate of reduced glutathione with the reactive metabolite is further transformed to cysteine and mercapturic acid conjugates, which are excreted. As the dose of paracetamol increases, hepatic glutathione stores are diminished and the glucuronidation and sulfation pathways

Fig. 1. Summary of metabolism of paracetamol based on data for different species[a]



[a]From Jollow *et al.* (1974), Wong *et al.* (1976) and Gemborys & Mudge (1981)

become saturated (Galinsky & Levy, 1981). A correlation has been demonstrated between species sensitivity to the hepatotoxicity of paracetamol and the balance between two pathways: (i) formation of glutathione conjugates and the corresponding hydrolysis products (indicative of the 'toxic' pathway) and (ii) metabolism *via* formation of glucuronide and sulfate esters (the 'detoxification pathway') (Gregus *et al.*, 1988). Paracetamol-induced liver toxicity and depletion of glutathione may be partially prevented by provision of dietary methionine (Reicks *et al.*, 1988; McLean *et al.*, 1989). At sufficiently high doses of paracetamol, glutathione is depleted and the reactive metabolite binds covalently to cell macromolecules. It has also been noted that paracetamol and *N*-acetyl-*para*-benzoquinoneimine may exert their cytotoxic effects *via* disruption of Ca^{2+} homeostasis secondary to the depletion of soluble and protein-bound thiols (Moore *et al.*, 1985). These data indicate that oxidative or free-radical reactions initiated by paracetamol have a role in the hepatotoxicity of this drug (Birge *et al.*, 1988).

Radiolabel was bound covalently to hepatocellular proteins following incubation of mouse, rat, hamster, rabbit or guinea-pig liver microsomes with ^{3}H-paracetamol; the degree of binding was correlated with the susceptibility of the species to hepatotoxicity *in vivo* (Davis *et al.*, 1974). Similar covalent binding of radiolabel to liver proteins of rats 48 h after administration of [*ring*-^{14}C]-paracetamol was proportional to the extent of liver damage (Davis *et al.*, 1976). Covalent binding of radiolabel to liver plasma membranes and microsomes was demonstrated 2.5 h after oral administration of ^{3}H-paracetamol at 2.5 g/kg bw to rats (Tsokos-Kuhn *et al.*, 1988).

Paracetamol is activated in the kidney by an NADPH-dependent cytochrome P450 mechanism to an arylating agent which can bind covalently to cellular macromolecules (McMurty *et al.*, 1978). Studies in several species have suggested that formation of *para*-aminophenol may be of importance with respect to paracetamol nephrotoxicity. *para*-Aminophenol was identified as a urinary metabolite in hamsters (Gemborys & Mudge, 1981); the deacetylation of paracetamol to *para*-aminophenol has also been demonstrated in mouse renal cortical slices (Carpenter & Mudge, 1981). In comparison to acetyl-labelled paracetamol, ring-labelled paracetamol was preferentially bound to renal macromolecules in Fischer rats, which are sensitive to paracetamol nephrotoxicity, whereas binding of ring- and acetyl-labelled paracetamol to renal macromolecules was similar in non-susceptible Sprague-Dawley rats (Newton *et al.*, 1985). This suggests that *para*-aminophenol may be responsible for paracetamol-induced renal necrosis in Fischer 344 rats (Newton *et al.*, 1982).

(ii) *Toxic effects*

The single-dose oral LD_{50} of paracetamol in male rats was 3.7 g/kg bw (Boyd & Bereczky, 1966); the 100-day LD_{50} in rats was 400 mg per day (Boyd & Hogan, 1968).

Hepatic necrosis following administration of paracetamol was first reported in rats (Boyd & Bereczky, 1966). The main signs are hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity (Dixon *et al.*, 1971). Paracetamol-induced hepatotoxicity varies considerably among species: hamsters and mice are most sensitive, whereas rats, rabbits and guinea-pigs are resistant to paracetamol-induced liver injury (Davis *et al.*, 1974; Siegers *et al.*, 1978). Toxic effects in dogs and cats given a single oral dose of paracetamol (maximal doses, 500 and 120 mg/kg bw, respectively) included hepatic centrilobular pathology in dogs, while cats, which do not glucuronidate exogenous compounds, had more diffuse liver pathological changes (Savides *et al.*, 1984).

The hepatotoxic effects of paracetamol administered in the diet to mice have been examined histologically. After continuous exposure at 10 000 mg/kg diet for 72 weeks (Hagiwara & Ward, 1986), severe chronic hepatotoxicity was observed, with centrilobular hepatocytomegaly, cirrhosis, lipofuscin deposition and hepatocyte necrosis varying from focal to massive. With the same dose, Ham and Calder (1984) observed macroscopically and microscopically deformed livers with extensive lobular collapse, foci of hepatic necrosis and lymphoid aggregation in portal tracts after 32 weeks. At a lower dose (5000 mg/kg bw) and a shorter exposure time (24 weeks), histological changes were mild. Ultrastructural changes in the livers of rats administered paracetamol at 10 000 mg/kg diet for up to 18 months have been described (Flaks *et al.*, 1985).

Histopathological review of liver sections from B6C3F1 mice of each sex fed paracetamol at 3000, 6000 or 12 500 mg/kg diet for 41 weeks and from NIH general-purpose mice of each sex fed paracetamol at 11 000 mg/kg diet for 48 weeks indicated severe liver injury, characterized by centrilobular necrosis in animals receiving more than 10 000 mg/kg diet (Maruyama & Williams, 1988).

A single subcutaneous dose of paracetamol at 750 mg/kg bw to male Fischer 344 rats produced renal tubular necrosis restricted to the upper part of the proximal tubule (McMurty *et al.*, 1978). Chronic cortical and medullary damage has been produced in uninephrectomized homozygous Gunn rats by single doses of various analgesic preparations containing paracetamol (Henry & Tange, 1984).

In fasted adult male mice given paracetamol at 600 mg/kg bw orally and killed within 48 h after treatment, degenerative and necrotic changes were detected in the bronchial epithelium and in testicular and lymphoid tissue, in addition to renal and hepatic effects (Placke *et al.*, 1987).

When male rats were given paracetamol at 500 mg/kg bw per day orally for 70 days, a significant decrease in testicular weight was observed (Jacqueson *et al.*, 1984).

(iii) *Effects on reproduction and prenatal toxicity*

In Sprague-Dawley rats administered paracetamol at 250 mg/kg bw orally on days 8 through 19 of gestation, embryo- and fetotoxic effects were not seen (Lubawy & Burriss Garret, 1977).

(iv) *Genetic and related effects*

Paracetamol was not mutagenic to *Salmonella typhimurium* at concentrations of up to 50 mg/plate in the presence or absence of an exogenous metabolic system (King *et al.*, 1979; Wirth *et al.*, 1980; Imamura *et al.*, 1983; Dybing *et al.*, 1984; Oldham *et al.*, 1986; Jasiewicz & Richardson, 1987). It did not induce mutations in a liquid pre-incubation test with *Escherichia coli* in the presence or absence of an exogenous metabolic system (King *et al.*, 1979). As reported in an abstract, paracetamol exhibited mutagenic activity towards *S. typhimurium* TA100 in the presence of an exogenous metabolic system (Tamura *et al.*, 1980).

Feeding of male *Drosophila melanogaster* with a 40-mM solution of paracetamol did not induce sex-linked recessive mutations (King *et al.*, 1979).

Treatment of Chinese hamster V79 cells with low concentrations (0.1-3.0 mM) of paracetamol inhibited DNA synthesis (Holme *et al.*, 1988; Hongslo *et al.*, 1988). Paracetamol at 10 mM had no effect on Reuber H4-II-E rat hepatoma cell DNA, as assayed by alkaline elution, but the toxic metabolite of paracetamol, *N*-acetyl-*para*-benzoquinoneimine, induced DNA strand breaks (Dybing *et al.*, 1984). Treatment of Chinese hamster V79 cells induced DNA strand breaks at 3 and 10 mM but not at 1 mM (Hongslo *et al.*, 1988). Analogous results were obtained with Chinese hamster ovary cells (Sasaki, 1986). Species specificity was observed in assays for unscheduled DNA synthesis *in vitro*. No unscheduled DNA synthesis was detected in Chinese hamster V79 cells (Hongslo *et al.*, 1988), in Syrian hamster or guinea-pig primary hepatocytes (Holme & Soderlund, 1986) or in rat hepatocytes (Milam & Byard, 1985; Sasaki, 1986; Williams *et al.*, 1989); however, a small but significant increase in unscheduled DNA synthesis was seen in rat primary hepatocytes and a marked increase in unscheduled DNA synthesis was observed in mouse hepatocytes (Holme & Soderlund, 1986).

Paracetamol did not induce mutations to ouabain-resistance in C3H/10T½ clone 8 mouse embryo cells (Patierno *et al.*, 1989). It was reported in an abstract that paracetamol did not induce mutations at the *hprt* locus in Chinese hamster V79 cells (Sawada *et al.*, 1985). It induced sister chromatid exchange in Chinese hamster V79 (Holme *et al.*, 1988; Hongslo *et al.*, 1988) and CHO cells (Sasaki, 1986). Micronuclei were induced by paracetamol in a rat kidney cell line (NRK-49F) at

concentrations above 10 mM (Dunn *et al.*, 1987). Paracetamol induced chromosomal aberrations in three different Chinese hamster cell lines (Sasaki *et al.*, 1980; Sasaki, 1986; Ishidate, 1988) and in human lymphocytes (Watanabe, 1982). It weakly transformed C3H/10T½ clone 8 mouse embryo cells (Patierno *et al.*, 1989).

Paracetamol given twice at a dose of 3 mM (450 mg/kg bw) either intraperitoneally or orally to NMRI mice did not induce micronuclei (King *et al.*, 1979). Oral treatment of female Sprague-Dawley rats with paracetamol at 500 and 1000 mg/kg bw induced aneuploidy in 12-day embryos (Tsuruzaki *et al.*, 1982). Oral treatment of Swiss mice with single or three consecutive daily doses of aqueous solutions of up to 2.5 mg/0.5 ml did not lead to chromatid breaks in bone-marrow cells (Reddy, 1984) or meiotic cells of male Swiss mice (Reddy & Subramanyam, 1985). [The Working Group noted that the description of the doses used in the two last studies was unclear.]

(*b*) *Humans*

(i) *Pharmacokinetics*

Following an oral dose, paracetamol is absorbed rapidly from the small intestine. The rate of absorption depends on the rate of gastric emptying (Clements *et al.*, 1978). First-pass metabolism of paracetamol is dose-dependent: systemic availability ranges from 90% (with 1-2 g) to 68% (with 0.5 g). Plasma concentrations of paracetamol in fasting healthy subjects peaked within 1 h after treatment with 0.5 or 1.0 g but continued to rise up to 2 h after treatment with 2.0 g (Rawlings *et al.*, 1977).

Paracetamol is rapidly and relatively uniformly distributed throughout the body fluids (Gwilt *et al.*, 1963). Binding to plasma proteins is considered insignificant (Gazzard *et al.*, 1973). The apparent volume of distribution of paracetamol in man is about 0.9 l/kg bw (Forrest *et al.*, 1982). The decrease in paracetamol concentrations in plasma is multiphasic both after intravenous injections and after oral dosing with 500 and 1000 mg. When the data from six healthy volunteers were interpreted according to a two-compartment open model, the half-time of the first exponential ranged from 0.15 to 0.53 h and that of the second exponential from 2.24 to 3.30 h. The latter value was in agreement with that found after oral dosing. Mean clearance (± SEM) after intravenous administration of 1000 mg was 352 (± 40) ml/min (Rawlings *et al.*, 1977). Renal excretion of paracetamol involves glomerular filtration and passive reabsorption, and the sulfate conjugate is subject to active renal tubular secretion (Morris & Levy, 1984). Both these metabolites have been shown to accumulate in plasma in patients with renal failure who are taking paracetamol (Lowenthal *et al.*, 1976).

Paracetamol crosses the placenta in unconjugated form, and excretion in the urine of an exposed neonate was similar to that of a two- to three-day-old infant (Collins, 1981).

Paracetamol passes rapidly into milk, and the milk:plasma concentration ratio ranges from 0.7 to 1.1 (Berlin *et al.*, 1980; Notarianni *et al.*, 1987).

Paracetamol is metabolized predominantly to the glucuronide and sulfate conjugates in the human liver. A minor fraction is converted by cytochrome P450-dependent hepatic mixed-function oxidase to a highly reactive arylating metabolite, which is postulated to be *N*-acetyl-*para*-benzoquinoneimine (Miner & Kissenger, 1979). This metabolite is rapidly inactivated by conjugation with reduced glutathione and eventually excreted in the urine as acetyl cysteine and mercapturic acid conjugates. Large doses of paracetamol can deplete glutathione stores, and the excess of highly reactive intermediate binds covalently with vital cell elements, which may result in acute hepatic necrosis (Mitchell *et al.*, 1973, 1974). Only 2-5% of a therapeutic dose was excreted unchanged in the urine. In young healthy subjects, about 55, 30, 4 and 4% of a therapeutic dose was excreted after hepatic conjugation with glucuronic acid, sulfuric acid, cysteine and mercapturic acid, respectively (Forrest *et al.*, 1982).

The fractional recovery of mercapturic acid and cysteine conjugates after ingestion of paracetamol at 1500 mg was 9.3% in Caucasians compared with only 4.4-5.2% in Africans (Critchley *et al.*, 1986). This may reflect different susceptibility to paracetamol hepatotoxicity.

(ii) *Adverse effects*

The toxic effects of paracetamol have been reviewed (Flower *et al.*, 1985).

Reports on the acute toxicity, and in particular hepatotoxicity, of paracetamol have continued to appear since the reporting of the first two cases in 1966 (Davidson & Eastham, 1966). Initial symptoms of overdose are nausea, vomiting, diarrhoea and abdominal pain. Clinical indications of hepatic damage become manifest within two to four days after ingestion of toxic doses; in adults, a single dose of 10-15 g (200-250 mg/kg bw) is toxic. Serum transaminases, lactic dehydrogenase and bilirubin concentrations are elevated, and prothrombin time is prolonged (Koch-Weser, 1976). The severity of hepatic injury increases with the ingested dose and with previous consumption of other drugs that induce liver cytochrome P450 enzymes (Wright & Prescott, 1973). Biopsy of the liver reveals centrilobular necrosis with sparing of the periportal area (James *et al.*, 1975). In nonfatal cases, the hepatic lesions are reversible over a period of months, without development of cirrhosis (Hamlyn *et al.*, 1977).

Heavy alcohol consumption has been stated in several case reports to be related to more severe paracetamol hepatotoxicity than in non- or moderate

drinkers (for review, see Black, 1984). Five cases of combined hepatocellular injury and renal tubular necrosis have been reported among patients with a history of chronic alcohol use who were receiving therapeutic doses of paracetamol (Kaysen *et al.*, 1985).

(iii) *Effects on reproduction and prenatal toxicity*

No association of paracetamol use with congenital abnormalities or stillbirths was observed in a study on drug use in approximately 10 000 pregnancies in the UK (Crombie *et al.*, 1970). In a case-control study of 458 mothers of malformed babies and 911 controls, there was no association of abnormalities with use of paracetamol during the first trimester (Nelson & Forfar, 1971). In the Collaborative Perinatal Project, in which drug intake and pregnancy outcome were studied in a series of 50 282 women in 1959-65, 226 women had been exposed to paracetamol during the first trimester of pregnancy. There were 17 malformed children in the exposed group, giving a nonsignificant standardized relative risk (RR) of 1.05 (Heinonen *et al.*, 1977).

In a study of 280 000 women belonging to a prepaid health plan in Seattle, WA (USA), all drug prescriptions and all pregnancy outcomes were monitored between July 1977 and December 1979. Among the liveborn babies of 6837 women, 80 (1.2%) had major congenital malformations. Three of the infants born to 493 women for whom paracetamol had been prescribed in the first trimester had major malformations (types not specified), giving a prevalence of 6 per 1000, which was not significantly different from the overall prevalence in the total population studied (12 per 1000). A second group of 328 women were exposed to paracetamol with codeine in the first trimester. Five of these had malformed babies, giving a prevalence of 15 per 1000, which was not significantly different from that in controls (Jick *et al.*, 1981).

In a second study of the same population, covering the period January 1980 to June 1982, 6509 women had pregnancies ending in livebirths; 105 (1.5%) of the infants had major congenital malformations. Two of the infants born to 350 women for whom paracetamol had been prescribed in the first trimester had major malformations (types not specified), giving a prevalence of 6 per 1000 compared with an overall prevalence in the entire group of 16 per 1000. Three of 347 women exposed to paracetamol with codeine had malformed babies, giving a prevalence of 9 per 1000 (not significant) (Aselton *et al.*, 1985).

(iv) *Genetic and related effects*

Eleven healthy volunteers were given paracetamol at 1000 mg three times over a period of 8 h. The frequency of chromatid breaks in peripheral blood lymphocytes was significantly increased after one day but returned to normal one week later (Kocisova *et al.*, 1988).

3.3 Case reports and epidemiological studies of carcinogenicity to humans

The Working Group considered only studies in which paracetamol was taken directly, either alone or in mixtures. Paracetamol may be taken by analgesic users who previously took phenacetin. Analgesic mixtures containing phenacetin are carcinogenic to humans; and phenacetin is probably carcinogenic to humans (IARC, 1980, 1987).

A population-based case-control study was conducted in Minnesota, USA, involving 495 cases of cancer of the renal parenchyma and 74 cases of cancer of the renal pelvis, diagnosed in 1974-79, and 697 controls (McLauglin *et al.*, 1983, 1984, 1985). An association between cancer of the renal pelvis and intensity and duration of use of paracetamol-containing drugs was seen in women (p for trend, < 0.05; RR in the highest exposure category, based on three exposed cases and eight exposed controls, 5.8; 95% confidence interval (CI), 0.8-40). [The Working Group noted that the trend test included unexposed cases and controls; if the unexposed are excluded, the trend is not statistically significant.] No other significant association was observed. Four of the five cases in the highest exposure category (two men, three women) who developed renal pelvic cancer had also taken phenacetin-containing analgesics; in the entire study, only two cases of cancer of the renal pelvis and seven controls had taken paracetamol alone.

Another population-based case control study was conducted among women aged 20-49 years in the state of New York (USA) involving 173 cases of bladder cancer diagnosed in 1975-79 and an equal number of controls matched for age and telephone area code (Piper *et al.*, 1985). A history of regular use of analgesics containing paracetamol (and not phenacetin) at least one year before diagnosis yielded a smoking-adjusted RR of 1.5 (95% CI, 0.4-7.2). In contrast, the risk for regular users of phenacetin-containing analgesics was significantly elevated whether they also regularly took paracetamol (RR, 3.8; 95% CI, 1.4-13.0) or not (RR, 6.5; 95% CI, 1.5-59.2).

A series of population-based case-control studies of urinary-tract cancer were conducted in New South Wales, Australia, involving cases identified in 1977-82 (McCredie *et al.*, 1983a,b, 1988; McCredie & Stewart, 1988). Ultimately, there were 360 cases of renal parenchymal cancer, 73 cases of renal pelvic cancer, 55 cases of ureteral cancer and 162 cases (women only) of bladder cancer. Controls (985 for renal parenchymal cancer and 689 for the other sites) were derived from electoral rolls. The only significant increase in risk for regular use of paracetamol (cumulative consumption of at least 0.1 kg) was with ureteral cancer (RR, 2.5; 95% CI, 1.1-5.9); this association was not further elevated in the subgroup with higher exposure (at least 1 kg; RR, 2.0; 95% CI, 0.8-4.5). The RR for cancer of the renal

pelvis was 1.2 (95% CI, 0.6-2.3). These analyses were adjusted for cigarette smoking and the presence of urological disease.

A further population-based case-control study was conducted in Los Angeles County, USA, based on 187 cases of cancer of the renal pelvis or ureter diagnosed in 1978-82 and an equal number of neighbourhood controls (Ross *et al.*, 1989). An association was found with use of nonprescription analgesics in general. The risks for use of analgesics containing paracetamol were nonsignificantly elevated, at 1.3 for use more than 30 days/year ($p = 0.34$) and 2.0 for use more than 30 consecutive days/year ($p = 0.08$). The analyses were controlled for cigarette smoking and history of urinary-tract stones. The authors noted that it was difficult to distinguish the effects of individual compounds in this study.

In a hypothesis-generating cohort study designed to screen a large number of drugs for possible carcinogenicity (described in detail in the monograph on ampicillin), 3238 persons to whom at least one prescription for paracetamol alone and 2612 to whom at least one prescription for paracetamol with codeine had been dispensed during 1969-73 were followed up for up to 15 years (Selby *et al.*, 1989). No significant association with cancer at any site was seen for use of paracetamol with codeine. For paracetamol alone, a positive association was noted for melanoma (seven cases observed, 1.7 expected; RR, 4.1; 95% CI, 1.7-8.5), and negative associations for cancer of the colon (four observed, 12.1 expected; RR, 0.33; 95% CI, 0.1-0.85) and cancer of the uterine corpus (one observed, 6.5 expected; RR, 0.15; 95% CI, 0-0.86); but no association was seen for any cancer of the urinary tract or for all cancers combined (Friedman & Ury, 1980, 1983; Selby *et al.*, 1989). [The Working Group noted that there was no information on non-prescription dispensing of paracetamol, which is the most common way that it is obtained. Since, as also noted by the authors, some 12 000 comparisons were made in this study, the associations should be verified independently. Data on duration of use were not provided.]

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Paracetamol has been used extensively as an analgesic and antipyretic since 1946.

4.2 Experimental carcinogenicity data

Paracetamol was tested for carcinogenicity by oral administration in mice and rats. In one strain of mice, a significant increase in the incidence of multiple liver

carcinomas and adenomas was observed in animals of each sex at a markedly toxic dose; in two studies on another strain, no increase in the incidence of any tumour was observed at a well-tolerated dose that was approximately half that in the preceding study. Administration of paracetamol to two different strains of rats did not increase tumour incidence. In a further strain of rats, the incidence of neoplastic liver nodules was increased in animals of each sex given the higher dose; the combined incidence of bladder papillomas and carcinomas (mostly papillomas) was significantly greater in high-dose male and in low-dose female rats. Although treatment increased the incidence of bladder calculi in treated rats, there was no relationship between the presence of calculi and of either hyperplasia or tumours in the bladder.

Oral administration of paracetamol to rats enhanced the incidence of renal adenomas induced by *N*-nitrosoethyl-*N*-hydroxyethylamine.

4.3 Human carcinogenicity data

A positive association between use of paracetamol and cancer of the ureter (but not of other sites in the urinary tract) was observed in an Australian case-control study. None of three other population-based case-control studies showed an association between paracetamol use and cancer in the urinary tract.

4.4 Other relevant data

One study provided no evidence that use of paracetamol in the first trimester of pregnancy is associated with an increase in the incidence of malformations. Paracetamol induced testicular atrophy in rats.

Hepatotoxicity has been reported repeatedly in people taking high doses of paracetamol; chronic alcohol users are particularly sensitive. Paracetamol is metabolized in humans and animals to reactive intermediates that bind to proteins. It is hepatotoxic to experimental animals and causes renal tubular necrosis in rats.

Paracetamol induced chromatid breaks in peripheral human lymphocytes *in vivo*. It induced aneuploidy in rat embryos treated transplacentally. It gave negative results in the micronucleus test in mice *in vivo*. It did not induce chromosomal aberrations in bone-marrow cells or spermatocytes of mice.

Paracetamol induced sister chromatid exchange and chromosomal aberrations in Chinese hamster cells, micronuclei in rat kidney cells and chromosomal aberrations in human lymphocytes *in vitro*. It did not induce point mutations in mouse or Chinese hamster cells. Paracetamol gave positive results in a transformation test in mouse cells *in vitro*. It induced unscheduled DNA synthesis in mouse and rat cells but not in Chinese or Syrian hamster or guinea-pig cells. Paracetamol did not induce sex-linked recessive lethal mutations in *Drosophila* and was not mutagenic to *Salmonella typhimurium* or *Escherichia coli*. (See Appendix 1.)

4.5 Evaluation[1]

There is *inadequate evidence* for the carcinogenicity of paracetamol in humans.

There is *limited evidence* for the carcinogenicity of paracetamol in experimental animals.

Overall evaluation

Paracetamol *is not classifiable as to its carcinogenicity to humans (Group 3).*

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[1]For description of the italicized terms, see Preamble, pp. 26–29.

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