PARACETAMOL

This substance was considered by a previous working group, in 1989 (IARC, 1990). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

### 1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 103-90-2
Deleted CAS Reg. No.: 8055-08-1
Chem. Abstr. Name: *N*-(4-Hydroxyphenyl)acetamide
IUPAC Systematic Name: 4′-Hydroxyacetanilide
Synonyms: 4-Acetamidophenol; acetaminophen; 4-acetaminophenol; 4-(acetylamino)-phenol; 4-(*N*-acetylamino)phenol; 4-hydroxyacetanilide; 4′-hydroxyacetanilide; *N*-(4-hydroxyphenyl)acetamide

1.1.2 *Structural and molecular formulae and relative molecular mass*
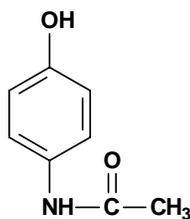
$C_8H_9NO_2$ Relative molecular mass: 151.17

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White crystalline powder (Verschueren, 1996)
(*b*) *Melting-point*: 170°C (Lide, 1997)
(*c*) *Density*: 1.293 g/cm³ at 21°C (Lide, 1997)
(*d*) *Solubility*: Insoluble in water; very soluble in ethanol (Lide, 1997)
(*e*) *Octanol/water partition coefficient (P)*: log P, 0.31 (Hansch *et al.*, 1995)
(*f*) *Conversion factor*: mg/m³ = 6.18 × ppm

1.2   Production and use

Information available in 1995 indicated that paracetamol was produced in 19 countries (Chemical Information Services, 1995).

Paracetamol is used as an analgesic and antipyretic, in the treatment of a wide variety of arthritic and rheumatic conditions involving musculoskeletal pain and in other painful disorders such as headache, dysmenorrhoea, myalgia and neuralgia. It is also indicated as an analgesic and antipyretic in diseases accompanied by generalized discomfort or fever, such as the common cold and other viral infections. Other uses include the manufacture of azo dyes and photographic chemicals, as an intermediate for pharmaceuticals and as a stabilizer for hydrogen peroxide (National Toxicology Program, 1991).

Demand for bulk paracetamol in the United States in 1997 was estimated to be 30 000–35 000 tonnes, more than half of worldwide consumption (Mirasol, 1998).

The conventional oral dose of paracetamol for adults is 325–1000 mg (650 mg rectally); the total daily dose should not exceed 4000 mg. For children, the single dose is 40–480 mg, depending on age and weight; no more than five doses should be administered within 24 h. For infants under three months of age, a dose of 10 mg/kg bw is recommended (Insel, 1996; Reynolds, 1996).

1.3   Occurrence

1.3.1   *Natural occurrence*

Paracetamol is not known to occur naturally.

1.3.2   *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 65 000 workers in the United States were potentially exposed to paracetamol. Occupational exposure may occur during its production and during its use as an analgesic and antipyretic, chemical intermediate or stabilizer.

1.3.3   *Environmental occurrence*

No information on the environmental occurence of paracetamol was available to the Working Group.

1.4   Regulations and guidelines

No international guidelines for paracetamol in drinking-water have been established (WHO, 1993).

2.   Studies of Cancer in Humans

Previous evaluation

An association between use of paracetamol and cancer of the ureter (but not of other sites in the urinary tract) was observed in one Australian case–control study. None of

three other case–control studies evaluated previously (IARC, 1990) showed an association between use of paracetamol and cancer in the urinary tract. Table 1 summarizes the results of these studies and those of case–control studies that have been published subsequently.

New studies

2.1   Cohort studies

A number of studies have been reported of patients with rheumatoid arthritis, osteoarthrosis, back pain or rheumatic disease, who were assumed to have greater than average use of analgesic and anti-rheumatoid drugs. As use specifically of paracetamol could not be separated from use of other drugs in these studies, they were considered not to be useful in evaluating the carcinogenicity of paracetamol.

2.2   Case–control studies

2.2.1   *Cancers of the urinary tract*

A population-based case–control study was conducted in Ontario, Canada, involving all histologically confirmed cases of renal-cell carcinoma newly diagnosed in 1986 or 1987 among residents aged 25–69 years (Kreiger *et al*., 1992). Cases were ascertained through the files of the Ontario Cancer Registry, and control subjects were randomly selected from the same geographic area and frequency-matched to cases by age and sex. A self-administered questionnaire was completed for 513 of the patients with renal-cell carcinoma (81%) and 1369 of the controls (72%) either directly by the subjects themselves or by their next-of-kin (10% of cases and 0.4% of controls). Only 21 cases (4.1%) and 75 controls (5.4%) reported use of paracetamol, defined as use of any type of a paracetamol-containing analgesic at least every other day for one month or more prior to 1980, yielding odds ratios (adjusted for age, smoking habits and body mass index) of 0.9 (95% confidence interval [CI], 0.4–1.8) for men and 0.6 (95% CI, 0.4–1.6) for women. [The Working Group noted that no estimates of cumulative lifetime use among exposed subjects were available.]

In a population-based case–control study in New South Wales, Australia (McCredie *et al*., 1993), all residents aged 20–79 years in whom cancer of the renal parenchyma or renal pelvis had been diagnosed during 1989–90 were identified from records of the New South Wales Central Cancer Registry, supplemented by surveillance of records from urological units of the State. Control subjects were selected from the electoral rolls and frequency matched on age. A personal or telephone interview was completed for 489 patients (310 men and 179 women) with renal-cell cancer (66% of all eligible patients), 147 (58 men and 89 women) with renal pelvis cancer (74% of eligible cases) and 523 (231 men and 292 women) of the control subjects (72% of eligible controls). The questionnaire sought information about lifetime consumption of prescription and non-prescription analgesics before 1987 in addition to other known or suspected risk factors for urinary tract cancer. Consumers of analgesics were defined as those who had taken this type of drug at least 20 times in their lifetime. Study subjects had often taken more

Table 1. Case–control studies of paracetamol by cancer site

Reference and location	Subjects	Estimated exposure (lifetime intake, kg)	Odds ratio (95% CI)	Comments
Renal-cell cancer				
McLaughlin et al. (1985)[a] Minnesota, United States	313 male patients 182 female patients 697 controls	Ever Men Women Regular, > 36 months Men Women	0.7 (0.5–1.0) 1.2 (0.8–1.9) 0.7 (0.1–3.4) 1.2 (0.3–4.6)	Adjusted for age and cigarette smoking
McCredie et al. (1988)[a] New South Wales, Australia	229 male patients 131 female patients 985 controls	Regular use (≥ 0.1 kg)	1.2 (0.8–1.8)	Adjusted for age, sex, smoking and intake of other analgesics
Kreiger et al. (1992) Ontario, Canada	312 male patients 201 female patients 1369 controls	Any[b] Men Women	0.9 (0.4–1.8) 0.6 (0.4–1.6)	Adjusted for age, cigarette smoking and body mass index
McCredie et al. (1993) New South Wales, Australia	310 male patients 179 female patients 523 controls	Any[c], men and women ≤ 0.48 kg 0.49–1.36 kg ≥ 1.37 kg	1.5 (1.0–2.3) 1.9 (1.0–3.6) 0.9 (0.4–1.9) 1.7 (0.9–3.3)	Adjusted for age, sex, cigarette smoking, obesity and intake of phenacetin
Mellemgaard et al. (1994) Denmark	226 male patients 142 female patients 396 controls	Any[d] Men Women > 1 kg Men Women	1.1 (0.5–3.0) 1.0 (0.4–2.5) 0.9 (0.2–4.0) 0.5 (0.1–1.8)	Adjusted for age, smoking, history of hypertension and socioeconomic status

Table 1 (contd)

Reference and location	Subjects	Estimated exposure (lifetime intake, kg)	Odds ratio (95% CI)	Comments
Renal-cell cancer (contd)				
Chow _et al._ (1994) Minnesota, United States	277 male patients 163 female patients 691 controls	Regular use[d], men 0.1–1 kg 1.1–5 kg > 5 kg Regular use[d], women 0.1–1 kg 1.1–5 kg > 5 kg	[0.8] [0.5–1.4] 0.7 (0.3–1.9) 0.7 (0.2–1.8) 0.4 (0.0–4.2) [1.0] [0.6–1.9] 1.0 (0.3–2.8) 0.9 (0.4–2.1) 0.9 (0.2–4.6)	Unadjusted Adjusted for age, smoking and body mass index Unadjusted Adjusted for age, smoking and body mass index
McCredie _et al._ (1995)[e] New South Wales (Australia), Sweden, Germany, Minnesota (USA), Denmark	1732 patients [distribution by sex not given] 2309 controls	Regular use[f] 0.1–1 kg 1.1–5 kg > 5 kg Men Women	1.1 (0.9–1.5) 1.1 (0.8–1.6) 0.9 (0.6–1.5) 1.9 (0.9–3.9) 1.0 (0.7–1.4) 1.3 (0.9–2.0)	Adjusted for centre, age, sex, cigarette smoking and body mass index
Rosenberg _et al._ (1998) United States	258 male patients 125 female patients 8149 non-cancer controls 6499 cancer controls	Non-cancer controls Regular use[d] < 5 years ≥ 5 years Cancer controls Regular use[d] < 5 years ≥ 5 years	1.2 (0.7–2.1) 1.3 (0.6–2.7) 1.1 (0.5–2.6) 1.1 (0.6–2.0) 1.2 (0.6–2.6) 1.1 (0.4–2.5)	Adjusted for age, sex, interview year and geographic area

Table 1 (contd)

Reference and location	Subjects	Estimated exposure (lifetime intake, kg)	Odds ratio (95% CI)	Comments
Renal cancer, not otherwise specified				
Derby & Jick (1996) United States	222 patients [distribution by sex not given] 885 controls	No. of prescriptions < 10 (< 0.2 kg) 10–19 (0.2–0.4 kg) 20–39 (0.5–0.9 kg) ≥ 40 (≥ 1 kg)	[1.2] [0.8–1.6] 1.3 (0.6–2.8) 1.9 (0.7–5.6) 2.6 (1.1–6.0)	Crude odds ratio
Renal pelvis				
McLaughlin *et al*. (1985)[a] United States	50 male patients 24 female patients 697 controls	Ever Men Women Regular use, > 36 months Men Women	1.2 (0.6–2.5) 2.2 (0.8–5.8) 2.5 (0.3–18) 5.8 (0.8–40)	Adjusted for age and cigarette smoking
McCredie & Stewart (1988)[a] New South Wales, Australia	31 male patients 42 female patients 689 controls	Regular use ≥ 0.1 kg ≥ 1 kg	1.2 (0.6–2.3) 0.8 (0.4–1.7)	Adjusted for sex, tobacco smoking and phenacetin use
McCredie *et al*. (1993) New South Wales, Australia	58 male patients 89 female patients 523 controls	Any[c], men and women ≤ 0.48 kg 0.49–1.36 kg ≥ 1.37 kg	1.3 (0.7–2.4) 0.9 (0.2–3.0) 0.9 (0.3–2.5) 2.0 (0.9–4.4)	Adjusted for age, sex, cigarette smoking, educational level and intake of phenacetin

Table 1 (contd)

Reference and location	Subjects	Estimated exposure (lifetime intake, kg)	Odds ratio (95% CI)	Comments
Renal pelvis and ureter				
Ross *et al.* (1989)[a] Los Angeles, United States	127 male patients 60 female patients 187 controls	Regular use (> 30 days/year)	1.3 [0.76–2.2]	Variables used in adjustment not indicated
Linet *et al.* (1995) United States	331 male patients 171 female patients 496 controls	Regular use ≤ 1 kg > 1 kg	1.0 (0.6–1.8) 1.1 (0.5–2.1) 1.0 (0.4–2.3)	Adjusted for age, sex, cigarette smoking and geographic area
Ureter				
McCredie *et al.* (1988)[a] New South Wales, Australia	39 male patients 16 female patients 689 controls	Regular use ≥ 0.1 kg ≥ 1 kg	2.5 (1.1–5.9) 2.0 (0.8–4.5)	Adjusted for sex, tobacco smoking and phenacetin use
Urinary bladder				
Piper *et al.* (1985)[a] New York, United States	173 female patients 173 controls	Regular use, only (> 30 days/year)	1.5 (0.4–7.2)	Matched-pair analysis
McCredie & Stewart (1988)[a] New South Wales, Australia	307 male patients 381 female patients	Regular use ≥ 0.1 kg ≥ 1 kg	0.7 (0.4–1.3) 0.7 (0.4–1.3)	Adjusted for sex, tobacco smoking and phenacetin use
Derby & Jick (1996) United States	504 patients [distribution by sex not given] 2009 controls	No. of prescriptions < 10 (< 0.2 kg) 10–19 (0.2–0.4 kg) 20–39 (0.5–0.9 kg) ≥ 40 (≥ 1 kg)	[1.0] [0.8–1.2] 1.1 (0.6–2.0) 1.1 (0.6–2.3) 1.3 (0.6–2.8)	Crude odds ratio

Table 1 (contd)

Reference and location	Subjects	Estimated exposure (lifetime intake, kg)	Odds ratio (95% CI)	Comments
Lower urinary tract (mainly bladder)				
Steineck *et al.* (1995) Stockholm, Sweden	325 patients [distribution by sex not given] 393 controls	Any use	1.6 (1.1–2.3)	Summary estimate, adjusted for sex, year of birth, smoking and use of other types of analgesics. According to the authors, 'no details in the exposure substantiated the finding'
Rosenberg *et al.* (1998) United States	384 male patients 114 female patients 8149 non-cancer controls 6499 cancer controls	Non-cancer controls Regular use[d] < 5 years ≥ 5 years Cancer controls Regular use[d] < 5 years ≥ 5 years	1.1 (0.6–1.9) 1.1 (0.5–2.3) 1.1 (0.5–2.6) 0.9 (0.5–1.6) 0.8 (0.4–1.8) 1.0 (0.4–2.4)	Adjusted for age, sex, interview year, and geographic area
Ovary				
Cramer *et al.* (1998) United States	563 patients 523 controls	Continuous use[g]	0.5 (0.3–0.9)	Adjusted for age, study centre, education, religion, parity, use of oral contraceptives and certain types of pain

[a] Evaluated previously (IARC, 1990)

[b] At least every other day for one month or more

[c] At least 20 times during lifetime

[d] At least twice weekly for a month

[e] Includes patient material from the studies of McCredie *et al.* (1993), Mellemgaard *et al.* (1994) and Chow *et al.* (1994)

[f] Lifetime intake of at least 0.1 kg

[g] At least once a week for at least six months

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than one type of analgesic, a substantial proportion having used phenacetin-containing compounds (21% of patients and 9% of controls). Use of paracetamol in any form was reported by 73 patients with renal-cell carcinoma (15%), 40 patients with cancer of the renal pelvis (27%) and by 55 controls (11%). When adjusted for age, sex, cigarette smoking, obesity, educational level and use of other types of analgesics, use of para-cetamol as a single drug was associated with a nonsignificant odds ratio for renal-cell carcinoma of 1.5 (95% CI, 0.9–2.4), and use of paracetamol in any form gave a non-significant odds ratio of 1.5 (95% CI, 1.0–2.3); the odds ratios for cancer of the renal pelvis were 1.3 (95% CI, 0.6–2.7) for paracetamol as a single drug and 1.3 (95% CI, 0.7–2.4) in any form. No dose–response relationship was found between lifetime con-sumption of paracetamol (highest category, ≥ 1.37 kg) taken in any form and the occurrence of either cancer. When the analysis was confined to the subset of subjects who had never taken phenacetin or aspirin analgesics, regular consumption of para-cetamol as a single drug was associated with a relative risk for renal-cell cancer of 1.6 (95% CI, 1.0–2.8) based on 38 exposed cases and 30 exposed controls. The authors noted that before 1968 in Australia most users of analgesics took phenacetin-containing prepa-rations, indicating that residual confounding from this drug may have influenced the results of this study.

In a population-based study, Mellemgaard *et al.* (1994) included all histologically confirmed cases of renal-cell carcinoma diagnosed between 1989 and 1991 in Danish inhabitants aged 20–79 years at the time of diagnosis. Control subjects were randomly selected from computerized files of the national Central Population Register with fre-quency matching by sex, age and place of residence at 1 April 1968. Personal interviews including detailed information on lifetime use of analgesics prior to diagnosis were com-pleted with 368 of the eligible patients (76%) and 396 of the eligible controls (79%). A drug–exposure matrix for prescribed and non-prescribed analgesics was applied in which information on past and current brand names obtained from study subjects was converted to information on active ingredients. Minimal consumption was defined as use twice weekly for a month. Any such consumption of paracetamol was reported for 22 cases (6%) and 24 controls (6%). The odds ratios for renal-cell carcinoma, adjusted for age, smoking, history of hypertension and socioeconomic status, were 1.1 (95% CI, 0.5–3.0) in men and 1.0 (95% CI, 0.4–2.5) in women. Among the study subjects with the highest life-long intake of paracetamol (> 1 kg), the odds ratios were 0.9 (95% CI, 0.2–4.0) for men and 0.5 (95% CI, 0.1–1.8) for women, based on a total of 10 exposed cases and 22 exposed controls.

Chow *et al.* (1994) studied 591 patients in the population of Minnesota, United States, aged 20–79 years, in whom renal-cell carcinoma had been newly diagnosed during 1988–90 and were identified through an existing State cancer surveillance system, and 691 control subjects, who were identified by random-digit dialling (aged 64 years or younger) or from the files of the Health Care Financing Administration of the State (aged 65 years or older) and matched to the cases by age and sex. Information on use of prescription and non-prescription analgesics, height, weight, medical conditions,

smoking habits, alcohol use and occupational histories was collected at personal
interviews with the study subjects or their next-of-kin. The response rates were 87% for
cases and approximately 85% for controls. Interviews were conducted with next-of-kin
for 151 (26%) of the cases and none of the controls; however, the risk estimates were
based only on information from the 440 (74%) directly interviewed cases and the 691
(100%) directly interviewed controls. Exposure to paracetamol, phenacetin and other
analgesic ingredients was assessed by means of a drug-exposure linkage system, in
which information on brand names obtained from study subjects was converted on an
annual basis into information on the type and amount of active ingredients. Total life-
long use was estimated by summing annual exposure of subjects before 1987. Any
regular use of paracetamol, defined as two or more times per week for one month or
longer, was reported by 43 patients (10%) and 70 controls (10%). When users were
compared with study participants who reported no or irregular use of any analgesics, use
of paracetamol was associated with crude odds ratios of [0.8] [95% CI, 0.5–1.4] for men
and [1.0] [95% CI, 0.6–1.9] for women. After adjustment for age, smoking status and
body mass index, there was no trend to increasing risk with increasing lifetime con-
sumption of paracetamol, salicylates or phenacetin for either men or women. Lifetime
intake of paracetamol amounting to 0.1–1, 1.1–5 or > 5 kg was associated with odds
ratios of 0.7 (95% CI, 0.3–1.9), 0.7 (95% CI, 0.2–1.8) and 0.4 (95% CI, 0.0–4.2),
respectively, among men and 1.0 (95% CI, 0.3–2.8), 0.9 (95% CI, 0.4–2.1) and 0.9
(95% CI, 0.2–4.6), respectively, among women. A statistically nonsignificant excess risk
was found for women (odds ratio, 2.1; 95% CI, 0.6–6.9) who had used only paracetamol-
containing analgesics, on the basis of seven exposed cases, but there was no correspon-
ding increase among men (odds ratio, 1.2; 95% CI, 0.5–3.2; eight exposed cases). [The
Working Group noted that different proportions of patients and controls were interviewed
directly and thus included in the analysis, which might have resulted in biased selection
of cases.]

McCredie *et al*. (1995) reported on the results of a large international case–control
study of renal-cell carcinoma which, in addition to the previously described study mate-
rials from New South Wales, Australia (McCredie *et al*., 1993), Denmark (Mellemgaard
et al., 1994) and Minnesota, United States (Chow *et al*., 1994), also included study
materials from Uppsala, Sweden, and from Berlin and Heidelberg, Germany. In the
Swedish study, patients aged 20–79 years with histologically confirmed renal-cell carci-
noma diagnosed in the Uppsala Health Care Region during 1989–91 were identified in a
population-based cancer registry, preceded by a rapid ascertainment system; patients in
the German study were identified by monitoring all hospitals and pathology departments
of the Berlin and Heidelberg study areas but keeping an upper age limit of 75 years.
Controls, frequency-matched to cases by age and sex, were taken from registries covering
the entire background population. The three sets of previously unpublished material from
Sweden and Germany comprised approximately 30% of the material in this pooled,
international analysis, which covered a total of 1732 patients with renal-cell carcinoma
and 2309 controls. These figures resulted from overall response rates of 72% and 75%

among cases and controls, respectively. Personal interviews were conducted with essentially the same questionnaire in all of the study centres, and estimates of lifetime intake of prescription and non-prescription paracetamol, phenacetin and other analgesics were derived from a jointly developed drug-exposure matrix specifying the amount and composition of active ingredients in each analgesic on sale in the five countries during the study period. Significant exposure to analgesics was defined as life-long intake of at least 0.1 kg which is equivalent to two 500-mg paracetamol tablets twice a week for one year. When compared with study participants who reported cumulative intake of less than 0.1 kg of any analgesic and with adjustment for centre, age, sex, cigarette smoking and body mass index, intake of 0.1 kg or more of paracetamol was associated with odds ratios of 1.0 (95% CI, 0.7–1.4; 55 exposed cases) for men and 1.3 (95% CI, 0.9–2.0; 64 exposed cases) for women and 1.1 (95% CI, 0.9–1.5) for the two sexes combined, on the basis of a total of 119 exposed patients and 142 exposed controls. Life-time intake of paracetamol of 0.1–1, 1.1–5 and > 5 kg (the two sexes combined) was associated with odds ratios of 1.1 (95% CI, 0.8–1.6), 0.9 (95% CI, 0.6-1.5) and 1.9 (95% CI, 0.9–3.9). The risk of the subset of women with a total consumption of > 5 kg paracetamol was statistically significantly increased, at 2.5 (95% CI, 1.0–6.2), based on 14 exposed cases and eight exposed controls, but there was no corresponding increase among men (odds ratio, 1.1; 95% CI, 0.3–4.0; six exposed cases). Adjustment for consumption of phenacetin did not alter the risk. The odds ratios for regular use exclusively of paracetamol were 0.6 (95% CI, 0.3–1.3) for men and 1.3 (95% CI, 0.6–2.6) for women; the risk did not increase with dose. [The Working Group noted that inclusion in the reference exposure category of cumulative doses up to 0.1 kg might have reduced the risk estimates.]

In a population-based study, Steineck et al. (1995) included incident cases of squamous- or transitional-cell carcinoma of the urinary tract diagnosed in the Stockholm area, Sweden, during the period 1985–87. Cases were identified from the files of the city hospitals, and controls, frequency-matched by sex and year of birth, were chosen at random from a computerized register covering the population of Stockholm. A postal questionnaire supplemented by a telephone interview was completed for 325 patients (78%), comprising 305 with cancer of the urinary bladder, six with cancer of the ureter, 11 with cancer of the renal pelvis and three with cancer at multiple sites in the lower urinary tract, and with 393 controls (77%). The questionnaire contained questions about use of paracetamol, aspirin and phenacetin, each categorized according to total intake (1–99 or ≥ 100 tablets) in each of three specified decades (1950–79), and about the subjects' medical history and smoking habits. Any use of paracetamol was reported by 119 cases (37%) and 119 controls (30%), yielding an odds ratio adjusted for sex, year of birth, tobacco smoking and other use of analgesics of 1.6 (1.1–2.3). The authors reported that analyses by duration and amount did not substantiate the finding [data were not given].

In a hospital-based study, Rosenberg et al. (1998) studied 498 patients with a histologically confirmed transitional-cell cancer of the urinary tract (440 patients with bladder cancer, 41 with renal pelvis cancer and 17 with cancer of the ureter or urethra) and 383 patients with a histologically confirmed renal-cell cancer from among the population of

adult patients under the age of 70 years who had been admitted to hospitals located in various cities of the United States over the years 1977–96 for any of a variety of malignant and non-malignant conditions. Two control groups were selected from the same population of adult patients under surveillance, i.e. one admitted for a non-malignant condition judged to be unrelated to use of paracetamol (8149 control patients) and the other for a cancer at one of a number of selected sites (6499 control patients). Information on use of prescription and non-prescription medications was obtained from patients and controls at personal interviews conducted by nurses during hospitalization on the basis of answers to questions about a number of indications for use of paracetamol and other analgesics, which included pain and muscle ache. Information on personal characteristics, medical and reproductive history and smoking habits was also collected. The overall response rate in the surveillance study was 95%. Any regular use of paracetamol, defined as use on two or more days a week for at least a month, that had begun at least one year before admission for the index disease was reported by 15 patients with transitional-cell cancer (3.0%), 14 patients with renal-cell carcinoma (3.7%), 294 non-cancer controls (3.6%) and 191 cancer controls (2.9%). The odds ratios for transitional-cell cancer, adjusted for age, sex, interview year and geographic area, were 1.1 (95% CI, 0.6–1.9) in the study with non-cancer controls and 0.9 (95% CI, 0.5–1.6) in the study with cancer controls when compared with study participants who reported no use of paracetamol. The corresponding relative risk estimates were 1.1 (95% CI, 0.5–2.6) and 1.0 (95% CI, 0.4–2.4) for five or more years of regular use and 1.1 (95% CI, 0.9–1.4) and 1.2 (95% CI, 0.9–1.5) for irregular use of paracetamol. The odds ratios for renal-cell cancer were 1.2 (95% CI, 0.7–2.1) in the study with non-cancer controls and 1.1 (95% CI, 0.6–2.0) in the study with cancer controls for regular use of paracetamol when compared with no use and 1.1 (95% CI, 0.5–2.6) and 1.1 (95% CI, 0.4–2.5) for long-term use (five years or more). Essentially identical relative risk estimates were seen for the group of irregular users of paracetamol. [The Working Group noted that hospital controls are more likely than population controls to have a condition that leads to use of paracetamol.]

In a population of about 380 000 members of a cooperative health insurance organization in Washington, United States, Derby and Jick (1996) studied 222 patients, aged 20 years or older, with cancer of the kidney and 504 with cancer of the urinary bladder, which had been diagnosed during the period 1980–91 at hospitals maintained by the insurance organization. Groups of 885 and 2009 control subjects were selected from among all members of the organization and matched to the cases of kidney and bladder cancer, respectively, on sex, age and duration of membership of the organization; they had to be free of cancer at the date of diagnosis of the corresponding case. Information on over-the-counter drugs and prescribed medications obtained by study subjects at one of the pharmacies maintained by the insurance organization was available from 1974 onwards. Information on smoking habits, coffee drinking, height, weight, occupation and history of urinary tract infections was taken from outpatient medical records, if available. Study subjects were categorized according to number of prescriptions for paracetamol

ever filled at the pharmacy, and the cumulative amount taken by the subject was esti-mated as follows: 1 prescription, 2–9 (< 0.2 kg), 10–19 (0.2–0.4 kg), 20–39 (0.5–0.9 kg) and > 40 (≥ 1 kg). The crude odds ratios were 0.9 (0.6–1.5), 1.3 (0.9–1.9), 1.3 (0.6–2.8), 1.9 (0.7–5.6) and 2.6 (1.1–6.0; nine exposed patients), respectively, compared with those with no prescription filled (*p* for trend, 0.01). Multivariate analysis with adjustment, whenever possible, for smoking, body mass index and history of urinary tract infection further increased the odds ratio for kidney cancer associated with 40 or more paracetamol prescriptions to 4.5 (95% CI, 0.7–30). The crude odds ratios for bladder cancer were 1.0 (0.8–1.4), 1.0 (0.8–1.3), 1.1 (0.6–2.0), 1.1 (0.6–2.3) and 1.3 (0.6–2.8) for subjects who filled 1, 2–9, 10–19, 20–39 and 40 or more paracetamol prescriptions, respectively, compared with those with no prescription filled. Analysis after adjustment for smoking, coffee drinking and occupation, whenever the information was available, gave essentially the same results. [The Working Group noted that potential confounders were not adjusted for in estimating several of the odds ratios reported above, including those for which the 95% confidence intervals excluded unity.]

In a multi-centre case–control study in New Jersey, Iowa and California, United States, Linet *et al.* (1995) included all histologically confirmed cases of cancer of the renal pelvis or ureter diagnosed between 1983 and 1986 among white residents aged 20–79 years. Controls for cases under the age of 65 at the time of diagnosis were chosen by random-digit dialling, while controls for cases aged 65 and older were selected from Medicare files maintained by the Health Care Financing Administration. Controls were frequency-matched to cases by age, sex and study centre. Personal interviews, conducted in the homes of subjects, were completed for 502 cases (62% of all eligible patients) and 496 controls (approximately 60% of eligible individuals), in which detailed information was sought on regular use of a large number of over-the-counter and prescription anal-gesics, excluding the five-year period before the interview. Lifetime cumulative use of paracetamol, salicylates and phenacetin was assessed by means of a drug-exposure matrix for analgesics in which information on past and current brand names obtained from study subjects was converted to information on amounts and types of active ingre-dients. Any regular use of paracetamol, defined as a minimum of two or more doses per week for at least one month, was reported for 35 patients (7%) and 31 controls (6.2%), which yielded an odds ratio for cancers of the renal pelvis and ureter, adjusted for age, sex, study centre and cigarette smoking, of 1.0 (95% CI, 0.6–1.8). Exclusive con-sumption of paracetamol was associated with an odds ratio of 0.8 (95% CI, 0.3–2.3), and lifetime intake of ≤ 1 kg or > 1 kg paracetamol was associated with odds ratios of 1.1 (95% CI, 0.5–2.1) and 1.0 (95% CI, 0.4–2.3), respectively. [The Working Group noted that, although 12 controls (2.4%) had had lifetime exposure to more than 1 kg phena-cetin, no association was seen in this study between cancers of the renal pelvis and ureter and use of phenacetin. Analgesic mixtures containing phenacetin are a recognized human carcinogen with the renal pelvis as the target tissue (IARC, 1987).]

2.2.2   *Ovarian cancer*

Cramer *et al*. (1998) reported on a population-based case–control study of women with a diagnosis of epithelial ovarian cancer or a lesion of borderline malignancy who were residents of eastern Massachusetts or all of New Hampshire (United States) at the date of diagnosis in 1992–97; the cases were identified from state cancer registries. Control subjects, matched to cases by age and geographical area, were identified through random-digit dialling, or—for cases in Massachusetts aged 60 years or older—from lists of all residents in towns. Of 1080 notified cases of ovarian cancer, only 563 (52%) were included in the analysis together with 523 control subjects [57%], after exclusion for various reasons. Interviews, usually completed at the subject's home, included questions about demographics, menstrual and reproductive history, medical and family history and personal habits. Continuous use of over-the-counter and prescribed analgesics, defined as at least once a week for at least six months, was assessed for paracetamol, aspirin and ibuprofen until one year before diagnosis. Any continuous use of over-the-counter paracetamol was reported by 26 patients with ovarian cancer (4.6%) and 46 control subjects (8.8%), yielding an odds ratio, adjusted for age, study centre, education, religion, parity, use of oral contraceptives and menstrual, arthritic or headache pain, of 0.5 (95% CI, 0.3–0.9). The lowest risks for ovarian cancer were seen for women who had used paracetamol daily (odds ratio, 0.4; 95% CI, 0.2–0.7; 16 cases, 35 controls), for more than 10 years (odds ratio, 0.4; 95% CI, 0.2–0.9; 10 cases, 24 controls) and for more than 20 tablet–years, defined as the number of tablets per day multiplied by the number of years of use (odds ratio, 0.5; 95% CI, 0.2–1.0; 10 cases, 20 controls). Inverse associations between use of paracetamol and risk were seen for all the histological subtypes of ovarian cancer included in the study. [The Working Group noted that the overall response rate among cases and controls was very low and that there were a number of substantial differences in demographic variables between cases and controls, suggesting that the results may have been influenced by biased selection of study subjects.]

3.   Studies of Cancer in Experimental Animals

Previous evaluation

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 also enhanced the incidence of renal adenomas induced by *N*-nitroso-*N*-ethyl-*N*-hydroxyethylamine (IARC, 1990). [The present Working Group noted that in the study in rats in which tumours were induced (Flaks *et al.*, 1985) no tumours were found in either male or female controls, which is a highly unusual finding and raises questions about the interpretation of the findings.]

New studies
3.1   Oral administration
3.1.1   *Mouse*
Groups of 50 male and 50 female B6C3F$_1$ mice, eight to nine weeks old, were given paracetamol (purity, > 99%) in the diet at concentrations of 0, 600, 3000 or 6000 mg/kg diet (ppm) for up to 104 weeks. There was no difference in survival between control and exposed mice, but a dose-related depression in body-weight gain was recorded in animals of each sex. Under the conditions of this study, there was no evidence of carcinogenic activity. The incidence of thyroid gland follicular-cell hyperplasia was increased in a dose-related manner in both males and females (National Toxicology Program, 1993).

3.1.2   *Rat*
Groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks old, were given paracetamol (purity, > 99%) in the diet at concentrations of 0, 600, 3000 or 6000 mg/kg diet (ppm) for up to 104 weeks. There was no difference in survival between control and exposed groups and no effect of treatment on body-weight gain. No treatment-related increase in tumour incidence was found in male rats, but an increase in the incidence of mononuclear cell leukaemia was observed in females at the high dose (9/50, 17/50, 15/50, 24/50 for the four groups respectively) (National Toxicology Program, 1993). [The Working Group noted the high and variable incidence of mononuclear-cell leukaemia between and within studies with Fischer rats and considered that this was not a treatment-related effect.]

3.2   Administration with known carcinogens or modifying factors
Rat: In a model of urinary bladder carcinogenesis, groups of 10 or 20 male Fischer 344 rats, six weeks of age, were treated with 0.1% *N*-nitrosodi(2-hydroxypropyl)amine in the drinking-water and 3.0% uracil in the diet for the first four weeks as an initiating schedule and, one week later, were given either a diet containing 0.8% paracetamol or a basal (control) diet for 35 weeks, at which point the animals were killed. Paracetamol did not significantly increase the incidences of tumours of the renal tubules, renal pelvis, ureter or urinary bladder when compared with the initiated control group (Shibata *et al.*, 1995).

In a model of intestinal carcinogenesis, six groups of 27 (controls) or 48 male Fischer 344 rats, approximately eight weeks of age, were given paracetamol (purity, 99%) in the diet at concentrations of 0, 250 or 5000 mg/kg (ppm) for 44 weeks, when the animals were killed. Commencing two weeks after the start of paracetamol feeding, 50 mg/kg bw

3,2-dimethyl-4-aminobiphenyl (DMAB) were injected subcutaneously once weekly for 20 weeks. Vehicle controls were given 1 mL/kg bw corn oil by the same route and for the same period as DMAB but no DMAB or paracetamol. There was also an untreated control group (no corn oil, paracetamol or DMAB), a group receiving paracetamol alone and a group receiving DMAB alone. Less than 50% of the animals receiving DMAB survived, the deaths being due mainly to intestinal obstruction from tumour growth. No intestinal tumours occurred in the controls or those receiving paracetamol alone. The incidence of intestinal tumours in those given DMAB alone was 73%, which was significantly reduced to 50% ($p < 0.05$) by paracetamol at each dose (Williams & Iatropoulos, 1997).

In a short-term model of fatty liver and liver cirrhosis, groups of 8–22 male Fischer 344 rats, six weeks of age, were fed either a choline-deficient or a choline-supplemented diet for four weeks, followed by a single oral gavage of paracetamol at a doses of 0, 0.5, 1 or 1.5 mg/kg bw in 0.2% tragacanth gum solution. Four hours after paracetamol treatment, the rats were subjected to a two-thirds hepatectomy, followed by a two-week recovery period, and then two weeks of feeding of 0.02% of 2-acetylaminofluorene coupled with a single gavage of carbon tetrachloride at the mid-point. The rats were killed nine weeks after the beginning of the study after an 18-h fast. A positive control group received a single intra-peritoneal injection of N-nitrosodiethylamine in the place of paracetamol. Foci of hepato-cellular alteration were assayed by immunohistochemical staining for γ-glutamyltrans-peptidase or the placental form of glutathione-S-transferase. Paracetamol did not significantly alter the number or size of liver foci when compared with the relevant control values (Maruyama *et al.*, 1990).

In a long-term model of fatty liver and liver cirrhosis, groups of 8–13 male Fischer 344 rats, six weeks of age, were fed either a choline-deficient diet for 27 weeks to produce liver cirrhosis or a choline-supplemented diet as a control group. From 27 weeks, the rats were fed a diet containing 0, 0.05, 0.45 or 0.9% paracetamol, basal diet or choline-supple-mented diet, until week 52, at which time the surviving animals were killed. Foci of hepatocellular alteration were assayed by immunohistochemical staining for γ-glutamyl-transpeptidase or the placental form of glutathione-S-transferase. Paracetamol did not alter the number or size of liver foci when compared with the relevant control groups (Maruyama *et al.*, 1990).

4.   Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1   Absorption, distribution, metabolism and excretion

4.1.1   *Humans*

After oral administration, paracetamol is absorbed rapidly from the small intestine, while absorption from the stomach is negligible. The rate of absorption depends on the rate of gastric emptying (Clements *et al.*, 1978). First-pass metabolism of paracetamol is dose-dependent: the systemic availability after oral administration ranges from 90% of

1–2 g to 63% of 0.5 g. The plasma concentrations of paracetamol in healthy subjects peaked within 1 h after ingestion of 0.5 or 1 g but continued to rise up to 2 h after oral intake of 2 g (Rawlins *et al*., 1977). Intramuscular administration of 600 mg paracetamol to healthy male volunteers resulted in a maximal average blood concentration of 7.5 μg/mL 1.7 h after dosing (Macheras *et al*., 1989).

A comparison of dogs and humans indicated that in humans paracetamol is rapidly and relatively uniformly distributed throughout the body fluids (Gwilt *et al*., 1963). After normal therapeutic doses of paracetamol, binding to plasma proteins is considered insignificant (Gazzard *et al*., 1973). The apparent volume of distribution of paracetamol in humans is about 0.9 L/kg bw (Forrest *et al*., 1982). The decrease in paracetamol concentrations in plasma is multiphasic after both intravenous injection and oral dosing with 500 or 1000 mg. When the data for six healthy volunteers were interpreted according to a two-compartment open model, the half-life of the first exponential ranged from 0.15 to 0.53 h and that of the second exponential from 2.2 to 3.3 h. The latter value was in agreement with that found after oral dosing. The mean clearance (± SEM) after intravenous administration of 1000 mg was 350 (± 40) mL/min (Rawlins *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 glucuronide and sulfate metabolites have been shown to accumulate in the plasma of patients with renal failure who are taking paracetamol (Lowenthal *et al*., 1976). Paracetamol crossed the placenta in an unconjugated form and was excreted in the urine of an exposed neonate in a similar composition to that found in the urine of a two- to three-day-old infant who had received an oral dose (Collins, 1981). During lactation, paracetamol passes rapidly into milk, and the milk:plasma concentration ratio increased from 0.9 to 1.3 between 45 min and 6 h after ingestion (Berlin *et al*., 1980; Notarianni *et al*., 1987).

Paracetamol is metabolized predominantly to the 4-glucuronide and 4-sulfate conjugates in human liver. In young healthy subjects, about 55 and 30% of a therapeutic dose of 20 mg/kg bw was excreted in the urine as the glucuronide and sulfate conjugates, respectively; the sum of cysteine conjugate and mercapturic acid resulting from glutathione conjugation was found to be 8% of the dose (Forrest *et al*., 1982).

Of the total radiolabel in urine of volunteers collected over 24 h after an oral dose of 1200 mg [³H]paracetamol, only 2% was unchanged paracetamol, while 52 and 41% were associated with sulfate and glucuronide conjugates, respectively. About 4% of the dose was associated with a mercapturic acid conjugate, probably through conversion of paracetamol by cytochrome P450 (CYP)-dependent hepatic mixed-function oxidase to the highly reactive *N*-acetyl-*para*-benzoquinone imine, which was subsequently inactivated by reaction with reduced glutathione and excreted as the mercapturic acid conjugate. Large doses of paracetamol can deplete glutathione stores, and the excess of the highly reactive intermediate binds covalently to vital cell elements, which may result in acute hepatic necrosis (Mitchell *et al*., 1974).

In a study to investigate the effect of inhibitors of oxidative metabolism on the bio-conversion of paracetamol, 10 healthy men (average weight, 77.6 kg) consumed 20 mg/kg bw

paracetamol dissolved in 400 mL of a soft drink over 2 min on three occasions at an interval of at least a week. On one occasion, the paracetamol was taken alone, on another with cimetidine and on the third occasion with ethanol (70° proof whisky). Cimetidine and ethanol were taken before the paracetamol and as maintenance doses for 8 h after intake. The plasma and urinary concentrations of paracetamol and its metabolites and plasma ethanol were determined. Cimetidine had no effect on the metabolism of paracetamol, but ethanol caused a highly significant decrease in the recovery of mercapturic acid and cysteine conjugates (Critchley *et al.*, 1983).

NADPH-dependent oxidation of [^{14}C]paracetamol to a reactive intermediate in microsomes from human liver obtained at autopsy was inferred from measurement of protein-bound radiolabel. The extent of inhibition of protein binding by 48 mmol/L ethanol was only one-fourth to one-half that found *in vivo* by Critchley *et al.* (1983) (Thummel *et al.*, 1989).

Liver microsomes from seven subjects converted 1 mmol/L paracetamol to a reactive intermediate detected as the paracetamol–cysteine conjugate. Antibody inhibition studies showed that two P450 isozymes, namely CYP2E1 and CYP1A1, catalyse nearly all of the paracetamol activation in human liver microsomes (Raucy *et al.*, 1989).

Microsomes isolated from four human livers were used to investigate the cytochrome P450 enzymes involved in paracetamol activation. The kinetics of the conversions of paracetamol to its glutathione conjugate suggests multiple K_m values, consistent with the involvement of several cytochrome P450s. Human cytochrome P450 enzymes expressed in HepG2 cells, CYP 2E1, CYP 1A2 and CYP 3A4, were all found to metabolize paracetamol to an activated metabolite identified as a glutathione conjugate. Because of the higher K_m of CYP 1A2, the authors proposed that CYP 3A4 and CYP 2E1 were of greater significance in the activation of paracetamol to *N*-acetyl-*para*-benzoquinone imine, and that this highly reactive intermediate is trapped by conjugation with glutathione (Patten *et al.*, 1993).

Purified human CYP 2E1 selectively oxidized paracetamol to the toxic metabolite *N*-acetyl-*para*-benzoquinone imine, whereas human CYP 2A6 selectively oxidized paracetamol to 3-hydroxyparacetamol. However, both isoforms of cytochrome P450 were capable of catalysing both reactions and the investigators concluded from their kinetic analysis that, at toxic doses of paracetamol, CYP 2A6 could contribute to formation of the toxic metabolite, although CYP 2E1 would be more efficient (Chen *et al.*, 1998).

4.1.2   *Experimental systems*

The proposed metabolic pathways of paracetamol are summarized in Figure 1. The major urinary metabolites (the glucuronide, sulfate and 3-mercapto derivatives) are observed in most species, although the percentages of these conjugates excreted in urine vary widely among species (Davis *et al.*, 1974). In rats, the proportion of biliary excretion due to the various metabolites of paracetamol increased from 20 to 49% as the intravenous doses were increased from 37.5 to 600 mg/kg bw. The glucuronide conjugate was the major metabolite recovered in bile at all doses (Hjelle & Klaassen, 1984). First-pass

Figure 1. Major pathways for the metabolism of paracetamol
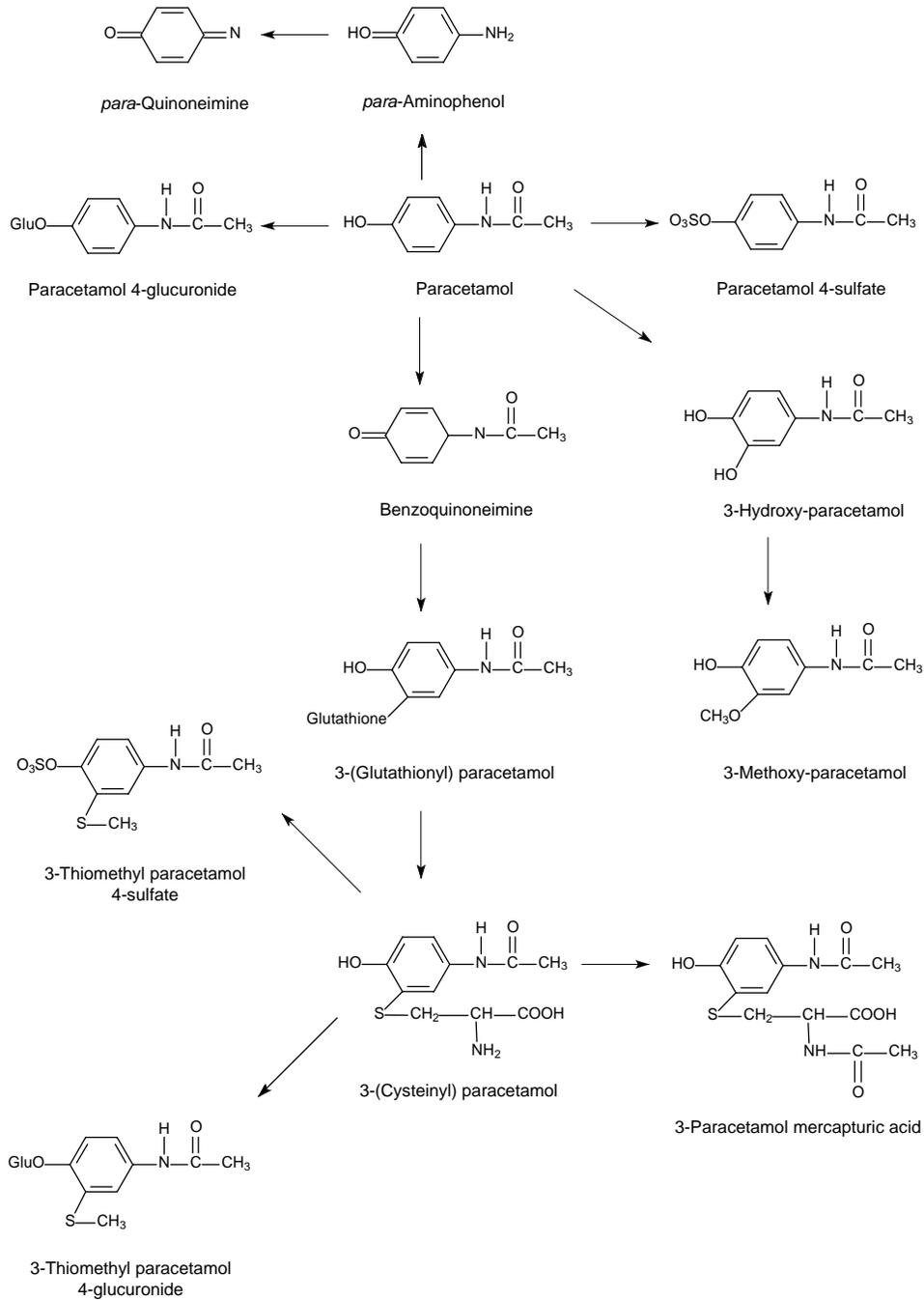
Modified from National Toxicology Program (1993)

metabolism by the intestine was reported to predominate, and saturation of paracetamol sulfate formation in the liver was suggested to be responsible for lower clearance at doses of 150 and 300 mg/kg bw administered intra-arterially and intravenously (Hirate *et al*., 1990).

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). *N*-Acetyl-*para*-benzoquinone imine was found to be formed as an oxidation product of paracetamol by purified P450 and cumene hydroperoxide. The reactive product was rapidly reduced back to paracetamol by a variety of reductants. Attempts to produce *N*-acetyl-*para*-benzoquinone imine from NADPH and microsomes were not successful owing to this rapid reduction; however, both purified *N*-acetyl-*para*-benzoquinone imine and paracetamol with an NADPH-generating system bound covalently to mouse liver microsomal protein. A subsequent reaction of *N*-acetyl-*para*-benzoquinone imine was found to be conjugation to glutathione, resulting in 3-*S*-glutathionylparacetamol (Dahlin *et al*., 1984).

Adult male Sprague-Dawley rats received an intravenous injection of 15, 30, 150 or 300 mg/kg paracetamol, and plasma and urine were assayed during 6–7 h after dosing for paracetamol and its glucuronide and sulfate conjugates. At each dose, the plasma concentrations of paracetamol decreased exponentially with time, but at the two higher doses total clearance and the fraction excreted as sulfate conjugate decreased. Both paracetamol glucuronide and paracetamol sulfate formation appeared to be capacity-limited (Galinsky & Levy, 1981). A correlation has been found 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'). The susceptible species (hamsters, mice) excreted 27–42% of the dose as metabolite in the toxication pathway, whereas the more resistant species (rats, rabbits, guinea-pigs) excreted only 5–7% of the dose via this route (Gregus *et al*., 1988). 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*-benzoquinone imine 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).

Prostaglandin *H* synthase catalysed the arachidonic acid-dependent polymerization of paracetamol and, in the presence of glutathione, also catalysed the formation of 3-(glutathion-*S*-yl)paracetamol. These reactions involved the overall 1- and 2-electron oxidation of paracetamol via formation of *N*-acetyl-*para*-benzosemiquinone imine and *N*-acetyl-*para*-benzoquinone imine (Potter & Hinson, 1987a). The polymerization reaction was also observed when cumene hydroperoxide was added to microsomes and paracetamol (Potter & Hinson, 1987b). These data indicate that oxidative or free-radical reactions initiated by paracetamol play a role in the hepatotoxicity of this drug (Birge *et al*., 1988).

The kinetics of the conversion of paracetamol to its glutathione conjugate suggested multiple equilibrium constants (K_m) consistent with the involvement of several P450s in

microsomes from the livers of Sprague-Dawley rats. Antibodies specific for rat CYP 2E1 and CYP 1A2 each inhibited approximately 40% of this biotransformation in microsomes from control male rats, but only slight inhibition was observed with CYP 3A1/2 antibodies in male or female rat microsomes. In microsomes from female rats induced with dexamethasone, the kinetics of paracetamol activation indicated a single K_m. In this case, the active enzyme was shown to be CYP 3A1, because antibodies against this enzyme inhibited activity by 80% (Patten *et al.*, 1993).

The role of cytochrome P450 isoforms in the acute liver toxicity of paracetamol was further established by the use of *cyp2e1* knock-out C57BL/6N mice. Mice that received single doses of paracetamol up to 400 mg/kg bw intraperitoneally survived, whereas 50% of the wild-type mice died. Significantly increased activities of the liver enzymes aspartate aminotransferase and alanine aminotransferase were found in wild-type when compared with knock-out mice given 200 or 400 mg/kg bw paracetamol. At the highest dose (800 mg/kg bw), most of the mice died and the aspartate aminotransferase activity was elevated in both wild-type and knock-out mice, suggesting a role of another cytochrome P450 such as CYP 1A2 (Lee *et al.*, 1996).

Paracetamol is activated in the kidney by an NADPH-dependent cytochrome P450 to an arylating agent, which can bind covalently to cellular macromolecules (McMurtry *et al.*, 1978). Studies in several species have suggested that formation of *para*-aminophenol may be of importance in the nephrotoxicity of paracetamol. In a study in hamsters, *para*-aminophenol was identified as a urinary metabolite (Gemborys & Mudge, 1981). When [*acetyl*-14C]paracetamol and [*ring*-14C]paracetamol were compared in binding studies with renal cortex protein, the amount of covalent binding of radiolabel from [*ring*-14C]paracetamol was four times higher in Fischer rats, which are sensitive to paracetamol nephrotoxicity. This suggests that deacetylation to *para*-aminophenol is the primary pathway of bioactivation. In contrast, binding of ring- and acetyl-labelled paracetamol to renal protein was similar in non-susceptible Sprague-Dawley rats, suggesting that oxidation may be an important metabolic route (Newton *et al.*, 1985). These data indicate that *para*-aminophenol may be responsible for paracetamol-induced renal necrosis in Fischer 344 rats (Newton *et al.*, 1982). Studies *in vitro* with microsomes from Sprague-Dawley rats and *in vivo* with Sprague-Dawley rats that received a 1100-mg/kg bw intraperitoneal dose of paracetamol in combination with specific inhibitors of deacetylation and oxidation suggested that both oxidative metabolism and deacetylation contribute to the nephrotoxicity of paracetamol (Mugford & Tarloff, 1995, 1997).

Exposure of CD-1 male mice to either 600 mg/kg bw paracetamol orally or 500 mg/kg bw *para*-aminophenol subcutaneously resulted in nephrotoxicity; prior inhibition of paracetamol deacylation by carboxylesterase inhibitors did not alter the hepatotoxicity or nephrotoxicity. Pretreatment of animals with the mixed-function oxidase inhibitor piperonyl butoxide decreased nephrotoxicity due to paracetamol but not that due to *para*-aminophenol. Immunohistochemical studies with antibodies directed against the *N*-acetyl moiety of paracetamol showed binding to renal tissues of mice that had received a nephrotoxic dose of paracetamol, but not of those that had received a nephrotoxic dose

of *para*-aminophenol. These findings led the investigators to conclude that in these mice deacylation was not required for P450-mediated activation of paracetamol-induced nephrotoxicity (Emeigh Hart *et al.*, 1991). The activity of CYP 2E1 in kidney microsomes isolated from male C3H/HeJ mice was 50-fold higher than that in females. An important role for activation of paracetamol by CYP 2E1 in the kidney was suggested by fact that renal damage by paracetamol was restricted to those areas in which the enzyme was localized, i.e. the proximal convoluted tubule. The extent of renal glutathione depletion in the male mice was significantly greater than that in the females, but the glutathione levels were restored much faster in males. Further studies in this strain indicated that paracetamol was nephrotoxic in female mice only when they were pretreated with testosterone, which resulted in induction of renal CYP 2E1 (Hu *et al.*, 1993). These effects were confirmed in a study with CD1 mice, which showed that renal rather than hepatic biotransformation of paracetamol is central to paracetamol-induced nephrotoxicity in the mouse (Hoivik *et al.*, 1995). Further studies in which toxicity was completely prevented by inhibition of organic-anion transport suggested that a paracetamol–glutathione conjugate may contribute to the observed nephrotoxicity (Emeigh Hart *et al.*, 1996).

Immunohistochemical analysis after exposure of male CD-1 mice to an oral dose of 600 mg/kg bw paracetamol showed correspondence between the location of bound paracetamol and CYP 2E1, and both corresponded to tissue damage in centrilobular hepatocytes, renal proximal tubules, lung Clara cells and Bowman gland and sustentacular cells in the olfactory epithelium. This suggests that toxicity in the major target tissues of mice is mediated by bioactivation of paracetamol *in situ* (Emeigh Hart *et al.*, 1995).

4.1.3   *Comparison of data for humans and rodents*

The experimental data on paracetamol point to similar pathways of liver biotransformation in humans and rodents. Although none of the rodent species studied resembled humans with respect to all of the urinary metabolites of paracetamol, the hamster was judged to be the best model with respect to the metabolites formed and susceptibility towards this compound. At therapeutic doses (up to 4 mg/kg), the glucuronidation and sulfation pathways predominate in humans, but the sulfation pathway becomes saturated at higher doses. In mice, the glucuronide pathway also predominates, but in rats at doses below 400 mg/kg, the sulfation pathway predominates and becomes saturated at higher doses. A minor pathway in both humans and rodents involves formation of the reactive intermediate *N*-acetyl-*para*-benzoquinone imine via CYP 2E1, CYP 1A2—and other isoforms including CYP 2A6 in humans—and prostaglandin *H* synthase. High doses of paracetamol deplete liver glutathione, which is protective at lower doses since it conjugates with the reactive intermediate *N*-acetyl-*para*-benzoquinone imine.

Three possible mechanisms for paracetamol-induced nephrotoxicity have been investigated. Paracetamol may be converted by cytochrome P450 in the kidney to the reactive intermediate *N*-acetyl-*para*-benzoquinone imine, which then forms the glutathione conjugate 3-(glutathionyl)paracetamol, or the metabolite may be formed in the liver and transported to the kidney. Alternatively, paracetamol may be deacetylated to *para*-aminophenol,

which has been shown to be nephrotoxic. Various species-, strain- and sex-related diffe-rences in these pathways in rodents have been investigated, but the pathway in humans has not been established (reviewed by Savides & Oehme, 1983).

4.2   Toxic effects

4.2.1   *Humans*

Reports on the acute toxicity, and in particular the hepatotoxicity, of paracetamol have continued to appear since the first two case reports in 1966 (Davidson & Eastham, 1966). The 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. The activities of serum transaminase and lactic dehydrogenase and bilirubin concentrations are elevated, and prothrombin time is prolonged (Koch-Weser, 1976). The severity of hepatic injury increases with the dose ingested and with previous consumption of other drugs that induce liver cytochrome P450 enzymes (Wright & Prescott, 1973). After an overdose of paracetamol, biopsy of the liver revealed a range of histological abnormalities, which varied from focal hyperplasia of Kuppfer cells and a few foci of hepatocytolytic necrosis to severe centrizonal necrosis, liver-cell loss, inflammation and features of active regeneration (James *et al.*, 1975). In non-fatal cases, the hepatic lesions are reversible over a period of months, without the 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 that 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).

In a multicentre case-control study in North Carolina, United States, 554 adults with newly diagnosed kidney disease (serum creatinine, $\geq$ 130 µmol/L) and 516 matched control subjects selected randomly from the same area were included. Histories of use of paracetamol, phenacetin and aspirin were obtained by telephone interview, with response rates of 78% and 73% among patients and controls, respectively. The information was supplied by next-of-kin for 55% of cases and 10% of controls. The odds ratios for renal disease, adjusted for consumption of the two other analgesics, were 1.2 (95% CI, 0.77–1.9) in subjects who used paracetamol weekly and 3.2 (95% CI, 1.0–9.8) in subjects who used it daily, when compared with subjects with none or occasional use (Sandler *et al.*, 1989).

In a liver specimen obtained within 1 h of death from a five-year-old girl who died of paracetamol poisoning, covalent binding of paracetamol to proteins was observed. With use of ^{125}I-conjugated goat anti-rabbit immunoglobulin G and purified anti-paracetamol antisera with sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting, the most prominent protein arylation was found in the cytosolic fraction in bands corresponding to 58 and 130 kD, with lesser bands at 38 and 62 kD. There was no apparent selective binding in the microsomal fraction. Immunohistochemical analysis revealed that the paracetamol binding was in the centrilobular regions (Birge *et al.*, 1990).

Administration of 4 g paracetamol daily for three days to 10 healthy female volunteers was found to decrease urinary excretion of prostaglandin E_2 and sodium. This effect was presumably due to inhibition of renal medullary prostaglandin E_2 synthesis. No effects were found on the glomerular filtration rate or effective renal plasma flow (Prescott *et al.*, 1990).

A case–control study was conducted in Maryland, Virginia, West Virginia, and Washington DC, United States, of the association between regular use of paracetamol, aspirin and other non-steroidal anti-inflammatory drugs. A total of 716 patients identified through a population-based registry of end-stage renal disease and 361 controls from the population of the study area were interviewed by telephone about their past use of medications containing the above-mentioned drugs. The response rates were 95% and 90% among identified patients and controls, respectively. The odds ratios for end-stage renal disease, adjusted for use of other analgesic drugs, were 1.4 (95% CI, 0.8–2.4) and 2.1 (95% CI, 1.1–3.7) for subjects whose annual average intake of paracetamol was categorized as moderate (105–365 pills per year) and heavy (366 or more pills per year), respectively, when compared with subjects whose annual average intake was categorized as light (0–104 pills per year). Similarly, the odds ratios for renal disease were 2.0 (95% CI, 1.3–3.2) and 2.4 (95% CI, 1.2–4.8) for subjects whose cumulative lifetime intake of paracetamol was 1000–4999 pills and ≥ 5000 pills, respectively, when compared with subjects whose cumulative intake was < 1000 pills (Perneger *et al.*, 1994).

A women with acute renal failure was admitted to hospital after exposure by self-medication to an estimated 15 g paracetamol over 3.5 days in combination with alcohol and several other pharmaceuticals. Acute renal tubular necrosis resulting from paracetamol poisoning occurs alone or in combination with hepatic necrosis. The margin of safety of paracetamol intake is lower in patients with risk factors such as starvation, chronic alcohol ingestion (which depletes glutathione) or use of medications (e.g. anticonvulsants) that activate the P450 system (Blakely & McDonald, 1995).

Acute renal failure without fulminant liver failure was reported after exposure to 7.5–10 g paracetamol over two days (case 1) and after exposure to an undetermined amount in a suicide attempt (case 2). In case 1, there was concomitant heavy use of alcohol and other drugs and pharmaceuticals, and in case 2 pharmaceuticals for treatment of schizophrenia and hypertension were also used. Other reported cases of acute renal failure without fulminant liver failure have been reviewed (Eguia & Materson, 1997).

4.2.2 *Experimental systems*

The LD_{50} of orally administered paracetamol in male rats was 3.7 g/kg bw (Boyd & Bereczky, 1966); the 100-day LD_{50} in rats was 765 mg/kg bw, and the maximal dose that caused no deaths was estimated to be 400 mg/kg bw per day over 100 days (Boyd & Hogan, 1968). Prior administration of β-carotene was found to protect mice against the acute toxicity of intraperitoneally administered paracetamol (Baranowitz & Maderson, 1995).

Hepatic necrosis after administration of paracetamol was first reported in rats (Boyd & Bereczky, 1966). The main signs are chromatolysis, hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity (Dixon *et al*., 1971). Sensitivity to paracetamol-induced hepatotoxicity varies considerably among species, hamsters and mice being the most sensitive and rats, rabbits and guinea-pigs being more resistant (Davis *et al*., 1974; Siegers *et al*., 1978). The toxic effects in dogs and cats given a single oral dose of paracetamol at maximal doses of 500 and 120 mg/kg bw, respectively, included hepatic centrilobular lesions in dogs and more diffuse pathological changes in the liver in cats, which have only a limited capacity to glucuronidate exogenous compounds (Savides *et al*., 1984).

Paracetamol was administered to CD-1(ICR)BR male mice at doses of 100, 300 or 600 mg/kg bw by gavage, and the mice were killed 2 or 18 h later. No histopathological changes or changes in glutathione concentrations were found at the lowest dose; the intermediate dose produced little necrosis but induced extensive glutathione depletion by 2 h after treatment; while 600 mg/kg bw caused both necrosis and glutathione depletion (Placke *et al*., 1987a).

The histological effects of paracetamol in the livers of B6C3F$_1$ mice given 10 000 mg/kg of diet for 72 weeks included centrilobular hepatocytomegaly, cirrhosis, lipofuscin deposition and focal to massive hepatocyte necrosis (Hagiwara & Ward, 1986). At the same dose, macroscopically and microscopically deformed livers were observed in C57BL/6J mice, with extensive lobular collapse, foci of hepatic necrosis and lymphoid aggregation in portal tracts after 32 weeks (Ham & Calder, 1984). In these grossly enlarged livers, ultrastructural changes such as proliferation of smooth endoplasmic reticulum, intracellular lipid accumulation and glycogen depletion were found in Leeds rats given diets containing 1% paracetamol for up to 18 months (Flaks *et al*., 1985).

Administration of butylated hydroxyanisole to female Swiss-Webster mice at a concentration of 1% in the diet for 12 days [600–800 mg/kg bw per day] prevented the hepatotoxicity of an intraperitoneal dose of 600 mg/kg bw paracetamol, as concluded from measurement of alanine aminotransferase and aspartate aminotransferase activity and histopathological examination. The rate of elimination of paracetamol from blood was 10-fold higher in the mice that had received butylated hydroxyanisole than in those that were given paracetamol only. Although the general pattern of excretion did not change, increases in the rate of formation of glucuronide, sulfate and glutathione conjugates were evident. Therefore, the protective effects of butylated hydroxyanisole were ascribed to enhanced conjugation (Hazelton, 1986).

In six-week-old male B6C3F$_1$ mice given 5000 or 10 000 mg/kg of diet paracetamol for up to 40 weeks, DNA synthesis and other parameters of cell proliferation and toxicity were evaluated in the liver and kidneys at 2, 8, 24 and 40 weeks. The high dose of paracetamol was very toxic, resulting in a 30–50% reduction in body weight and severe hepatic necrosis. The survival rates were 68% at 24 weeks and 45% at 32 weeks. The weights of the liver and kidney were statistically significantly increased in mice at 10 000 ppm in comparison with total body weights. Histopathological alterations in mice

at the high dose included focal-to-lobular, massive hepatocyte necrosis, hepatocellular degeneration and hepatocytomegaly. At both doses, increased labelling indices and thymidine kinase activities were seen in the liver at two weeks, and an increased labelling index was seen at 40 weeks only in the low-dose group. No significant histopathological lesions or increased labelling indices or changes in thymidine kinase were seen in the kidney, except for a small decrease at both doses at eight weeks (Ward *et al*., 1988).

Histopathological review of liver sections from B6C3F$_1$ mice of each sex fed paracetamol at 0.3, 0.6 or 1.25% in the diet for 41 weeks and from NIH outbred, white mice of each sex fed paracetamol at 1.1% in the diet for 48 weeks indicated severe liver injury, characterized by centrilobular necrosis, in animals receiving the highest doses. In the mice receiving lower doses, no significant compound-related liver lesions were found (Maruyama & Williams, 1988).

Paracetamol was tested in Fischer 344 rats and B6C3F$_1$ mice by dietary exposure. In a 13-week study, rats received up to 25 000 mg/kg of diet paracetamol. The body weights of animals of each sex at 12 500 and 25 000 ppm were significantly lower than those of controls, and two animals in each of these groups died. Hepatotoxicity and inflammation were observed in rats of each sex exposed to 25 000 ppm and in males exposed to 12 500 ppm. An increase in the severity of spontaneous age-related chronic progressive nephropathy was seen in animals of each sex exposed to 25 000 ppm. Testicular atrophy was reported in all male rats at the highest dose. No significant changes were seen in animals exposed to 6200 ppm. In a two-year study in which rats were exposed to 600, 3000 or 6000 ppm paracetamol, the only toxic effects reported were increased severity of the spontaneous age-related chronic progressive nephropathy and hyperplasia of the parathyroid gland in males. In mice exposed to up to 25 000 ppm paracetamol for 13 weeks, the body weights of mice of each sex at 12 500 and 25 000 ppm were depressed, with accompanying histopathological lesions of the liver in these groups and in males receiving 6200 ppm. In a two-year study in which mice were exposed to 600, 3000 or 6000 ppm paracetamol, follicular hyperplasia of the thyroid gland was the only reported finding (National Toxicology Program, 1993).

Inhibition of cellular respiration due to impairment of mitochondrial function, decreased cellular ATP and glutathione depletion were found in isolated mouse hepatocytes exposed to toxic concentrations of paracetamol, which were ≥ 0.5 mmol/L as determined by release of lactic dehydrogenase (Burcham & Harman, 1990).

Loss of genomic DNA, evidence of DNA fragmentation and an increase in nuclear Ca^{2+} concentration were found in the livers of Swiss mice after an intraperitoneal dose of 600 mg/kg bw paracetamol, indicating that activation of endonucleases may be an early event in necrosis (Ray *et al*., 1990). This was confirmed in studies with paracetamol-treated mouse liver cells *in vitro*, which showed formation of a 'ladder' of DNA fragments characteristic of Ca^{2+}-mediated endonuclease activation prior to cytotoxicity and the development of necrosis (Shen *et al*., 1992).

Pretreatment of male Wistar rats with the glutathione reductase inhibitor 1,3-bis(2-chloroethyl)-1-nitrosourea before paracetamol treatment was reported greatly to enhance

the sensitivity of hepatocytes to paracetamol in liver subcellular fractions derived from these rats (*ex vivo*). A similar result was obtained with primary cultures of rat hepatocytes treated *in vitro* with this nitrosourea and paracetamol (Ellouk-Achard *et al*., 1992). Pretreatment of male ICR mice with the adrenergic agonist phenylpropanolamine, which depleted glutathione by a moderate 30–50%, enhanced liver necrosis produced by intraperitoneal administration of 400 mg/kg bw paracetamol (James *et al*., 1993).

Intraperitoneal administration of 1 g/kg bw paracetamol to male Sprague-Dawley rats had no effect on the levels of diene conjugates in liver mitochondria or microsomes but inhibited the formation of thiobarbituric acid-reactive substances (TBARS) by hepatocytes *in vitro*. It was concluded that lipid peroxidation does not necessarily correlate with paracetamol-induced hepatotoxicity in Sprague-Dawley rats (Kamiyama *et al*., 1993).

In male BALB/c mice injected intraperitoneally with 500 mg/kg bw paracetamol, the concentration of TBARs in hepatic microsomes was decreased, and the activities of glutathione *S*-transferase and glutathione peroxidase were increased. In contrast, the concentration of TBARS was increased and the activities of glutathione and glutathione *S*-transferase were decreased in whole-liver homogenates. The authors concluded that glutathione-dependent enzyme defence mechanisms protect microsomes against oxidative stress (Özdemirler *et al*., 1994).

In a time–course study, 375 mg/kg bw paracetamol were administered intraperitoneally to female Swiss mice, and biochemical changes in the liver were measured within 1 h. The initial changes after 15 min included increased spontaneous in-situ chemiluminescence and hydrogen peroxide concentration, decreased glutathione and increased oxidized glutathione levels and decreased non-Se-glutathione peroxidase activity. At 60 min, which was the last time at which measurements were made, the changes included decreased reduced and oxidized glutathione, decreased non-Se-glutathione peroxidase and decreased Se-glutathione peroxidase. Over the 60-min period, histopathological evidence of liver damage and the activity of marker enzymes for hepatic damage (lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase) increased (Arnaiz *et al*., 1995).

In a 6-h study, 400 mg/kg bw paracetamol were administered intraperitoneally to male ICR mice. The hepatic concentrations of glutathione had decreased to 7% of baseline levels by 1 h, the concentrations of TBARS started to increase at 1 h and reached maximal values at 3 h, and the endogenous concentrations of reduced coenzyme Q_9 and Q_{10} decreased steadily over the 6-h period. Plasma alanine aminotransferase activity increased during the course of the experiment. Pretreatment with the lipid-soluble antioxidants coenzyme Q_{10} and α-tocopherol protected against lipid peroxidation but did not completely prevent hepatic injury (Amimoto *et al*., 1995).

When male Wistar rats were injected intraperitoneally with 500 mg/kg bw paracetamol, 24-day-old animals formed 24 times more liver glutathione conjugates than three- to four-month-old animals. The younger animals also showed greater glutathione depletion and had somewhat lower initial glutathione concentrations. The investigators

suggested that the increased rate of glutathione conjugation in young rats is due to more rapid saturation of competing elimination pathways, such as glucuronidation and sulfation (Allameh *et al.*, 1997).

Radiolabel was bound covalently to hepatocellular proteins after incubation of mouse, rat, hamster, rabbit or guinea-pig liver microsomes with [3H]paracetamol; the degree of binding correlated with the susceptibility of the species to paracetamol-induced hepatotoxicity *in vivo* (Davis *et al.*, 1974). Similarly, covalent binding of radiolabel to liver proteins of rats 48 h after administration by gavage of [ring-14C]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 [3H]paracetamol at 2.5 g/kg bw to rats (Tsokos-Kuhn *et al.*, 1988).

Binding of paracetamol to liver proteins in three-month-old male Crl:CD-1(CR)BR mice was demonstrated 4 h after oral administration of 600 mg/kg bw paracetamol. With use of 125I-conjugated goat anti-rabbit immunoglobulin G and purified anti-paracetamol antisera with sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting, the most prominent protein arylation was found in the cytosolic fraction in bands corresponding to 44, 58 and 130 kD. There was also selective binding to a 44-kD protein in the microsomal fraction (Birge *et al.*, 1990). Several specific proteins have now been identified as paracetamol-binding proteins, which could disrupt hepatic functions such as ammonia trapping, oxidative phosphorylation, mitochondrial function and—possibly—transmethylation reactions (reviewed by Cohen *et al.*, 1997). Paracetamol–protein binding has been identified by immunodetection not only in the liver but also in kidney, skeletal muscle, heart, lung, pancreas and stomach of male CD-1 and C57BL/6J mice that received an intraperitoneal injection of 600 mg/kg bw paracetamol (Bulera *et al.*, 1996).

Protein adducts were found in B6C3F$_1$ mice given 400 mg/kg bw paracetamol, but there was no evidence of oxidative protein damage as determined by protein aldehyde formation (Gibson *et al.*, 1996).

Diallyl sulfide, which inhibits CYP 2E1, protected rats treated with 750 or 2000 mg/kg bw paracetamol [route not specified] against death and hepatic and renal toxicity, as measured by serum lactate dehydrogenase activity and creatinine concentration. In liver microsomes *in vitro*, diallyl sulfide inhibited formation of the glutathione conjugate of *N*-acetyl-*para*-benzoquinone imine (Hu *et al.*, 1996).

The depletion of glutathione induced in female Fischer 344 rats by an oral dose of 600 mg/kg bw paracetamol was inhibited by guaiazulene, which inhibits lipid peroxidation by scavenging hydroxyl radicals. The investigators concluded that the significant protection conferred by guaiazulene against paracetamol-induced glutathione depletion and hepatic damage could be ascribed to its antioxidant activity (Kourounakis *et al.*, 1997).

In male ICR mice given doses of 10, 50, 100 or 400 mg/kg bw [3H]paracetamol, the substance showed apparent binding to hepatic and renal DNA 2 h after exposure. 32P-Postlabelling did not show readily detectable DNA adducts after exposure to 400 mg/kg bw

paracetamol, although a few faint spots were reported after 24 h. Binding of [^{3}H]*N*-acetyl-*para*-benzoquinone imine to purified DNA was greatly enhanced by lowering the pH of the reaction mixture to < 4 or by the addition of alternative nucleophiles such as cysteine (at 0.1 mol/L but not 1 mol/L) or by the presence of the proteins in isolated nuclei or nuclear chromatin. Apparent DNA binding was also generated *in vitro* by horseradish peroxidase and hydrogen peroxidase and, to a much lesser extent, by hepatic microsomes in the presence of NADPH (Rogers *et al.*, 1997).

Intraperitoneal administration of 1 g/kg bw paracetamol to male Long Evans hooded rats resulted in expression of inducible nitric oxide synthase protein that was correlated with damage to centrilobular regions of the liver and increases in serum transaminase activity. Hepatocytes isolated from rats exposed to paracetamol were found to produce more nitric oxide than those from untreated controls. Pretreatment of rats with the inducible nitric oxide synthase inhibitor aminoguanidine prevented the hepatotoxicity of paracetamol (Gardner *et al.*, 1998).

A single subcutaneous dose of 750 mg/kg bw paracetamol to male Fischer 344 rats produced renal tubular necrosis restricted to the distal portions of the proximal tubule (McMurtry *et al.*, 1978). Continuous exposure of female Fischer 344 rats to 140–210 mg/kg bw paracetamol for up to 83 weeks did not produce nephrotoxicity, as determined by light and electron microscopy. In comparison, similar doses of aspirin caused renal papillary necrosis and decreased urinary concentrating ability (Burrell *et al.*, 1991).

In C57BL/6NIA mice given an intraperitoneal dose of 375 mg/kg bw paracetamol, the glutathione and cysteine concentrations were decreased to a greater extent in 31-month-old mice than in 12- or 3-month-old mice. Older mice also lost the ability to recover from the paracetamol-induced depletion of glutathione (Richie *et al.*, 1992).

The nephrotoxic effects of a single oral dose of 1500 mg/kg bw paracetamol to adult male Wistar rats were not prevented by administration of methionine or *N*-acetylcysteine, which protect against hepatotoxicity. Depletion of glutathione was observed in the liver but not in the kidney. Diethyldithiocarbamate, which is an inhibitor of microsomal mono-oxygenase, protected against both nephrotoxicity and hepatotoxicity (Möller-Hartmann & Siegers, 1991).

In other experiments, it was shown that paracetamol-induced renal toxicity may be due to a direct effect or be mediated by hepatic metabolites of paracetamol. In the isolated perfused kidney of male Wistar rats, haemodynamic and tubular function were affected directly by paracetamol at concentrations of 5–10 mmol/L; however, in these rats *in vivo,* inhibition of γ-glutamyltranspeptidase, an enzyme involved in the processing of hepatically derived glutathione conjugates, was protective against the nephrotoxicity induced by an intraperitoneal dose of 1000 mg/kg bw paracetamol (Trumper *et al.*, 1992, 1995, 1996).

In fasted adult male mice given an oral dose of 600 mg/kg bw paracetamol 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.*, 1987b).

A study of the effects of 0.5, 1 or 1.5% paracetamol in the diet on urothelial cell proliferation in male Sprague-Dawley rats showed transient increases in the labelling index and hyperplasia of the renal pelvis and bladder at six weeks (Johansson *et al.*, 1989).

The roles of various biochemical factors in paracetamol-induced hepatotoxicity and nephrotoxicity have been investigated, including metallothionein (Waalkes & Ward, 1989), cytokines (Blazka *et al.*, 1995), lysosomal enzymes (Khandkar *et al.*, 1996), the nuclear transcription factors NF-κB and NF-IL6 (Blazka *et al.*, 1996a), tumour necrosis factor α and interleukin 1α (Blazka *et al.*, 1996b) and AP-1 transcription factors (Blazka *et al.*, 1996c). Knock-out mice in which the gene coding for tumour necrosis factor α was inactivated were found to respond to paracetamol no differently from wild-type mice (Boess *et al.*, 1998).

4.3   Reproductive and developmental effects

4.3.1   *Humans*

The teratogenicity of paracetamol in humans has been reviewed and reported to be non-existent, on the basis of the frequency of congenital anomalies reported in multiple large cohort and case–control studies. The risk for adverse reproductive outcomes (e.g. spontaneous abortions, a variety of malformations, fetal distress and hepatic and renal toxicity in infants) was, however, considered to be significant in situations of paracetamol overdose during pregnancy (Friedman & Polifka, 1994).

In a case–control study involving 538 cases and 539 normal controls in California (United States) between 1989 and 1991, the odds ratio for neural tube defects associated with maternal use of paracetamol was 1.0 (95% confidence interval, 0.80–1.4); 144 of the patients and 141 controls had been exposed. Simultaneous adjustment for maternal race, ethnicity, age, educational level, body mass index and vitamin use did not substantially change the results (Shaw *et al.*, 1998).

In a prospective study conducted by the Teratology Information Service and the London National Poisons Information Service of the pregnancy outcomes of 300 women who took an overdose of paracetamol, either alone or in combination with other drugs, in 1984–92 fetal toxicity was not increased, even though 53% of the mothers had required treatment for the overdose. Thirty-eight per cent of the fetuses had been exposed during the first trimester. There were 219 liveborn infants with no malformations (61 of whom had been exposed in the first trimester), 11 with malformations (none with first-trimester exposure), 18 spontaneous abortions, two late fetal deaths and 54 elective terminations (McElhatton *et al.*, 1997).

4.3.2   *Experimental systems*

Paracetamol was evaluated for teratogenicity in frog embryos. In the absence of bioactivation, the 96-h median lethal concentration and the concentration inducing gross malformations in 50% of the surviving embryos were 191 and 143 mg/L, respectively, to yield a teratogenic index of 1.3. In the presence of a microsomal activating system from Arochlor-induced rat liver, these concentrations decreased by 3.9- and 7.1-fold,

respectively, which suggests that a reactive metabolite was involved in the developmental toxicity noted in this test system (Fort *et al.*, 1992).

As reported in an abstract, exposure of female Sprague-Dawley rats to 0, 125, 250 or 500 mg/kg paracetamol on day 10 of gestation gave rise to enhanced immunohistochemical staining of glucose-6-phosphate dehydrogenase and reduced staining of NAD-linked isocitrate dehydrogenase in frozen sections of embryos, including the decidual mass, at all doses on day 11. The authors concluded that rat embryos are sensitive to metabolic perturbation caused by maternal exposure to paracetamol (Beck *et al.*, 1997). In another abstract, it was reported that direct exposure of gestation day-11 rat embryos in culture to 500 μmol/L paracetamol resulted in a dramatic loss of intracellular glutathione histofluorescence (Harris *et al.*, 1997).

CD-1 mice were fed diets containing paracetamol (purity, 99.7%) at doses of 0, 0.25, 0.5 and 1% (equivalent to intakes of 0, 360, 720 and 1400 mg/kg bw per day). The parental generation was exposed for 14 weeks during cohabitation, and the number of litters produced was used as a primary index of reproductive capacity. Feed consumption was reduced in the females at the highest concentration. No changes were noted in the number of pups per litter, pup viability, or adjusted [for litter size] pup weight, but 6/19 pairings at the high dose did not result in a fifth litter, and this accounted for the significantly decreased number of litters per pair in this group. The fifth litter that was produced by 13 pairs at the high dose contained fewer pups. The last litter produced by the parental generation was maintained on exposure through adulthood. A reduction in body-weight gain (range, 6–18%) was seen in treated animals of each sex throughout mating. An increase in the percentage of abnormal sperm was observed in males of the F_1 generation at the high dose. In the F_2 generation, a reduction in pup weight adjusted for litter size (11%) was seen at the high dose (Reel *et al.*, 1992; National Toxicology Program, 1997).

Groups of 24–26 male B6C3F_1/BOM M mice received intraperitoneal injections of 0 or 400 mg/kg bw paracetamol daily for five consecutive days. Treated mice lost weight during the exposure, but there was no difference between control and treated animals throughout the remainder of the post-treatment period. The relative testis weights were reduced by 16–18% by paracetamol 27 and 33 days after treatment, but the only alteration in histological appearance up to 10 days was an approximately 6% reduction in the diameter of the seminiferous tubules five days after treatment. DNA synthesis (measured by [³H]thymidine incorporation in the testis was reduced for several hours immediately after the fifth dose, and this response was seen with concentrations as low as 100 mg/kg bw per day. Flow cytometric analysis of testicular ploidy 26 days after the last dose of 400 mg/kg bw per day revealed alterations in the cell types indicative of altered transit through the cell cycle, but no effect on the chromatin structure of late-maturing spermatids (Wiger *et al.*, 1995).

4.4   Genetic and related effects

The genotoxicity of paracetamol has been reviewed (Rannug *et al.*, 1995).

4.4.1   *Humans*

Ten healthy volunteers ingested three doses of 1 g paracetamol over a period of 8 h. Blood samples were taken before and 24 h after ingestion. The frequency of sister chromatid exchange was significantly increased (0.19 ± 0.03 per chromosome before, 0.21 ± 0.024 per chromosome after exposure), as was the mean frequency of chromatid breaks (from 0.33 ± 0.50 per 100 cells before to 2.2 ± 1.3 per 100 cells after exposure). Exposure of human lymphocytes *in vitro* showed that concentrations > 0.1 mmol/L paracetamol inhibited replicative DNA synthesis, while exposure to 1–10 mmol/L paracetamol for 2 h increased the frequency of sister chromatid exchange. An increased frequency of chromatid and chromosome breaks was found after exposure of lymphocytes to 0.75–1.5 mmol/L paracetamol for 24 h (Hongslo *et al*., 1991).

The clastogenic activity of paracetamol was determined in a group of 11 volunteers who received three doses of 1000 mg of the drug during 8 h. Blood samples were taken 0, 24, 72 and 168 h after ingestion of the first dose. The frequency of aberrant cells increased from 1.7% at 0 h to 2.8% at 24 h, and the number of breaks per cell from 0.018 (control) to 0.03. These effects had disappeared by 168 h. No increase in lipid peroxidation was observed after ingestion of paracetamol, as determined in the thiobarbituric acid assay; however, simultaneous ingestion of ascorbic acid increased lipid peroxidation (Kocišová *et al*., 1988).

In a similar study, 11 volunteers received three doses of 1000 mg paracetamol in the course of 8 h, with or without three doses of 1000 mg ascorbic acid. Blood samples and buccal mucosa cells were collected 0, 24, 72 and 168 h after ingestion of the first dose. Unscheduled DNA synthesis induced by 1-methyl-3-nitro-1-nitrosoguanidine was determined in peripheral lymphocytes by a liquid scientillation method, and micronucleus formation was determined in buccal mucosa cells by staining with Light Green. The level of nitrosoguanidine-induced unscheduled DNA synthesis was significantly decreased 24 h after ingestion of paracetamol, and simultaneous intake of ascorbic acid prolonged this effect. A significant increase in micronucleus formation in buccal mucosa cells was observed at 72 h (Topinka *et al*., 1989). No increase in the frequency of micronucleated buccal cells was observed, however, when the cytokinesis block micronucleus method was used in a study of 12 volunteers who took three doses of 1000 mg paracetamol over an 8-h period (Kocišová & Šrám, 1990).

In a study to investigate further the clastogenicity of paracetamol in which both participants and investigators were unaware of the treatment and placebo status of individuals, volunteers were pre-screened for normal liver function; they were all non-smokers, and their diet and environmental exposure were controlled during the study. One group of 12 volunteers received two 500-mg tablets of paracetamol three times during an 8-h time interval, and the other 12 volunteers received placebo. Blood was drawn from each volunteer on the day before and on days 1, 3 and 7 after exposure. No significant increase in the frequency of structural chromosomal aberrations was found when pre-treatment values were compared with post-treatment values or when the treated group was compared with the placebo group (Kirkland *et al*., 1992).

The ability of paracetamol to induce chromosomal aberrations in peripheral lymphocytes was evaluated in volunteers who received a single oral dose of 3 g paracetamol, in patients who had received 2 g paracetamol by intravenous infusion every 6 h for at least seven days and in self-poisoned patients who had ingested more than 15 g paracetamol. In none of these groups did paracetamol affect chromosomes in peripheral lymphocytes (Hantson *et al.*, 1996).

4.4.2   *Experimental systems* (see Table 2 for references)

Paracetamol did not induce gene mutation in *Salmonella typhimurium* TA100, TA102, TA1535, TA1537, TA1538 or TA98 or in *Escherichia coli* with or without exogenous metabolic activation, except in one study in which a metabolic activation system from hamster was used. It induced chromosomal aberrations in *Allium cepa*. Paracetamol did not induce sex-linked recessive lethal mutation in *Drosophila melanogaster*. It gave negative or weakly positive results for the induction of DNA strand breaks in rodent cells *in vitro*. The chemical induced unscheduled DNA synthesis in mouse hepatocytes; it gave inconsistent results in rat hepatocytes and did not induce this effect in hamster or guinea-pig hepatocytes or in Chinese hamster lung cells *in vitro*. Paracetamol did not induce gene mutation in mammalian cells *in vitro*. It induced sister chromatid exchange in Chinese hamster cells and in mouse cells with and without exogenous metabolic activation *in vitro*. It induced micronuclei in a rat kidney cell line *in vitro* but not in primary rat hepatocytes without exogenous metabolic activation. It induced chromosomal aberrations in Chinese hamster and—weakly—in mouse cells *in vitro*. Paracetamol weakly induced cell transformation in mouse cells. It induced sister chromatid exchange and chromosomal aberration in human lymphocytes without exogenous metabolic activation *in vitro*.

Paracetamol inhibited DNA synthesis in several tissues of mice and rats treated *in vivo*. It induced DNA single-strand breaks in mouse liver cells but not in mouse kidney or rat liver or kidney cells *in vivo*. Paracetamol did not induce micronuclei in mice *in vivo* but did induce chromosomal aberrations in mouse bone-marrow cells. Paracetamol induced aneuploidy in rat embryo cells *in vivo*.

5.   Summary of Data Reported and Evaluation

5.1   Exposure data

Paracetamol (acetaminophen) is widely used as an analgesic and antipyretic at daily doses up to 4000 mg, with minor uses as an intermediate in the chemical and pharmaceutical industries. Occupational exposure may occur during its production and its use.

5.2   Human carcinogenicity data

In the previous monograph on paracetamol, a positive association with cancer of the ureter (but not of other sites in the urinary tract) was observed in an Australian case–control study. None of the other three case–control studies showed an association with

Table 2. Genetic and related effects of paracetamol

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	3624 µg/plate	King *et al.* (1979)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	1000 µg/plate	Wirth *et al.* (1980)
Salmonella typhimurium TA100, TA98, TA102, reverse mutation	NT	–	3020	Dybing *et al.* (1984)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	5000 µg/plate	Oldham *et al.* (1986)
Salmonella typhimurium TA100, TA98, TA97, TA1535, TA1537, TA1538, reverse mutation	–	–	10 000 µg/plate	Jasiewicz & Richardson (1987)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, reverse mutation	–	–	10 000 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	755 µg/plate	Camus *et al.* (1982)
Escherichia coli K12/343/113, forward or reverse mutation	–	–	4530	King *et al.* (1979)
Allium cepa, chromosomal aberrations	+	NT	5000	Reddy & Subramanyam (1981)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		6040	King *et al.* (1979)
DNA damage, Reuber H4-II-E rat hepatoma cells *in vitro*	–	NT	1510	Dybing *et al* (1984)
DNA damage, Chinese hamster lung V79 cells *in vitro*	(+)	NT	1510	Hongslo *et al.* (1988)
Gene mutation, mouse C3H 10T1/2 clone 8 cells *in vitro*	–	NT	1000	Patierno *et al.* (1989)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	151	Holme *et al.* (1988)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	+	453	Hongslo *et al.* (1988)
Sister chromatid exchange, mouse TA_3H cells *in vitro*	+	NT	151	Hongslo *et al.* (1990)

Table 2 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	(+)	(+)	150	National Toxicology Program (1993)
Micronucleus formation, rat kidney cells *in vitro*	+	NT	1510	Dunn *et al.* (1987)
Micronucleus formation, rat primary hepatocytes *in vitro*	−	NT	151	Mueller-Tegethoff *et al.* (1995)
Chromosomal aberrations, Chinese hamster lung cells *in vitro*	(+)	NT	60	Ishidate *et al.* (1978)
Chromosomal aberrations, Chinese hamster Don-6 cells *in vitro*	+	NT	75.5	Sasaki *et al.* (1980)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	+	47.8	Mueller *et al.* (1991)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	+	1257	National Toxicology Program (1993)
Chromosomal aberrations, mouse TA$_3$H cells *in vitro*	(+)	NT	151	Hongslo *et al.* (1990)
Cell transformation, C3H10T1/2 clone 8 cells	(+)	(+)	1000	Patierno *et al.* (1989)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	151	Hongslo *et al.* (1991)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	200	Watanabe (1982)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	226	Hongslo *et al.* (1991)
DNA single-strand breaks, mouse liver cells *in vivo*	+		600 ip × 1	Hongslo *et al.* (1994)
DNA single-strand breaks, mouse kidney cells *in vivo*	−		600 ip × 1	Hongslo *et al.* (1994)
DNA single-strand breaks, rat liver and kidney cells *in vivo*	−		600 ip × 1	Hongslo *et al.* (1994)
Micronucleus formation, NMRI mouse bone-marrow cells *in vivo*	−		453 ip or po × 2	King *et al.* (1979)
Chromosomal aberration, Swiss mouse bone-marrow cells *in vivo*	(+)		2.5/mouse po × 3	Reddy (1984)
Chromosomal aberration, mouse bone-marrow cells *in vivo*	+		100 ip × 1	Severin & Beleuta (1995)

Table 2 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, mouse testis *in vivo*	–		2.5/mouse po × 3	Reddy & Subramanyam (1985)
Aneuploidy, rat embryos *in vivo*	+		500 po × 25	Tsuruzaki *et al.* (1982)
Binding (covalent) to DNA, ICR mouse liver *in vivo*	+		300 ip × 1	Hongslo *et al.* (1994)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, in-vitro test, μg/mL; in-vivo test, mg/kg bw per day; ip, intraperitoneal; po, oral

cancer in the urinary tract. Nine new—mainly population-based—case–control studies of cancers of the urinary tract have been published, many of which addressed more than one subsite.

None of six studies from Australia, Europe and North America, including a very large international study, found a consistent association between renal-cell cancer and regular intake of paracetamol at any level. In one study from the United States which included patients with renal cancer (type not specified), the risk increased with increasing cumulative intake of paracetamol, to reach statistical significance at the highest exposure; however, this result was not adjusted for intake of other analgesics.

In one study from Australia which included patients with cancer of the renal pelvis, a nonsignificant twofold increase in risk was seen among people in the highest exposure category, with no excess risk in the two lower exposure categories. Another, large case–control study of cancer of the renal pelvis and ureter from the United States showed no association with regular intake of paracetamol.

Of the three new studies that included patients with urinary bladder cancer, that conducted in Sweden showed an elevated risk without providing details. The other two (both from the United States) showed only a slight or no association.

5.3   Animal carcinogenicity data

Paracetamol was tested for carcinogenicity by oral administration in mice and rats. An early study indicated an increased incidence of liver adenomas and carcinomas at a markedly toxic dose in mice of one strain; however, this result was not corroborated in a later study in mice, also at a dose greater than the maximal tolerated dose. A more recent, well-conducted study showed no evidence of a carcinogenic effect in mice. Paracetamol had no carcinogenic effect in rats of several strains, but in rats of one inbred strain, increased incidences of liver and bladder neoplasms were recorded in males at the high dose and an increased incidence of bladder tumours in females at the low dose. A more recent, well-conducted study showed no treatment-related carcinogenic effect in rats. Paracetamol did not promote urinary bladder carcinogenesis in rats and reduced the incidence of intestinal tumours in a two-stage model of intestinal carcinogenesis in rats. It enhanced the incidence of renal adenomas induced by one renal carcinogen but not those induced by another.

5.4   Other relevant data

Activation of a relatively small percentage of paracetamol to *N*-acetyl-*para*-benzo-quinone imine by cytochrome P450, predominantly CYP 2E1, has been found to be involved in the mechanism of hepatic and, perhaps, renal toxicity. Most paracetamol is metabolized by glucuronidation, sulfation and conjugation with glutathione, which protects the liver at therapeutic doses. Doses of 300 mg/kg bw per day paracetamol and higher saturate conjugation reactions, deplete glutathione and result in binding of the benzoquinone imine to cellular proteins; this has been proposed to be the mechanism of hepatocellular injury in rodents and humans. Several protein adducts have been found in

humans and rodents *in vivo* after exposure to paracetamol. DNA adducts were not observed in mice.

In humans, an association was reported in two case–control studies between daily use of paracetamol and renal disease; however, a causal relationship has not been established. Humans and rodents exposed to doses of paracetamol well above the therapeutic range have experienced centrilobular hepatotoxicity and nephrotoxicity involving the proximal renal tubule. In experimental animals, hepatic, renal and testicular damage occurred only at oral doses that exceeded 300 mg/kg bw per day in rats and 900 mg/kg bw per day in mice. At lower doses, toxic effects in rodents are minimal or absent.

Paracetamol does not present a teratogenic risk to humans at doses associated with severe maternal toxicity. It did not affect reproductive performance of mice in a continuous breeding protocol, although growth and birth weights were reduced. Sperm abnormalities have been observed in mice.

The results of studies of the cytogenetic effects of paracetamol in humans are inconclusive. Paracetamol induced sister chromatid exchange in human cells *in vivo*, and it was aneugenic and induced chromosomal aberrations but not micronuclei in mammalian cells *in vivo*. It induced DNA single-strand breaks in mice treated *in vivo*. Paracetamol induced sister chromatid exchange and chromosomal aberrations in human cells *in vitro*. It weakly induced cell transformation in a mouse cell line. It induced chromosomal aberrations, micronuclei and sister chromatid exchange in mammalian cells *in vitro*. It did not induce gene mutation, and the results of tests in mammalian cells *in vitro* for unscheduled DNA synthesis and DNA damage were inconclusive. Overall, paracetamol was genotoxic in mammalian cells *in vivo* and *in vitro*. It was not mutagenic to insects but was clastogenic in plant cells. It was not mutagenic in any standard assay in bacteria.

5.5   Evaluation

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

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

Overall evaluation

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

6.   References

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