Indomethacin

1. Chemical and Physical Characteristics

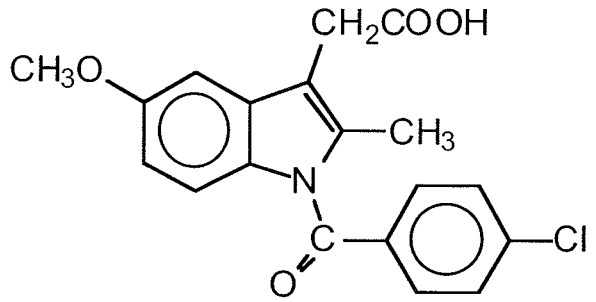
1.1 Name

Chemical Abstracts Services Registry Number
53-86-1

UPAC Systematic Chemical Name
1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid

Synonyms
1-(4-Chlorobenzoyl)-5-methoxy-2-methylindolo-3-acetic acid; 1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid; 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3 acetic acid

1.2 Structural and molecular formulae and relative molecular mass



$C_{19}H_{16}ClNO_4$ Relative molecular mass: 357.81

1.3 Physical and chemical properties

From Budavari *et al.* (1996) and Reynolds and Prasad (1982), unless otherwise stated.

Description
White to yellow–tan, odourless crystalline powder

Melting-point
Indomethacin exhibits polymorphism, with a melting-point of ~155 °C for one form and ~162 °C for the other.

Solubility
Soluble in ether, acetone and castor oil; practically insoluble in water

Spectroscopy data
Absorbance spectrum in ethanol has maxima at 230, 260 and 319 nm

Stability
Stable in neutral or slightly acid media; decomposed by strong alkali.

Impurities
α–Substituted monoglyceryl esters of 4-chlorobenzoic acid and indomethacin are formed through chemical interaction of the drug with glycerin present in suppositories. Only trace or undetectable amounts of these and other impurities were recorded after analysis of bulk samples or after formulation as capsules (Curran *et al.*, 1980).

1.4 Technical products

Amuno, Argun, Artracin, Artrinovo, Artrivia, Bonidon, Catlep, Chibro-Amuno, Chrono-Indocid-75, Confortid, Dolcidium-PL, Dolovin, Durametacin, Elmetacin, Idomethine, Imbrilon, Inacid, Indacin, Indocid, Indocil, Indocin, Indocollyre, Indomecol, Indomed, Indomee, Indometacin, Indometacine, Indomethacine, Indomethine, Indomod, Indo-Phlogont, Indoptic, Indorektal, Indo-Rectalmin, Indo-Tablinen, Indoxen, Indren, Inflazon, Infrocin, Inteban SP, Lausit, Metacen, Metastril, Methazine, Metindal, Mezolin, Mikametan, Mobilan, Reumacide, Rheumacin LA, Sadoreum, Tannex, Vonum.

2. Occurrence, Production, Use, Analysis and Human Exposure

2.1 Occurrence

Indomethacin is not known to occur naturally.

2.2 Production

Indomethacin may be synthesized by several routes (Shen & Winter, 1977), which are generally modifications of the original procedure (Shen *et al.*, 1963) in which 5-methoxy-2-methylindole-3-acetic acid was

used as the starting material. Technical details about its current commercial production were not available to the Working Group.

2.3 Use

The only known use of indomethacin is as a pharmaceutical agent. The drug is formulated as capsules or suppositories (Watanabe *et al.*, 1993). Experimental formulations of the drug are being investigated (Tsuji *et al.*, 1993). Indomethacin has analgesic, anti-inflammatory and anti-pyretic properties and is used extensively in the treatment of rheumatic disorders at doses of 25 mg and 50 mg two to four times daily up to 100–200 mg daily (Waller, 1983).

2.4 Analysis

In general, methods for the analysis of indomethacin are restricted to its determination in pharmaceutical preparations and in body fluids. Most of the methods involve high-performance liquid chromatography. Indomethacin can be determined in pharmaceutical preparations in the presence of other non-steroidal anti-inflammatory drugs (NSAIDs) (Rau *et al.*, 1991). Methods exist for its determination in plasma and urine (Hubert & Crommen, 1990; Singh *et al.*, 1991; Johnson & Ray, 1992).

2.5 Human exposure

Indomethacin is a commonly used NSAID (Griffin *et al.*, 1991; Langman *et al.*, 1994). Since its introduction in 1962, it has been used extensively for the treatment of acute and chronic arthritis and other inflammatory disorders. Although indomethacin is normally taken by mouth, it can be administered rectally in order to reduce the occurrence of gastrointestinal side-effects. This approach has been investigated in persons with familial adenomatous polyposis and remaining rectal segments (Hirata *et al.*, 1994; Hirota *et al.*, 1996). When it is used for the treatment of rheumatological disease, similar clinical benefits are seen with oral and rectal doses of indomethacin, although most patients prefer the oral route of administration (Huskisson *et al.*, 1970).

Indomethacin is currently available in five dosage formulations: a sterile solution containing 1 mg for intravenous administration, a conventional gelatin capsule, (25 or 50 mg) for oral administration, a 75-mg sustained release capsule; an oral suspension containing 25 mg indomethacin per 5 ml and a 50-mg suppository for rectal use (Billups & Billups, 1992).

3. Metabolism, Kinetics and Genetic Variation

3.1 Human studies

3.1.1 Metabolism

In adults, indomethacin undergoes extensive hepatic biodegradation by *O*-demethylation and *N*-deacylation reactions (Duggan *et al.*, 1972), and only a small amount is excreted in the urine unchanged (Helleberg, 1981) (Fig. 1). These metabolites lack anti-inflammatory activity (Shen, 1965).

Indomethacin is demethylated to form demethylindomethacin through the cytochrome P450 microsomal pathway (Duggan *et al.*, 1972). Leemann *et al.* (1993) showed in human liver microsomes, that a single cytochrome P450 monooxygenase plays a critical role in the elimination of indomethacin by the liver. Analysis of inhibition by indomethacin in comparison with other of NSAIDs suggested that a common isoenzyme, CYP2C9, catalyses oxidation of NSAIDS by human liver.

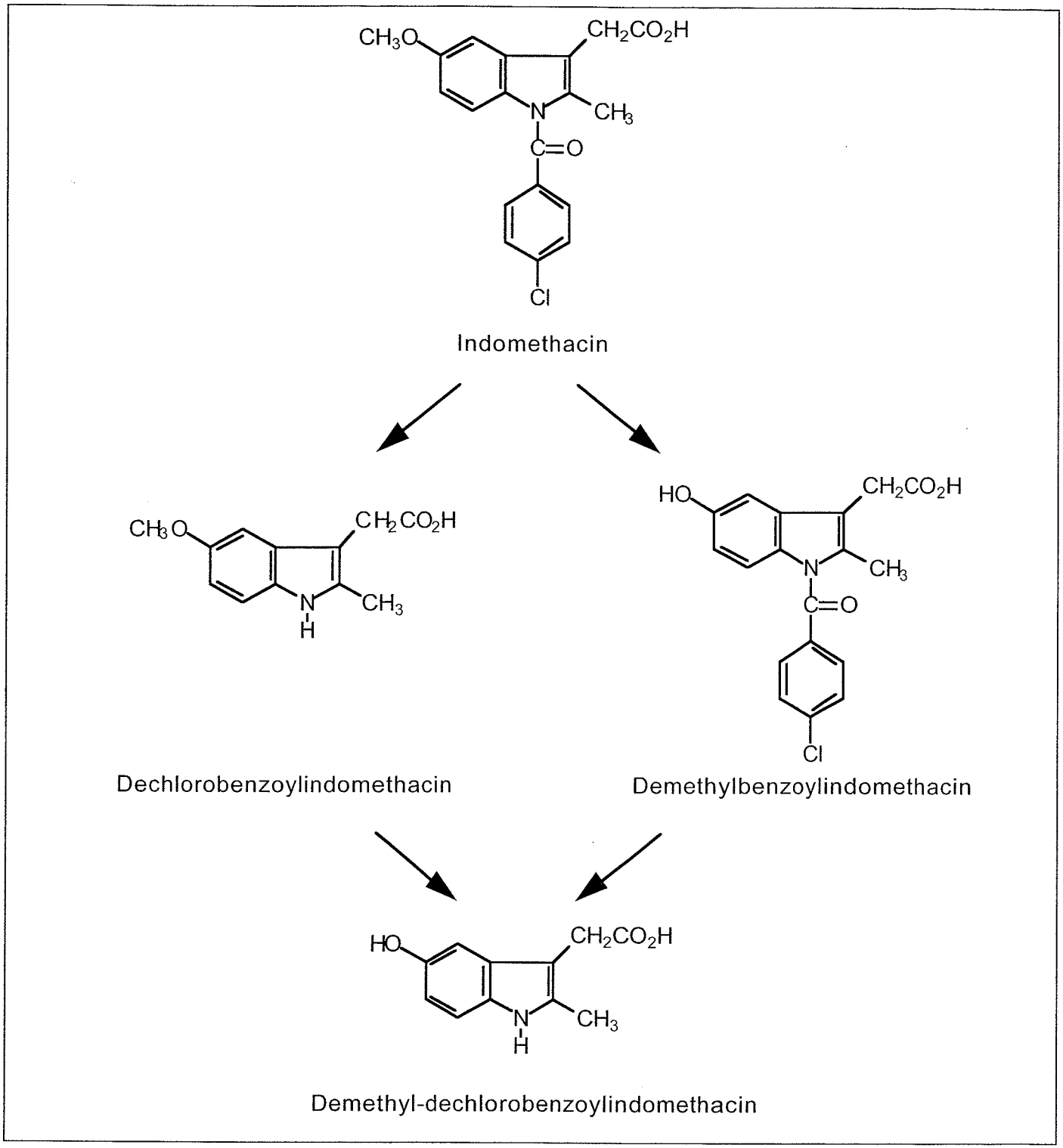
3.1.2 Pharmacokinetics

The pharmacokinetics of indomethacin in humans has been extensively studied and reviewed (Alvan *et al.*, 1975; Helleberg, 1981; Waller, 1983; Yeh, 1985).

(a) Absorption

Conventional indomethacin capsules are readily and completely absorbed after oral administration, with an estimated mean bioavailability of 85–122% (Duggan *et al.*, 1972; Alvan *et al.*, 1975; Kwan *et al.*, 1976; Yeh, 1985). Concomitant ingestion of foods may affect the absorption of indomethacin: total absorption is greater and the rate of absorption is generally quicker in fasting than in non-fasting subjects when delays of up to 4 h have been reported (Arnold & Brynger, 1970; Turakka & Airaksinen, 1974). Absorption of indomethacin from

Figure 1. 1-(p-Chlorobenzyl)-5-methoxy-2-methylindole-3-acetic acid (indomethacin) and its main metabolites



50-mg gelatin capsules was nearly twice as long when taken with food than in fasting subjects (Rothermich, 1966; Emori *et al.*, 1976). Diets high in carbohydrates appear to delay absorption most, followed by high-protein and high-lipid diets (Wallusch *et al.*, 1978). The presence of antacids or antidiarrhoeal medications may also decrease the rate of absorption of orally administered indomethacin (Rothermich, 1966; Emori *et al.*, 1976). The overall bioavailability of indomethacin, is not however, influenced by the presence of food (Kwan *et al.*, 1976; Wallusch *et al.*, 1978), and similar values are reported in fasting and non-fasting

subjects (Alvan *et al.*, 1975). Despite the possible effects on absorption, indomethacin and other NSAIDs are commonly taken with meals or antacids in order to lessen the gastric side-effects.

Comparisons of the bioavailability and pharmacokinetic profiles of sustained-release indomethacin and conventional capsules have been reported (Helleberg, 1981; Yeh *et al.*, 1982; Waller, 1983). The 75-mg sustained-release capsule contains 25 mg of an immediate-release fraction, and the remaining 50 mg are polymer-coated to ensure gradual release. The formulation is administered once or twice daily, as opposed to the less convenient three times daily dosing regime with conventional 25- or 50-mg capsules.

Use of the sustained-release formulation is associated with a slower rate of absorption and lower peak plasma concentrations, although the overall plasma concentration–time relationship is generally comparable to that of the conventional oral formulation. Waller (1983) reported peak plasma times of 2.0 ± 0.9 h with the sustained-release capsule and 1.0 ± 0.4 h with conventional capsules, and a 55% reduction in peak plasma concentrations. Schoog *et al.* (1981), however, in a cross-over study, found significant differences in the plasma concentration-time curves with 75-mg sustained-release and conventional capsules. The greatest differences were seen between 1 and 5 h after administration.

The peak plasma levels associated with rectal administration are generally lower than those with orally administered indomethacin capsules and are achieved earlier (Holt & Hawkins, 1965), although at least one author has disagreed on this point (Alvan *et al.*, 1975). Studies with volunteers showed that the maximal plasma concentrations of indomethacin after administration of suppositories (50–100 mg) were achieved within 60–80 min, with mean peak plasma concentrations of 1.5–2.8 μg/ml (Holt & Hawkins, 1965; Arnold & Brynger, 1970; Kwan *et al.*, 1976). The overall bioavailability of rectally delivered indomethacin is similar to that of orally administered ormulations, with values ranging from 80 to 100% (Alvan *et al.* 1975; Kwan *et al.*, 1976).

Circadian variation in the pharmacokinetics of indomethacin has been described (Clench *et al.*, 1981; Aronson *et al.*, 1993). In nine healthy volunteers, absorption of a single 100-mg oral dose of indomethacin was more rapid when it was taken at 7:00 or 11:00 h than at 15:00, 19:00 or 23:00 h (Clench *et al.*, 1981). Diurnal variations in the rate of gastric emptying may partially explain this effect, since the rate is significantly slower in the evening. Similarly, the transport mechanisms in the small bowel, where most indomethacin is absorbed, may be more efficient before midday. No diurnal variation was reported after rectal administration of a 100-mg suppository (Taggart *et al.*, 1987).

(b) Distribution

Like most other acidic NSAIDs, indomethacin readily binds to human serum albumin and other plasma proteins (Hucker *et al.*, 1966; Hultmark *et al.*, 1975; Rane *et al.*, 1978; Zini *et al.*, 1979), with binding affinities similar to those of other NSAIDs (Hultmark *et al.*, 1975). The lack of binding to erythrocytes reported in earlier studies (McArthur *et al.*, 1971) was later disproved (Bruguerolle *et al.*, 1986), when a more sensitive detection technique was used. In this study, uptake of indomethacin by erythrocytes represented approximately 2.4% of the total blood indomethacin levels in both young and older volunteers.

The numbers of indomethacin-binding sites on human serum albumin have been calculated to be between four (Hultmark *et al.*, 1975) and 15 (Hvidberg *et al.*, 1972), with an association constant of 0.86 x 10^3 litre/mol (Hvidberg *et al.*, 1972), and the actual percentage of indomethacin bound to albumin has been reported to range from 92 to 99% (Mason & McQueen, 1974; Hultmark *et al.*, 1975). All of the studies showed consistent binding over the therapeutic dose range of indomethacin and at higher levels (Hucker *et al.*, 1966; Hvidberg *et al.*, 1972; Mason & McQueen, 1974; Hultmark *et al.*, 1975). At therapeutic doses, binding to albumin is independent of concentration (Rane *et al.*, 1978).

Binding of indomethacin is not altered in uraemic patients with chronic renal failure

(Sjoholm *et al.*, 1976); however, decreased protein binding has been observed in cancer patients with active disease, probably reflecting lower serum albumin levels in those patients (Raveendran *et al.*, 1992).

Indomethacin readily penetrates body tissues (Hucker *et al.*, 1966; Kohler *et al.*, 1981) and has been recovered in synovial fluid from rheumatoid arthritis patients (Caruso, 1971; Emori *et al.*, 1973) and in fatty tissue, muscle and bone (Kohler *et al.*, 1981). Indomethacin enters the synovial fluid slowly and exceeds plasma levels after 5 h (Emori *et al.*, 1973). Only trace amounts of indomethacin have been detected in saliva (Rothermich, 1971) and in brain tissue (Hucker *et al.*, 1966). Indomethacin readily crosses the human placenta (Traeger *et al.*, 1973; Moise *et al.*, 1990) and is distributed in fetal tissues (Parks *et al.*, 1977). Placental transfer is independent of gestational age (Moise *et al.*, 1990).

In women given indomethacin for pain relief, *post partum* negligible levels of indomethacin have been recovered from breast milk (Takyi, 1970; Lebedevs *et al.*, 1991). In one study, six of seven breast-fed infants had plasma indomethacin concentrations of < 20 μg/ml, below the detection limit of the assay, after maternal doses of 0.94–4.3 mg/kg bw per day (Lebedevs *et al.*, 1991). Since the average milk:plasma ratio of indomethacin in subjects with measurable levels was only 0.37, it was concluded that only small amounts of indomethacin could be ingested via breast milk.

(c) Elimination

Most administered indomethacin is excreted in the urine either unchanged or in the form of conjugated and unconjugated metabolites which include demethylindomethacin, dechlorobenzoylindomethacin and demethyldechlorobenzoylindomethacin (Duggan *et al.*, 1972). After oral administration, these metabolites represent 19–42% of the dose recovered in faeces, whereas an average of nearly 60% appears in the urine as the parent drug and its glucuronide conjugates (Hucker *et al.*, 1966; Duggan *et al.*, 1972; Kwan *et al.*, 1976). In these studies, the amount of unchanged indomethacin in urine was between less than 5% and up to 18%. No differences in the excretion pattern of indomethacin or its metabolites were found after oral, rectal or intravenous administration (Kwan *et al.*, 1976).

Indomethacin is also eliminated in bile, where it undergoes extensive enterohepatic recycling (Kwan *et al.*, 1978). Once discharged into the bile, indomethacin is subsequently hydrolysed and re-enters the circulation through the gastrointestinal tract (Hucker *et al.*, 1966). It has been estimated that 24–115% of a given dose is reabsorbed into the circulation by this mechanism (Kwan *et al.*, 1976). The sporadic nature of biliary clearance may be responsible for the wide fluctuations in plasma indomethacin levels and plasma half-lives reported in the literature. The lack of correlation between plasma indomethacin levels and clinical therapeutic effects further supports this theory. Biliary recycling and the presence of unchanged indomethacin in bile may con-tribute to the production of intestinal lesions in some patients.

A two-compartment, open kinetic model has been proposed to describe the pharmacokinetic profiles of individuals participating in single-dose studies and patients undergoing long-term therapy. Dissolution of indomethacin from the plasma follows a biexponential pattern, with an initial rapid phase lasting up to 8 h followed by a slower secondary phase lasting 2.6–11 h (Alvan *et al.*, 1975).

Linear pharmacokinetics have been demonstrated with oral doses of 25–75 mg, with typical peak plasma concentrations of 1.1–4.4 μg/ml within 30–60 min (Emori *et al.*, 1976). The half-life in plasma is extremely variable, ranging from 2 to 11 h, perhaps because of enterohepatic cycling (Flower *et al.*, 1985). Comparable measurements of the area under the curve of plasma concentration–time were observed in subjects given 25 mg indomethacin orally or intravenously (Alvan *et al.*, 1975). Marked variability in the peak plasma concentration between subjects and in the same subjects tested on three separate occasions were reported by Emori *et al.* (1976), while few differences in plasma levels have been noted by other investigators (Alvan *et al.*, 1975).

No evidence of altered elimination patterns after long-term treatment with indomethacin have been documented.

(d) Effects of age

The total plasma clearance rates of indomethacin in adults are highly variable, ranging from 44 to 109 ml/h per kg bw (Alvan *et al.*, 1975); in premature infants, a substantially lower clearance rate of 7.6 ± 3.0 ml/h per kg bw has been reported after intravenous administration (Vert *et al.*, 1980). In children aged one year, the total clearance of indomethacin is substantially higher, at about 192 ml/h per kg bw (Olkkola *et al.*, 1989). Indomethacin has been widely used as a non-surgical treatment of patent ductus arteriosus in premature infants, at oral doses of 0.1–0.3 mg/kg bw. Plasma half-lives are considerably longer in premature newborns (11–90 h) than in adults, and wide variation is seen (Bhat *et al.*, 1979, 1980; Bianchetti *et al.*, 1980).

Some investigators have postulated that the plasma half-life is inversely correlated with gestational age (Evans *et al.*, 1979; Vert *et al.*, 1980). Decreased renal function or lower hepatic metabolism may explain the lower rate of elimination of indomethacin in premature infants. Alternatively, the differences related in half-life related to gestational age may correspond to maturation of drug metabolism systems (Evans *et al.*, 1981).

The pharmacokinetics of indomethacin in the elderly population has been described (Traeger *et al.*, 1973; Kunze *et al.*, 1974; McElnay *et al.*, 1992). No differences in absorption rate or peak plasma levels were seen between young volunteers and groups of healthy elderly subjects (McElnay *et al.*, 1992). One study, however, reported twofold higher levels in elderly patients than in young adults after a single 75-mg dose of indomethacin (Bruguerolle *et al.*, 1986), although the elimination rate of indomethacin was the same in the two groups. The clinical relevance of these data may be that untoward effects after indomethacin administration occur more frequently in patients over 60 years of age (Castleden & Pickles, 1988).

Age does not appear to influence the protein-binding capacity of indomethacin. After oral administration, more than 90% of a dose of indomethacin is bound to protein (Hultmark *et al.*, 1975) comparable values were found in premature newborns (Evans *et al.*, 1979), full-term infants (Friedman *et al.*, 1978) and the elderly (Bruguerolle *et al.*, 1986).

Some age-related alterations in excretion capacity have been noted. In one study, lower levels of unchanged indomethacin were recovered with increasing age, which were correlated with a reduction in renal function (Kunze *et al.*, 1974). The elimination kinetics in this group, were not affected, however. A 40% reduction in renal clearance of indomethacin was observed in a study of 12 healthy 36–50-year-old subjects in comparison with 15 healthy 19–34-year-old subjects (Wichlinski *et al.*, 1983). This study suggests that a reduction in renal clearance may accompany advancing age.

3.2 Experimental models

As in humans, biodegradation of indomethacin in most animal species involves deacylation and demethylation pathways (Harman *et al.*, 1964; Hucker *et al.*, 1966). While there is no evidence of demethylation reactions or metabolites in hamsters, both metabolic pathways have been shown in the rats, rabbits and guinea-pigs (Rowe & Carless, 1982). In monkeys, extensive metabolism of indomethacin into dechlorobenzoylindomethacin and excretion in the urine have been reported, whereas in rats indomethacin is metabolized principally into demethylindomethacin (Yesair *et al.*, 1970a).

Interspecies variations in the metabolism of drugs and in their binding affinities to plasma protein must be considered before data on the pharmacokinetics of indomethacin can be extrapolated. Marked differences in the absorption, plasma half-life, metabolism and excretion rate of indomethacin have been documented among animal species and in comparison with humans (Yesair *et al.*, 1970a).The plasma concentration is also influenced by the route of administration (Hucker *et al.*, 1966).

In an early study, higher plasma levels of ^{14}C-indomethacin were reported in dogs and rats than in rhesus monkeys or guinea-pigs after intravenous administration. The tissue distribution of the radiolabel was highest in

guinea-pigs, which had a faster plasma clearance rate than the other species. The plasma half-lives of indomethacin were several hours in rats and minutes in monkeys and dogs (Hucker *et al.*, 1966). In horses, a peak plasma level of about 125 ng/ml was seen within 1 h after a 250-mg oral dose of indomethacin (Phillips *et al.*, 1980).

In horses given 100 mg indomethacin rectally, maximal urinary levels were observed 2 h later, with peak concentrations of 19–81 μg/ml (Delbeke *et al.*, 1991). In rabbits, peak plasma levels appeared within 30–45 min after rectal administration of a 100-mg indomethacin suppository (Kuroda *et al.*, 1983), comparable to the time in humans. In beagle dogs, gastric acidity influenced the absorption rate of a sustained-release indomethacin capsule: low gastric acid resulted in faster absorption, but bioavailability was reduced (Yamada *et al.*, 1990).

As in humans, placental transfer of indomethacin has been documented. When given during late gestation, indomethacin readily crosses the placenta in rats (Sharpe *et al.*, 1975), rabbits (Parks *et al.*, 1977) and sheep (Levin *et al.*, 1979; Anderson *et al.*, 1980). In one study in rats, the maternal indomethacin plasma levels were 37–66 times higher than fetal levels when the drug was administered on days 11 and 12 of gestation, whereas only a three- to fourfold difference between maternal and fetal values was seen when it was given on day 21 (Klein *et al.*, 1981). Progressive decreases in the maternal:fetal ratio of the concentration of indomethacin plasma with advancing gestational age have been confirmed in rats (Momma & Takao, 1987). In other animal models, such as the rabbit and sheep, fetal plasma levels exceeded maternal levels when the drug was given late in gestation (Parks *et al.*, 1977; Harris & Van Petten, 1981).

Differences in the protein binding capacity of maternal and fetal blood may affect indomethacin transport throughout gestation. In rabbits, Parks *et al.* (1977) noted that the fetal levels of indomethacin increased as the maternal levels increased and there was always a substantial difference between the maternal and fetal concentrations, probably due to protein binding of indomethacin. Anderson *et al.* (1980) found no difference in maternal and fetal plasma protein binding affinity in sheep. Evidence to support the theory that the plasma half-life of indomethacin is inversely correlated with gestational age was provided in a study in neonatal rats, in which an age-related increase in cytochrome P450 activity was reported (Clozel *et al.*, 1986).

The increase in microsomal activity may be partially explained by an increased affinity of the enzyme for its substrate with age. An alternative theory is that the low affinity in the neonate is due to the presence of competitive inhibitors, as has been shown in neonatal rabbit liver (Evans *et al.*, 1981).

The kidneys play a significant role in the elimination of indomethacin in some animal species, except dogs. In dogs, at least 80% of an administered dose of ^{14}C-indomethacin was excreted in the faeces as the parent compound, and large amounts were also present in bile. It was estimated that about 50% of the amount of indomethacin excreted in bile is reabsorbed by the intestines in dogs (Hucker *et al.*, 1966). Enterohepatic recycling has been described in both rats (Hucker *et al.*, 1966; Yesair *et al.*, 1970a,b) and monkeys (Yesair *et al.*, 1970a). Most excreted indomethacin is reabsorbed slowly by the intestine in rats (Hucker *et al.*, 1966; Liss *et al.*, 1968; Yesair *et al.*, 1970b) and is thought to be involved in the occurrence of intestinal lesions in this species (Baer *et al.*, 1974).

3.3 Genetic variation

No genetic variation in the pharmacokinetics of indomethacin has been described among different population groups.

4. Cancer-preventive Effects

4.1 Human studies

4.1.1 Studies of cancer occurrence

(a) Colorectal cancer

There are no studies that specifically address the risk for colorectal cancer after use of indomethacin alone. Studies that included separate estimates of NSAIDs other than aspirin and those in which aspirin and other NSAIDs were considered together are summarized in the chapter on aspirin.

(b) Breast cancer

Indomethacin was included in a hypothesis-generating cohort study designed to screen 215 drugs for possible carcinogenicity, which covered more than 140 000 subscribers enrolled in July 1969 to August 1973 in a prepaid medical care programme in northern California (USA). Computer records of persons to whom at least one drug prescription had been dispensed were linked to cancer records from hospitals and the local cancer registry. The observed numbers of cancers were compared with expected numbers, standardized for age and sex, for the entire cohort. Three publications summarized the findings for follow-up periods of up to seven years (Friedman & Ury, 1980), nine years (Friedman & Ury, 1983) and 15 years (Selby *et al.*, 1989). Among 4867 persons who received indomethacin, there was a significant ($p < 0.01$) deficit of breast cancer (12 observed, 26 expected) in the seven-year follow-up. No negative or positive association with use of indomethacin was reported in the 15-year follow-up.

4.1.2 Studies of other relevant end-points

(a) Sporadic adenomatous polyps in the colon

There are no controlled studies of the risk for sporadic adenomatous polyps of the colon and use of indomethacin alone. Studies of combined non-aspirin NSAIDs in this respect are summarized in the chapter on aspirin.

(b) Adenomatous polyps in patients with familial adenomatous polyposis

Hirata *et al.* (1994) reported on two patients with familial adenomatous polyposis who had residual polyps in the rectal remnant after undergoing total colectomy and ileoproctoscopy. They were treated with 50-mg indomethacin suppositories once or twice daily and showed regression of polyps (both size and numbers) within three months. In one patient, polyps recurred after cessation of therapy and regressed again with reinstitution.

Eight patients with familial adenomatous polyposis who had undergone total colectomy with ileorectal anastomosis were given a 50-mg indomethacin suppository once or twice daily for four or eight weeks (Hirota *et al.*, 1996). In six patients, the polyps regressed, but recurred on cessation of therapy.

(c) Case studies of treatment for desmoid tumours

Waddell and Gerner (1980) reported three patients with refractory desmoid tumours who responded to indomethacin. Waddell *et al.* (1983) described two additional patients with desmoid tumours, one of whom responded to indomethacin. Klein *et al.* (1987) reported six patients treated with indomethacin for these tumours: one regressed completely, but no response was seen in the other five patients. Of four patients with desmoid tumours treated by Tsukada *et al.* (1992), one had complete remission of the tumour, but the others did not respond. Itoh *et al.* (1988) reported one patient with recurrent abdominal desmoid tumours who did not respond to indomethacin. Thus, of 16 patients reported, six responded to indomethacin.

4.2 Experimental models

4.2.1 Experimental animals

(a) Colon

Studies on the prevention of colon carcinogenesis in rats treated with indomethacin are summarized in Table 1.

Eight-week-old male Donryu rats received intraperitoneal injections of 20 mg/kg bw methylazoxymethanol acetate once a week for six weeks. One group of rats received an intrarectal instillation of a 1-ml solution of indomethacin (macrogolum powder; 7.5 mg/kg bw) once a week on weeks 27, 28 and 29. One control group received instillations of 1.0 ml water (vehicle control), and another was untreated. Colon tumours were counted in week 30. The incidence of colon tumours in indomethacin-treated rats (15/30) was significantly lower than that in the vehicle control group (19/23) and in the untreated controls (26/30) ($p < 0.05$). Indomethacin treatment reduced the mean number of colon tumours per tumour-bearing rat (2.0) from that in the vehicle control group (3.5) and in untreated controls (3.3) ($p < 0.05$). Treatment also reduced the incidence of small intestine adenocarcinomas

Table 1. Prevention of colon tumourigenesis by indomethacin

Species, strain, sex	No. of animals/group	Carcinogen (dose)	Indomethacin		Preventive efficacy	Reference
			Dose (route)	Treatment relative to carcinogen		
Rat, Donryu, male	30	MAM (20 mg/kg bw)	7.5 mg/kg bw (intrarectally)	After	Incidence, 42% ($p < 0.05$); multiplicity, 39%	Kudo *et al.* (1980)
Rat, Sprague-Dawley male	10	DMH (30 mg/kg bw)	20 mg/l (water)	3 d after 12 d after 35 d after	Multiplicity, 83% ($p < 0.01$) Multiplicity, 65% ($p < 0.01$) Multiplicity, 77% ($p < 0.01$)	Pollard & Luckert (1980)
			0.2 mg/kg bw (gavage)	After	Multiplicity, 48% (NS)	
Rat, Sprague-Dawley, male	7/8	NDMA-OAc (13 mg/kg bw)	20 mg/l (water)	After	Multiplicity, 90% ($p < 0.05$)	Pollard & Luckert (1981a)
Rat, Lobund Sprague-Dawley, male	9	DMH (30 mg/kg bw)	20 mg/l (water)	After	Incidence, 75% ($p < 0.01$); multiplicity, 83% ($p = 0.01$)	Pollard & Luckert (1981b)
	7	MAM (30 mg/kg bw)	20 mg/l (water)	After	Incidence, 81% ($p < 0.01$); multiplicity, 89% ($p < 0.01$)	
Rat, F344, female	29	MNU (2 mg/rat)	2.5 mg/kg (intraperitoneal)	After	Incidence, 55% ($p < 0.002$); multiplicity, 59% ($p < 0.02$)	Narisawa *et al.* (1981)
Rat, F344, female	30	MNU (2 mg/rat)	20 ppm (water)	After	Incidence, 75% ($p < 0.01$); multiplicity, 78% ($p < 0.01$)	Narisawa *et al.* (1982)
	30		10 ppm (water)	After	Incidence, 79% ($p < 0.01$); multiplicity, 80% ($p < 0.01$)	
Rat, F344, female	27 27 27	MNU (3 x 4 mg/rat)	10 ppm (water)	During 2–30 weeks 11 weeks after	Incidence, 58% ($p < 0.05$) Incidence 76% ($p < 0.01$) None	Narisawa *et al.* (1983)
Rat, Lobund Sprague-Dawley, male	10–27	DMH (2 x 30 mg/kg bw) MAM (30 mg/kg bw)	20 ppm (water)	After	Incidence, 81% Incidence, 79%	Pollard & Luckert (1983)

Table 1 (contd)

Species, strain, sex	No. of animals/group	Carcinogen (dose)	Indomethacin		Preventive efficacy	Reference
			Dose (route)	Treatment relative to carcinogen		
Rat, Sprague-Dawley, male	30	DMH (20 mg/kg bw)	20 mg/L (water)	During and after	Incidence, 36% ($p < 0.005$)	Metzger *et al.* (1984)
Rat, Sprague-Dawley, male	50	NDMA-OAc (2 mg/kg bw)	10 ppm (water)	During After During and after	None Multiplicity, 32% ($p < 0.05$ Multiplicity, 55% ($p < 0.05$	Narisawa *et al.* (1984a)
Rat, ACI/N, male	14	1-HA (1.5% in the diet)	16 ppm (water)	During and after	Incidence of colon adenoma + carcinoma, 100% ($p < 0.002$); of colon adenoma + adenocarcinoma, 100% ($p < 0.01$); of squamous cell papillomas of the forestomach, 72% ($p < 0.01$)	Tanaka *et al.* (1991)
Rat, F344, male	19–20	NDEA (100 mg/kg bw); MNU (20 mg/kg bw); NBHBA (500 ppm); DMH (40 mg/kg bw); NBHP (1000 ppm)	20 ppm (water)	After	Adenoma incidence, 100% Tumour incidence, 100% ($p < 0.05$)	Shibata *et al.* (1995)

MAM, methylazoxymethanol acetate; DMH, 1,2-dimethylhydrazine; NS, not significant; NDMA-OAc, *N*-nitrosodimethylacetoxyamine; F344, Fischer 344; MNU, *N*-methyl-*N*-nitrosourea; 1-HA, 1-hydroxyanthraquinone; NDEA, *N*-nitrosodiethylamine; NBHBA, *N*-nitrosobutyl(4-hydroxybutyl)amine; NBHP, *N*-nitrosobis-2-hydroxypropylamine

to 4/30, from 10/23 in vehicle controls and 12/30 in untreated controls ($p < 0.05$) (Kudo *et al.*, 1980).

Male Sprague-Dawley rats received five weekly intragastric administrations of 30 mg/kg bw 1,2-dimethylhydrazine as a solution of the hydrochloride in sterile saline, freshly prepared before administration. Three groups of 10 rats were given 20 mg/litre indomethacin in the drinking-water *ad libitum* starting 3, 12 or 35 days after the last dose of 1,2-dimethylhydrazine, and three control groups were given tap-water that had been neither chlorinated nor acidified. The numbers of colon tumours were counted 20 weeks after the start of carcinogen treatment. Treatment with indomethacin reduced the number of tumours per rat from 5.3 to 0.9 ($p = 0.002$) when given three days after the carcinogen, from 2.6 to 0.9 ($p = 0.0065$) when given after 12 days and from 2.2 to 0.5 ($p = 0.007$) when given after 35 days. In the last group, the number of rats with tumours was reduced from 9/10 to 4/9 ($p = 0.0016$). Indomethacin treatment had no effect on body weight, and no lesion was observed in any other organ. In a second protocol, two groups of 10 rats received intragastric administrations of 1,2-dimethylhydrazine as above. Seven days after the fifth dose, one group received daily intragastric administrations of 0.25 mg/kg bw indomethacin obtained from commercial capsules (Indocin®), and the second group received an intragastric administration of the vehicle (1% cornstarch). Twenty weeks after the onset of treatment, a nonsignificant reduction in the number of tumours per rat, from 2.7 to 1.4, was seen ($p = 0.08$) (Pollard & Luckert, 1980).

Two groups of seven and eight male weanling Sprague-Dawley rats received a single intraperitoneal injection of 13 mg/kg bw *N*-nitrosomethyl(acetoxymethyl)amine. Two weeks later, the first group was given 20 mg/ml (3 mg/kg bw) indomethacin in the drinking-water for 18 weeks, at which time the experiment was terminated. The number of rats with tumours was reduced from 6/8 to 1/7 in the first experiment and from 8/10 to 0/5 in the second. [The statistical significance of these differences was not reported.] The number of intestinal tumours per rat was reduced from 1.5 to 0.14 ($p < 0.05$). In a duplicate experiment with groups of five indomethacin-treated and 10 control rats, the number of tumours per rat was reduced from 1.4 to none (Pollard & Luckert, 1981a).

Weanling male Lobund strain Sprague-Dawley rats received a single dose of 30 mg/kg bw 1,2-dimethylhydrazine by gavage; 34 days later, indomethacin was given in the drinking-water (20 mg/l) and continued until the end of the experiment at 20 weeks. Indomethacin treatment reduced the incidence of colon tumours from 9/10 to 2/9 ($p < 0.01$) and the multiplicity of tumours from 1.30 to 0.22 ($p = 0.01$). Indomethacin also reduced the average body weight by 7% ($p < 0.05$). In a second experiment, rats were injected subcutaneously with methylazoxymethanol acetate (30 mg/kg bw) and 7 or 35 days later given indomethacin in the drinking-water (20 mg/l). The numbers of intestinal tumours in indomethacin-treated and untreated rats were determined at week 20. Treatment with indomethacin seven days after carcinogen treatment reduced the incidence from 7/9 to 1/7 ($p < 0.01$) and the multiplicity from 1.3 to 0.14 ($p < 0.01$). Treatment 35 days after carcinogen treatment reduced the incidence from 3/5 to 0/5 and the multiplicity from 1.4 to 0. No significant reduction in body-weight gain was seen with either protocol (Pollard & Luckert, 1981b).

Nine-week-old female Fischer 344 rats were given an intrarectal instillation of a 0.5-ml solution (2 mg) of 13.3 mg/kg bw *N*-methyl-*N*-nitrosourea three times a week in weeks 1–5. Groups of 29 and nine rats received intraperitoneal injections of 2.5 mg/kg bw indomethacin solution three times a week in weeks 11–25. Animals in the first group were killed at 25 weeks, and those in the second group were subjected to endoscopic examination and kept for an additional 10 weeks. A third group of nine rats was given indomethacin in weeks 26–35.The tumour incidences in the three groups were 9/29, 3/9 and 7/9, respectively, and the numbers of tumours per rat were 0.45, 0.33 and 1.0, respectively. Three control groups of 20, 30 and nine rats were treated with *N*-methyl-*N*-nitrosourea as above; one group

then received intraperitoneal injections of 0.1 ml of the vehicle (methylcellulose); the other two groups were not treated. The tumour incidences in the three groups were 14/20, 20/30 and 6/9, respectively, and the numbers of tumours per rat were 1.1, 1.0 and 1.3 [p value not given]. Thus, indomethacin did not inhibit existing tumours when administered between 25 and 35 weeks (Narisawa *et al.*, 1981).

Three groups of 30 female Fischer 344 rats received intrarectal instillations of 2 mg *N*-methyl-*N*-nitrosourea in weeks 1–5. Two groups were given indomethacin at concentrations of 20 or 10 mg/l [20 or 10 ppm] in weeks 11–25 [consumption of water was not documented]; the third group was given tap-water. All rats were killed at week 26. Three rats given the high dose of indomethacin died before the end of treatment and a total of 8/90 rats died of pneumonia or lung abscess. In rats treated with 20 mg/l [20 ppm] of indomethacin, a reduction in the incidence of colon tumours was seen, from 18/27 to 4/24 ($p < 0.01$) and a reduction in the multiplicity from 1.04 to 0.23 tumours per rat ($p > 0.01$). With a dose of 10 mg/l [10 ppm] indomethacin, the reduction in incidence was from 18/27 to 4/28 ($p < 0.01$) and the reduction in multiplicity from 1.04 to 0.21 ($p < 0.01$) (Narisawa *et al.*, 1982).

Colonic tumours were induced in female Fischer 344 rats by intrarectal administration of 4 mg per rat of a freshly prepared 0.5-ml solution of *N*-methyl-*N*-nitrosourea on days 3, 5 and 7. Indomethacin was given at a concentration of 0.001% [10 ppm] in drinking-water for various periods. The incidence of tumours in control rats killed at week 31 was 12/27. Administration of indomethacin on days 1–7 reduced the incidence to 5/27 ($p < 0.05$), and administration in weeks 2–30 to week 30 reduced the incidence to 3/28 ($p < 0.01$). The reduction in the incidence (6/28) of colon tumours when indomethacin was given in weeks 11–30 was not significant. The incidence of colon tumours in rats given indomethacin in weeks 2–30 and killed on week 41 (10/22) was significantly higher ($p < 0.01$) than in rats receiving identical treatment with indomethacin but killed in week 31 (Narisawa *et al.*, 1983).

Groups of 10–27 male weanling Lobund Sprague-Dawley rats received two intragastric administrations of 30 mg/kg bw 1,2-dimethylhydrazine hydrochloride at seven-day intervals or a single subcutaneous injection of 30 mg/kg bw methylazoxymethanol acetate. Treatment with 20 mg/l indomethacin in the drinking-water (estimated intake, 2.5 mg/kg bw per day) was initiated 14 or 63 days after 1,2-dimethylhydrazine treatment and 14 or 77 days after methylazoxymethanol acetate treatment. The development of intestinal tumours was prevented or retarded significantly with indomethacin in comparison with that of control animals. In the rats treated with 1,2-dimethylhydrazine, the incidence of colon tumours was reduced from 20/25 to 4/27 ($p < 0.05$) after 14 days and from 12/12 to 10/13 ($p < 0.05$) after 63 days. In those treated with methylazoxymethanol acetate, the incidence of colon tumours was reduced from 16/17 to 3/15 ($p < 0.05$) after 14 days and from 10/10 to 9/10 after 77 days (Pollard & Luckert, 1983).

Two groups of 30 male Sprague-Dawley rats, with an average weight of 120 g, were given subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine hydrochloride once a week for 20 weeks. One group of rats was given 20 mg/l [20 ppm] indomethacin in drinking water during the initiation and post-initiation phases. The rats were killed 32 weeks after the start of carcinogen treatment. Indomethacin had no significant effect on food intake or water consumption, and the average body weights of the two groups were similar. The incidence of colonic tumours was reduced from 88 to 56% ($p < 0.005$). The numbers of metastases to lymph nodes were comparable (Metzger *et al.*, 1984).

Male Sprague-Dawley rats, eight weeks old, were given Altromin-1320 chow and an intrarectal instillation of 2 mg/kg bw *N*-nitrosodimethyl(acetoxymethyl)amine once a week in weeks 1–10. Three groups of 50 rats were given 0.001% [10 ppm] indomethacin in the drinking-water in weeks 1–10, 11–20 and 1–20. Two control groups were given 0.1% ethanol in water or water only. Indomethacin given after the carcinogen treatment reduced the multiplicity of colon tumours from 4.7 to

3.2 ($p < 0.05$); when it was given during and after the carcinogen it reduced the multiplicity from 4.7 to 2.1 ($p < 0.05$) (Narisawa *et al.*, 1984).

Six-week-old male ACI/N rats were fed a diet containing 1.5% 1-hydroxy-anthraquinone [purity and diet consumption unspecified] for 48 weeks. A second group was given 1-hydroxy-anthraquinone as above plus 16 ppm indomethacin in the drinking-water for 48 weeks [water intake unspecified]. A control group received basal diet and tap-water only. Treatment with 1-hydroxyanthraquinone decreased body weight by 7% ($p < 0.02$) and increased the relative liver weight by 15% ($p < 0.001$). The number of rats with adenomas or adenocarcinomas in the colon (12/27) was reduced to 0 by co-administration of indomethacin ($p < 0.01$), and the number of squamous-cell papillomas of the forestomach was reduced from 14/27 to 2/14 ($p < 0.01$) (Tanaka *et al.*, 1991).

(b) Oesophagus

These studies are summarized in Table 2. In a first experiment, groups of 24 and 45 three-month-old C57BL male mice were given *N*-nitrosodiethylamine [purity unspecified] at concentration of 0.04 µl/ml [37.7 ppm] and indomethacin at a concentration of 16 mg/l [16 ppm] in the drinking-water daily for two weeks and then three times a week for 18 weeks [water intake not monitored]. The number of oesophageal tumours per centimetre was reduced from 6.04 to 3.74 ($p < 0.001$). In a second experiment, groups of eight and 16 mice were given the same treatments daily for 30 days and then twice a week for 16 weeks. The number of oesophageal tumours per centimetre was reduced from 4.10 to 2.66 ($p < 0.01$). In a third experiment, 16 mice were given the carcinogen daily for 30 days and then twice a week for four months, and 19 mice received indomethacin in the drinking-water four months after the beginning of carcinogen treatment. Both groups were killed eight months after that date. The number of tumours per centimetre was reduced from 6.10 to 4.74 ($p < 0.01$) (Rubio, 1984). [The Working Group noted that body weights and gastrointestinal toxicity were not documented.]

Groups of 18–61 female C57BL mice, three months of age, were given *N*-nitrosodiethylamine [purity unspecified] in the drinking-water at a concentration of 0.4 mg/l [0.4 ppm] daily for three months. The first group was killed at the end of treatment and the second group three months after the end of treatment; the third group was given indomethacin at a concentration of 1.6 mg/l [1.6 ppm] for three months from the end of carcinogen treatment. Indomethacin treatment reduced the number of tumours per centimetre of oesophageal mucosa from 5.01 to 2.85 ($p < 0.01$). In a second experiment, two

Table 2. Prevention of oesophageal tumourigenesis by indomethacin

Species, strain, sex	No. of animals/ group	Carcinogen (dose)	Indomethacin		Preventive efficacy	Reference
			Dose (route)	Treatment relative to carcinogen		
Mouse, C57BL, male	24 + 45	NDEA (37.7 ppm)	16 ppm	During	38%; $p < 0.001$	Rubio (1984)
	8 + 16		(drinking-water)	During	35%; $p < 0.01$	
	16 + 19			During and after	22%; $p < 0.01$	
Mouse, C57BL, female	18–61	NDEA (0.4 ppm)	1.6 ppm	After	43%; $p < 0.01$	Rubio (1986)
	18 + 19		(drinking-water)	After	21%; p 0.05	
					32%; $p < 0.01$	
	35 + 39			After	32% $p < 0.01$	
Rat, LIO, male	35 + 58	NSEE (50 mg mg bw)	25 ppm (diet)	After	63%; $p < 0.05$	Bespalov *et al.* (1989)

NDEA, N-nitrosodiethylamine; NSEE, N-nitrososarcosine ethyl ester

groups of 19 and 18 mice were given the same concentration of *N*-nitrosodiethylamine in the drinking-water for three months. The first group was killed six months after treatment and the second was given indomethacin in the drinking-water for six months starting at the end of carcinogen treatment, after which they were killed. Indomethacin treatment reduced the tumour index from 4.79 to 3.78 ($p < 0.05$). In a third experiment, three groups of 39, 35 and 36 mice were given the carcinogen treatment for four months. The first group was killed at the end of treatment and the second three months after treatment; the third group was given 1.6 mg/l [1.6 ppm] indomethacin for three months after carcinogen treatment. A reduction in the tumour index from 5.23 to 3.55 ($p < 0.01$) was observed with indomethacin treatment (Rubio, 1986). [The Working Group noted that body weights and gastrointestinal toxicity were not reported.]

A group of male outbred LIO rats, weighing 120–130 g, received *N*-nitrososarcosine ethyl ester [purity unspecified] by gavage at a dose of 50 mg/kg bw, five times per week for 16 weeks. After this treatment, a control group of 58 rats was given basal diet, and a second group of 35 rats received a diet containing 25 mg/kg [25 ppm] indomethacin [intake not documented]. All rats were killed 32 weeks after the beginning of carcinogen treatment, and tumours of the oesophagus were examined macroscopically and histologically. Three rats died with a perforating gastric ulcer. Indomethacin treatment reduced the incidence of oesophageal tumours from 89.7 to 65.7% ($p < 0.05$), and their multiplicity from 4.3 ± 0.6 to 1.6 ± 0.4. The incidence of forestomach tumours was also decreased, from 41.4 to 14.3% ($p < 0.05$), and their multiplicity from 0.9 ± 0.1 to 0.4 ± 0.3 (Bespalov *et al.*, 1989).

(c) Mammary gland

These studies are summarized in Table 3. Female Sprague-Dawley rats, 50 days of age, received a single intragastric administration of 5 mg 7,12-dimethylbenz[*a*]anthracene (DMBA). Three days later, 32 rats were given a low-fat diet (5% corn oil) and 34 rats a high-fat diet (18% corn oil), with or without indomethacin. Indomethacin was added to the diet at a concentration of 0.004% (w/w) [40 ppm] [food intake unspecified]. This treatment did not change the body weights significantly. In rats fed the low-fat diet, the reductions in the incidence, multiplicity and size of mammary tumours were not significant. In rats fed the high-fat diet, indomethacin had no significant effect on the incidence or multiplicity of mammary tumours, but the mean tumour size was reduced from 4.08 to 1.46 g ($p < 0.01$) (Carter *et al.*, 1983).

Female Sprague-Dawley rats, 50 days old, received intragastric administrations of 16 mg/rat DMBA in 1 ml sesame oil. Four groups of 25 rats were given indomethacin at 25 or 50 mg/kg diet [25 or 50 ppm] from weeks -2 to +1 or from week +1 for 150 days. When administered from weeks –2 to +1, indomethacin delayed the appearance of mammary tumours by 10–20 days. After 150 days of indomethacin treatment, the total number of tumours per rat was reduced from 8.66 to 5.79 with 50 ppm ($p < 0.01$) and to 5.94 with 25 ppm ($p < 0.01$). When administered at 50 ppm from weeks +1 to the end, indomethacin reduced the multiplicity of mammary carcinomas from 6.40 to 3.71 ($p < 0.01$), that of benign tumours from 2.26 to 0.40 ($p < 0.01$) and that of all tumours from 8.66 to 4.11 ($p < 0.01$). Administration of the lower dose of indomethacin during the same period changed none of the three parameters. The experiment was repeated with a dose of 8 mg DMBA per rat. The results of the two experiments were comparable (McCormick *et al.*, 1985).

Virgin female Sprague-Dawley rats, 36 days old, received an intragastric administration of 10 mg DMBA in 1 ml of sesame oil. Indomethacin was mixed into the diet at a concentration of 50 mg/kg diet [50 ppm], which was given either from weeks –2 to +1 or weeks +1 to the end of the experiment at 27 weeks. Treatment had no effect on body weights. Administration of indomethacin from weeks –2 to +1 did not reduce the multiplicity of mammary cancers or of all tumours, but administration from weeks +1 to the end of experiment reduced mammary carcinoma multiplicity from 3.46 to 2.56 ($p < 0.05$) and total tumour multiplicity from 3.65 to 1.88 ($p < 0.01$) (McCormick & Wilson, 1986).

Table 3. Prevention of mammary tumourigenesis by indomethacin

Species, strain, sex	No. of animals/ group	Carcinogen (dose)	Indomethacin		Preventive efficacy	Reference
			Dose (route)	Treatment relative to carcinogen		
Rat, Sprague-Dawley, female	32–34	DMBA (5 mg/rat)	40 ppm (diet)	After	None	Carter *et al.* (1983)
Rat, Sprague-Dawley, female	25	DMBA (16 mg/rat)	25 or 50 ppm (diet)	Before and during After	Benign tumours, multiplicity, 81% ($p < 0.01$) Carcinomas, multiplicity, 42% ($p < 0.01$)	McCormick *et al.* (1985)
Rat, Sprague-Dawley, female	25	DMBA (10 mg/rat)	50 ppm (diet)	Before and during After	None Carcinoma multiplicity, 26% ; $p < 0.05$	McCormick & Wilson (1986)
Rat, Sprague-Dawley, female	28–29	DMBA (10 mg/rat)	40 ppm (diet)	After	None	Abou-El-Ela *et al.* (1989)
Rat, Sprague-Dawley, female	32–33	DMBA (5 mg/rat)	50 ppm (diet)	After	High fat: tumour incidence, 63% ($p < 0.01$) Low fat: none	Noguchi *et al.* (1991)
Rat, LIO, female	21	MNU (12 mg/rat)	25 ppm (diet)	After	Tumour incidence, 37%; $p < 0.05$	Bespalov *et al.* (1992)

DMBA, 7,12-dimethylbenz[*a*]anthracene; MNU, *N*-methyl-*N*-nitrosourea

Virgin female Sprague-Dawley rats, 50 days old, received a single intragastric administration of 10 mg DMBA. Three weeks later, groups of 28–29 rats were placed on high-fat diets (20% corn oil) and 0.004% (w/w) [40 ppm] indomethacin until the end of the experiment at 16 weeks. Indomethacin had no significant effect on body weight or food intake and did not reduce the incidence, latency or number of mammary tumours per tumour-bearing rat (Abou-El-Ela *et al.*, 1989).

Four groups of 32 or 33 virgin female Sprague-Dawley rats received an intragastric administration of 5 mg DMBA. Seven days later, two groups were given a high-fat diet (20% corn oil), and the two other groups received a low-fat diet (0.5% corn oil). One group on each diet was given 0.005% (w/w) [50 ppm] indomethacin seven days after DMBA up to the end of the experiment, 20 weeks after DMBA administration. Indomethacin treatment reduced the number of rats with tumours from 26/32 to 10/33 in the high-fat group ($p < 0.01$) but increased the number of tumour-bearing rats from 9/33 to 11/32 in the group on the low-fat diet. Indomethacin reduced the multiplicity of mammary tumours in the high-fat groups from 2.3 to 0.9 ($p < 0.001$) but had no preventive effect in rats fed the low-fat diet (Noguchi *et al.*, 1991).

Female LIO (outbred) albino rats, weighing 200–230 g, were given one dose of 1 mg *N*-methyl-*N*-nitrosourea in 0.1 ml saline solution into each of the 12 mammary glands (total dose, 12 mg/rat). A control group of 25 rats then received basal diet, and 21 rats were given indomethacin at 25 mg/kg in the diet [25 ppm] for six months, when all animals were killed [feed intake unspecified]. Most of the mammary tumours were adenocarcinomas. Indomethacin reduced the incidence of mammary tumours from 19/25 to 10/21 ($p < 0.05$) but did not affect their multiplicity (1.36 and 1.14, respectively) (Bespalov *et al.*, 1992).

(d) Tongue

Two groups of 17 and 13 six-week-old male ACI/N rats, weighing 120 g, were given 4-nitroquinoline-1-oxide at 10 ppm in the drinking-water for 12 weeks. One group was switched to tap-water and the second to 10 ppm indomethacin for 24 weeks [water consumption unspecified]. No difference between the two groups in body weight or relative liver weight was seen. The incidence of all tumours (squamous-cell papilloma or carcinoma) was reduced from 12/17 to 3/13 ($p < 0.02$) and the incidence of carcinoma from 12/17 to 2/13 ($p < 0.005$) (Tanaka *et al.*, 1989); see also Table 4).

(e) Oral cavity

Two groups of five female and five male Syrian golden hamsters, eight weeks of age, received applications of a 0.5% solution of DMBA in mineral oil three times a week on the left buccal pouch. One of the groups simultaneously received a 0.5% solution of indomethacin (1 mg/animal) in the mouth daily until killing. A group of five male and five female animals was left untreated. One female and one male from each treated group were killed in weeks 8, 10, 12, 13 and 14. No morphological changes were seen in untreated hamsters. Indomethacin treatment reduced the multiplicity of tumours from 6.4 to 2.8 ($p < 0.05$) (Perkins & Shklar, 1982). [The Working Group noted the small number of animals per group.]

Three groups of 10–11 male Syrian golden hamsters, three to four months old, were treated with either mineral oil, a 0.5% solution of DMBA in mineral oil, DMBA in mineral oil plus an indomethacin suspension or mineral oil plus the indomethacin suspension. DMBA was applied to the right buccal pouch three times a week for 14.5 weeks; four drops of a suspension of indomethacin (1 mg/animal) was administered orally daily. All hamsters were killed 16.5 weeks after the beginning of treatment. Indomethacin had no effect on the incidence of all oral tumours or of carcinomas or on the latency or multiplicity of tumours (Gould *et al.*, 1985).

Two groups of six to 12 male Syrian hamsters, four to six weeks old, received applications of a 0.5% solution of DMBA in liquid paraffin on both cheek pouches three times a week for 10 weeks. During weeks 12–22 (time of terminal kill), one group of hamsters received a daily oral dose of 1 mg/animal (0.1 ml solution) sodium indomethacin trihydrate; the second group was not treated. The total number of

Table 4. Prevention of tumorigenesis in other organs by indomethacin

Sex, species, strain	No. of animals/ group	Carcinogen (dose)	Organ	Indomethacin		Preventive efficacy	Reference
				Dose (route)	Treatment relative to carcinogen		
Rat, ACI/N, male	13	4-NQO (10 ppm)	Tongue	10 ppm (water)	After	Incidence: total tumour, 67% ($p < 0.002$); carcinoma, 78% ($p < 0.005$)	Tanaka *et al.* (1989)
Hamster, Syrian golden, male and female	10	DMBA (unspecified)	Oral cavity	1 mg (oral)	During	Multiplicity, 56% ($p < 0.02$)	Perkins & Shklar (1992)
Hamster, Syrian golden, male	10–11	DMBA (unspecified)	Oral cavity	1 mg (oral)	During	0	Gould *et al.* (1985)
Hamster, Syrian golden, male	6–12	DMBA (unspecified)	Oral cavity	1 mg (oral)	During	0	Franklin & Craig (1987)
Rat, ACI/N, male	10	AAF (200 ppm)	Liver	10 ppm (water)	Before, during and after	Incidence: adenoma, 86% ($p < 0.01$); carcinoma, 100% ($p < 0.001$); multiplicity, 97% ($p < 0.001$)	Tanaka *et al.* (1993)
Hamster, Syrian golden, female	20–30	BOP (10 mg/kg)	Pancreas	20 ppm (water)	After	Multiplicity, 51% ($p < 0.05$)	Takahashi *et al.* (1990)
Mouse, BDF, male	78–80	OH-BBN (8 doses of 7.5 mg/mouse)	Urinary bladder	7.5 ppm (diet)	Before, during and after	Incidence, 79% ($p < 0.05$)	Grubbs *et al.* (1993)
	78–84			15.0 mg/kg (diet)	Before, during and after	Incidence, 100% ($p < 0.01$)	
Mouse, Swiss, female	26	3-Methylcholanthrene	Cervix	40 ppm (diet)	Before, during and after	Incidence, 71% ($p < 0.01$)	Rao & Hussain (1988)

Table 4. (contd)

Sex, species, strain	No. of animals/ group	Carcinogen (dose)	Organ	Indomethacin		Preventive efficacy	Reference
				Dose (route)	Treatment relative to carcinogen		
Mouse, albino outbred, female	30–61	DMBA (8 doses of 25 μg)	Vagina and cervix	20 mg/l (water)	After	Carcinoma incidence, 40.5% ($p < 0.05$)	Bespalov *et al.* (1992)
Mouse, Balb/c, male	6	BaP (100 μg/rat)	Skin	8.4 μg/mouse (topically)	Before and during	Tumour weight reduced by 46%	Andrews *et al.* (1991)
	6	DMBA unspecified	Skin			None	
Mouse, *hr/hr*, female	20	UV radiation	Skin	1.8 mg/kg (water)	During	0	Haedersdal *et al.* (1995)
Rat, LIO, male and female	25	ENU (75 mg/kg bw)	Brain, kidney	20 mg/l (water) postnatally	After	Incidence: brain, 35% ($p < 0.05$); kidney, 29% ($p > 0.05$)	Alexandrov *et al.* (1996)

4-NQO, 4-nitroquinoline-1-oxide; 2-AAF, 2-acetylaminofluorene; OH-BBN, *N*-nitrosobutyl(4-hydroxybutyl)amine; DMBA, 7,12-dimethylbenz[*a*]anthracene; BaP, benzo[*a*]pyrene; BOP, *N*-nitrosobis(2-oxopropyl)amine; UV; ultraviolet; ENU, *N*-ethyl-*N*-nitrosourea

small and large tumours of the oral cavity in the two groups was similar (Franklin & Craig, 1987).

(f) Liver

Two groups of 10 five-week-old male inbred ACI/N rats were fed a diet containing 200 ppm 2-acetylaminofluorene in weeks 0–6 and basal diet in weeks 17–36. One group was given 10 ppm indomethacin in the drinking-water starting one week before carcinogen treatment to week 17. The experiment was terminated in week 36. Treatment with 2-acetylaminofluorene increased the liver weight from 3.47 to 4.75 g/100 g bw ($p < 0.001$); and administration of indomethacin reduced the weight to 3.69 g/100 g bw ($p < 0.05$). Indomethacin treatment also reduced the incidence of hepatic adenomas from 7/10 to 1/10 ($p < 0.01$) and that of hepatic carcinomas from 8/10 to 0/10 ($p < 0.001$). The number of all hepatic neoplasms per rat was reduced from 4.00 to 0.10 ($p < 0.001$) (Tanaka *et al.*, 1993; see also Table 4).

(g) Pancreas

Outbred female Syrian golden hamsters, five weeks old, received five weekly doses of 10 mg/kg bw *N*-nitrosobis(2-oxopropyl)amine subcutaneously. One group of 20 hamsters was given 20 ppm indomethacin in weeks 6–32 (after which they were killed), and a control group of 30 hamsters was given tap-water. Indomethacin treatment had no effect on body-weight gain but induced a nonsignificant reduction in the incidence of pancreatic tumours, from 20/28 to 10/19, and a significant reduction in the multiplicity of pancreatic adenocarcinomas, from 1.29 to 0.63 ($p < 0.05$) (Takahashi *et al.*, 1990).

(h) Urinary bladder

Groups of 78, 80 and 84 male C57BL x DBA/2F$_1$ mice, 49 days of age, received 7.5 mg *N*-nitrosobutyl(4-hydroxybutyl)amine [purity unspecified] dissolved in 0.1 ml/l ethanol:water (20:80) by intragastric administration once a week for eight weeks. The first group of mice was given the carcinogen alone, and the second and third groups received diets containing indomethacin at 7.5 and 15 mg/kg diet [7.5 and 15 ppm] starting one week before carcinogen treatment and continuing until the end of the experiment at 180 days; the higher concentration was determined to be the maximal nontoxic dose. Indomethacin reduced the incidence of urinary bladder tumours from 14 to 4% ($p < 0.05$) when given at 7.5 ppm and to 0 ($p < 0.01$) when given at 15 ppm. The incidence of all bladder tumours, including papillomas, was reduced from 24 to 5% ($p < 0.05$) and 0 ($p < 0.01$), respectively (Grubbs *et al.*, 1993; see also Table 4).

(i) Cervix and vagina:

These studies are summarized in Table 4. Random-bred, 10–12-week-old virgin Swiss mice were treated with 3-methylcholanthrene [purity unspecified] by the insertion of sterile, double cotton threads impregnated with beeswax containing approximately 600 µg 3-methylcholanthrene into the uterine cervix [levels of exposure unspecified]. Four groups of 25 mice were given 0, 10, 20 or 40 mg/kg of diet [0, 10, 20 or 40 ppm] indomethacin starting two weeks before carcinogen treatment up to 16 weeks, when all animals were killed. Control groups of 15 mice received beeswax-impregnated threads and the same diets as described above [dietary intake not documented]. Indomethacin treatment did not reduce body-weight gain. No cervical tumours were observed in the mice treated with beeswax-impregnated thread. In 3-methylcholanthrene-treated mice, indomethacin at 40 ppm reduced the cervical tumour incidence from 21/23 to 6/23 ($p < 0.01$). The reduction at lower doses of indomethacin was not significant (Rao & Hussain, 1988). [The Working Group noted that the linearity of the preventive efficacy in relation to the doses of indomethacin was not documented.]

Groups of 30 outbred albino (SHR) virgin female mice, 12 weeks old received polymer sponge tampons impregnated with a 0.1% triethylene glycol solution of DMBA intravaginally. The average dose of DMBA was 25 µg per application. The tampons were changed twice weekly for eight weeks, for a total of 16 applications. Nine weeks after the start of DMBA treatment, 61 mice received no further treatment and 30 received indomethacin in the drinking-water at 20 mg/l [20 ppm; water consumption unspecified] for a further 28 weeks. All surviving

mice were killed 36 weeks after the start of the experiment, but 30–60% died with progressing tumours of the vagina and cervix before that time. All tumours were examined histologically. The total incidence of vaginal and cervical (papillomas plus carcinomas) was similar in the two groups (63–72%), but the ratio of carcinomas to papillomas was lower in mice given DMBA followed by indomethacin (12 carcinomas and 7 papillomas) than in mice given DMBA alone (41 carcinomas and 3 papillomas; carcinoma:papilloma ratio, 14). Significantly more control than indomethacin-treated mice died before the end of the experiment (61% and 37%, respectively; $p < 0.05$) (Bespalov *et al.*, 1992).

(j) Skin

Two groups of six male Balb/c mice, aged six to eight weeks, received two weekly applications of 20 µl benzo[*a*]pyrene [purity unspecified] dissolved in 0.5% acetone (total dose, 100 µg per rat). One group was given 8.4 µg indomethacin dissolved in acetone at a concentration of 0.42 mg/ml 20 min before the benzo[*a*]pyrene application on the same area of the shaved dorsal trunk. Treatment lasted for six months. Indomethacin pretreatment increased the time of tumour onset from 19.8 to 24.8 weeks ($p < 0.05$) but reduced the mean weight of tumours from 0.57 to 0.31 g. Two other groups received weekly applications of DMBA dissolved in lanolin plus liquid paraffin [dose of DMBA unspecified], and one group was treated weekly with 16.8 µg indomethacin 20 min before DMBA application, as described above. No difference in tumour onset or weight was seen between the two groups (Andrews *et al.*, 1991; see also Table 4).

Two groups of 20 female *hr/hr* C3H/Tif mice, 14–15 weeks of age, were exposed to ultraviolet radiation at a daily dose of 12.6 kJ/m^2 for 8 min/day on four days per week. Indomethacin was given in the drinking-water [concentration unspecified] at an intake estimated to be 1.8 mg/kg bw per day. Mice were treated until they were killed by tumours. Indomethacin delayed the time of appearance of the first tumours ($p < 0.001$) but increased the mortality rate ($p < 0.0005$) (Haedersdal *et al.*, 1995).

(k) Transplacental carcinogenesis

Groups of 6–10 LIO outbred albino rats, three to four months of age, were injected intravenously on day 21 of gestation with 75 mg/kg bw *N*-ethyl-*N*-nitrosourea in saline; there were 12-16 pregnant controls. Two groups of 42 and 25 pups of each sex were treated with water alone or with 20 mg/l [20 ppm] indomethacin in the drinking-water throughout postnatal life. The daily intake of indomethacin was estimated to be 1.6 mg/kg bw. The incidence of brain tumours was reduced from 36/42 to 14/25 ($p < 0.05$) and their multiplicity was decreased from 1.95 to 0.92 ($p < 0.05$). The multiplicity of kidney tumours was reduced from 0.45 to 0.32 ($p < 0.05$). There was no significant difference in body weights between the groups [exact data not provided] (Alexandrov *et al.*, 1996; see also Table 4).

(l) Multi-organ carcinogenesis

Groups of 19–20 male Fischer 344 rats, six weeks of age, were treated sequentially with five carcinogens, as follows: a single intraperitoneal injection of 100 mg/kg bw *N*-nitrosodiethylamine on day 1; intraperitoneal injections of 20 mg/kg bw *N*-methyl-*N*-nitrosourea on days 3, 9 and 12; administration of *N*-nitrosobutyl(4-hydroxybutyl)amine in the drinking-water (0.05%) [500 ppm] during weeks 1 and 2; subcutaneous injections of 40 mg/kg bw 1,2-dimethylhydrazine on days 17, 20, 23 and 26; administration of *N*-nitrosobis-2-hydroxypropylamine in the drinking-water (0.1%) [1000 ppm] during weeks 3 and 4. Groups of rats were given indomethacin in the drinking-water [20 ppm] and basal diet in weeks 6–28. Indomethacin did not reduce body-weight gain or food or water consumption. The incidences and multiplicities of lung adenomas were significantly decreased in indomethacin-treated rats. Indomethacin treatment did not reduce the incidence or multiplicity of urinary bladder papillomas but did decrease the development of preneoplastic lesions. The incidence of adenomas of the large intestine and the number of rats bearing tumours were decreased in the indomethacin-treated group in comparison with control. Only the decrease in tumour incidence was statistically significant ($p < 0.05$) controls (Shibata *et al.*, 1995; see also Table 1).

4.2.2 *In-vitro models*

(a) Cultured mammalian cells

Non-regressing, premalignant, nodule-like alveolar lesions can be induced in cultured mouse mammary organs by DMBA. Female Balb/c mice were pretreated with oestradiol and progesterone for nine days to stimulate hormones, and then their mammary glands were excised and cultured for 10 days with insulin, prolactin, aldosterone and hydrocortisone to promote growth. During this period, the cultures were exposed to 2 µg/ml DMBA for 72–96 h. After the promotion period, all of the hormones except insulin were withdrawn to induce regression of the lobular alveolar structures. Half of the mammary glands were also treated with indomethacin at doses of (10^{-9} to 10^{-5} mol/l during the first 10 days of culture. The average incidence of mammary gland lesions in the DMBA-treated group was 63%; indomethacin inhibited the formation of DMBA-induced lesions, the most effective dose of 10^{-6} mol/l causing 77% inhibition (Mehta *et al.*, 1991).

Inhibition of TPA-induced early antigen of Epstein-Barr virus in lymphoblastoid Raji cells has been used to screen for anti-tumour promoters. Indomethacin inhibited the induction in a dose-related manner; the effective concentration resulting in 50% inhibition was 13 µg/ml (Saito *et al.*, 1986).

Indomethacin was a competitive inhibitor of dihydrodiol dehydrogenase in isolated hepatocytes from uninduced Sprague-Dawley rats. Preincubation of cells with 30 µmol/l indomethacin before addition of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene prevented the formation of benzo[*a*[pyrene-7,8-dione, which may be an activated metabolite of the carcinogen benzo[*a*]pyrene. (Flowers-Geary *et al.*, 1995).

(b) Antimutagenicity in short-term tests

The antimutagenicity effects of indomethacin are summarized in Table 5 and are displayed graphically as an activity profile in Figure 2.

Indomethacin reduced aflatoxin-induced mutagenicity in *Saccharomyces cerevisiae* (Niggli *et al.*, 1986) and clastogenicity in human lymphocytes (Amstad *et al.*, 1984). It inhibited arachidonic acid-induced sister chromatid exchange in Chinese hamster ovary cells co-cultured with human leukocytes (Weitberg, 1988). Indomethacin inhibited the induction of sister chromatid exchange by arachidonic acid in combination with benzo[*a*]pyrene or DMBA in a human tumour-derived cell line and inhibited sister chromatid exchange induction by the last two compounds in a rat tumour-derived cell line (Abe, 1986). Indomethacin completely inhibited the mutagenicity of 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene in V79 cells pretreated with arachidonic acid (Sevanian & Peterson, 1989). In *Salmonella typhimurium* strain TA98, indomethacin reduced the mutagenicity of *N*-acetylbenzidine, but it enhanced benzidine-induced mutagenicity. It inhibited DNA binding induced by benzidine or benzidine analogues in a microsomal activation system initiated by arachidonic acid but had no effect when the activation system was initiated by hydrogen peroxide (Petry *et al.*, 1988). It partially inhibited benzidine- or diethylstilboestrol-induced sister chromatid exchange in cell lines derived from rat and human hepatomas (Buenaventura *et al.*, 1984; Grady *et al.*, 1986), and it inhibited sister chromatid exchange induced by diethylstilboestrol in mouse cells (Hillbertz-Nilsson & Forsberg, 1989). It also reduced the frequency of chromium chloride-induced chromosomal aberrations in human lymphocytes (Friedman *et al.*, 1987). It inhibited DNA single-strand breaks induced by the tumour promoter, fecapentane-12, but it did not inhibit hydrogen peroxide-induced breaks (Plummer *et al.*, 1995). Pretreatment of rats with indomethacin prevented hydralazine hydrochloride-induced unscheduled DNA synthesis in hepatocytes *in vitro* (Martelli *et al.*, 1995). Indomethacin did not inhibit mitomycin C-induced sister chromatid exchange in human lymphocytes (Ekmekci *et al.*, 1995). It inhibited DNA binding induced by phenylhydroquinone *in vitro* (Pathak & Roy, 1993), and it inhibited the effects of the tumour promoter, TPA, including chromosomal aberrations in human lymphocytes and sister chromatid exchange in Chinese hamster ovary cells co-cultured with human leukocytes (Emerit & Cerutti, 1982; Weitberg, 1988). Indomethacin enhanced the frequency of styrene- and styrene

Table 5. Antimutagenic effects of indomethacin

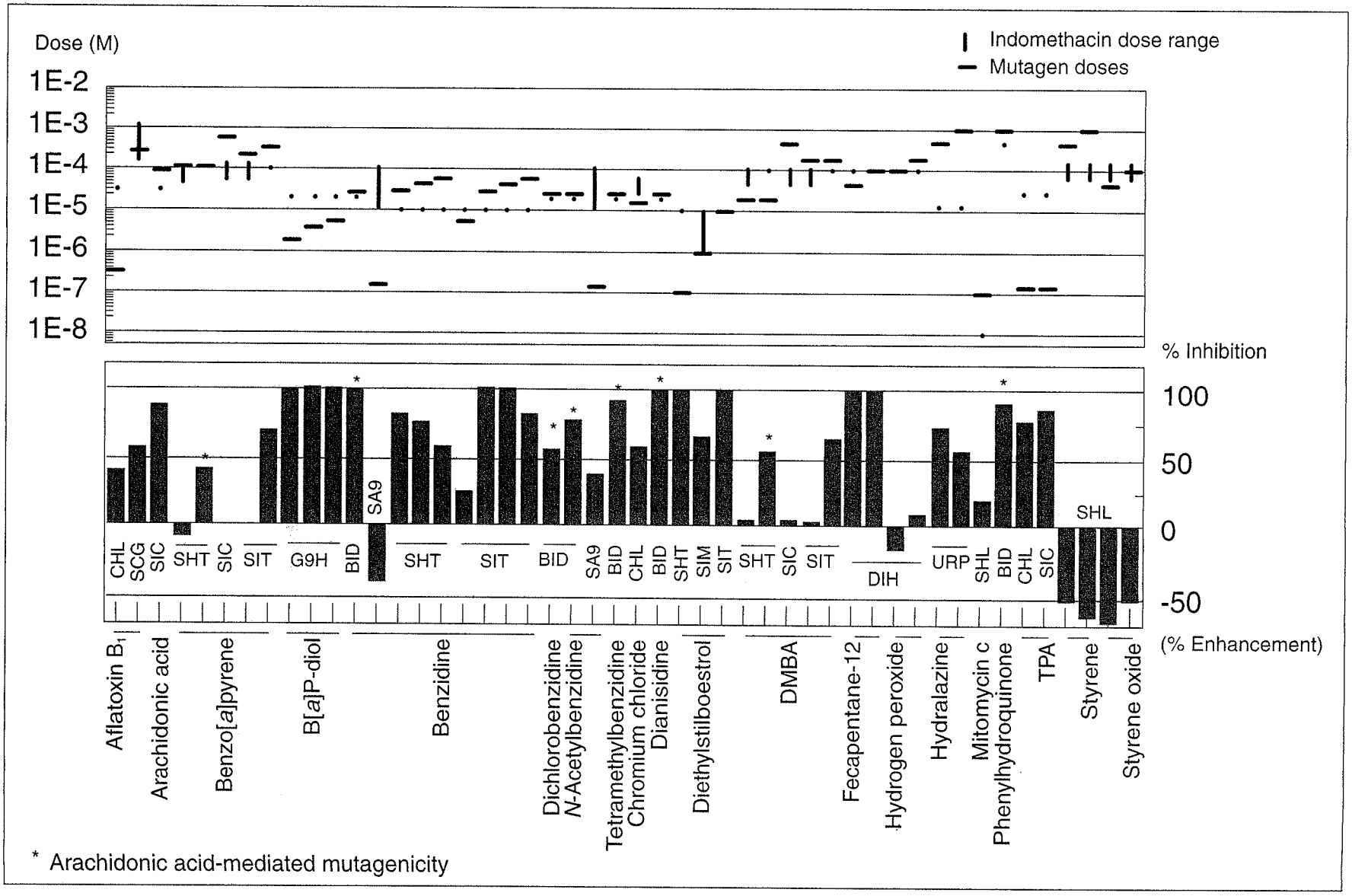
Mutagen	Dose of mutagen (μg/ml)	Test code[a]	% Inhibition[b] (% enhancement)	Dose range (μg/ml)		Reference
				Low	High	
Aflatoxin B1	0.09	CHL	38	10.0	10.0	Amstad *et al.* (1984)
Aflatoxin B1	78.0	SCG	55	0.1	0.7	Niggli *et al.* (1986)
Arachidonic acid	24.3	SIC	86	10.7	10.7	Weitberg (1988)
Benzo[*a*]pyrene + arachidonic acid	25.2	SHT	(9)	18.0	36.0	Abe (1986)
Benzo[*a*]pyrene	25.2	SHT	40	36.0	36.0	Abe (1986)
Benzo[*a*]pyrene	126	SIC	0	18.0	36.0	Abe (1986)
Benzo[*a*]pyrene	50.4	SIT	0	18.0	36.0	Abe (1986)
Benzo[*a*]pyrene	75.6	SIT	69	36.0	36.0	Abe (1986)
B[*a*]P-diol	0.5	G9H	100	7.2	7.2	Sevanian & Peterson (1989)
B[*a*]P-diol	1.0	G9H	100	7.2	7.2	Sevanian & Peterson (1989)
B[*a*]P-diol	1.5	G9H	100	7.2	7.2	Sevanian & Peterson (1989)
Benzidine + arachidonic acid	4.6	BID	99	7.2	7.2	Petry *et al.* (1988)
Benzidine	0.028	SA9	(42)	3.6	36.0	Petry *et al.* (1988)
Benzidine	5.0	SHT	80	3.6	3.6	Grady *et al.* (1986)
Benzidine	7.5	SHT	75	3.6	3.6	Grady *et al.* (1986)
Benzidine	10.0	SHT	56	3.6	3.6	Grady *et al.* (1986)
Benzidine	1.0	SIT	25	3.6	3.6	Grady *et al.* (1986)
Benzidine	5.0	SIT	100	3.6	3.6	Grady *et al.* (1986)
Benzidine	7.5	SIT	100	3.6	3.6	Grady *et al.* (1986)
Benzidine	10.0	SIT	80	3.6	3.6	Grady *et al.* (1986)
Dichlorobenzidine	6.3	BID	55	7.2	7.2	Petry *et al.* (1988)
N-Acetylbenzidine	5.6	BID	76	7.2	7.2	Petry *et al.* (1988)
N-Acetylbenzidine	0.034	SA9	37	3.6	36.0	Petry *et al.* (1988)
Tetramethylbenzidine	6.0	BID	93	7.2	7.2	Petry *et al.* (1988)
Chromium chloride	2.5	CHL	57	10.7	21.0	Friedman *et al.* (1987)
Dianisidine	6.1	BID	99	7.2	7.2	Petry *et al.* (1988)
Diethylstilboestrol	0.027	SHT	100	3.6	3.7	Buenaventura *et al.* (1984)
Diethylstilboestrol	0.27	SIM	65	0.4	3.6	Hillbertz-Nilsson & Forsberg (1989)
Diethylstilboestrol	2.7	SIT	100	3.6	3.6	Buenaventura *et al.* (1984); Shamon *et al.* (1994)
DMBA	5.1	SHT	5	17.9	36.0	Abe (1986)
DMBA + arachidonic acid	5.1	SHT	54	35.8	36.0	Abe (1986)
DMBA	128	SIC	5	17.9	36.0	Abe 1986)
DMBA	51	SIT	4	17.9	36.	Abe (1986)
	51	SIT	64	35.8	36.0	Abe (1986)
Fecapentane-12	12.5	DIH	100	35.8	36.0	Plummer *et al.* (1995)
Fecapentane-12	25.0	DIH	100	35.8	36.0	Plummer *et al.* (1995)
Hydrogen peroxide	3.4	DIH	(17)	35.80	36.0	Plummer *et al.* (1995)
Hydrogen peroxide	6.8	DIH	10	35.80	36.0	Plummer *et al.* (1995)
Hydralazine	110	URP	73	5.0	5.0	Martelli *et al.* (1995)
Hydralazine	200	URP	55	5.0	5.0	Martelli *et al.* (1995)
Mitomycin C	0.03	SHL	21	0.0	0.0	Ekmekci *et al.* (1995)
Phenyl hydroquinone	186	BID	92	179	179	Pathak & Roy (1993)
TPA	0.1	CHL	79	10.0	10.0	Emerit & Cerutti (1982)
TPA	0.1	SIC	88	10.7	10.7	Weitberg (1988)
Styrene	52.0	SHL	(52)	26.9	53.7	Lee & Norppa (1995)
Styrene	104	SHL	(63)	26.9	53.7	Lee & Norppa (1995)
Styrene oxide	6	SHL	(181)	26.9	53.7	Lee & Norppa (1995)
Styrene oxide	12.0	SHL	(51)	26.9	53.7	Lee & Norppa (1995)

B[*a*]P-diol, 7,8-dihydroxy-7,8-dihydrobenz[*a*]pyrene; DMBA, 7,12-dimethylbenz[*a*]anthracene; TPA, 12-*O*-tetradecanoylphorbol 13-acetate

[a] The test codes are defined in Appendix 2

[b] A positive value is the percentage inhibition of the effect induced by the mutagen in the test; a negative value (in parentheses) is the percentage enhancement of the effect.

Figure 2. Antimutagenicity profile of indomethacin



oxide-induced sister chromatid exchange in human lymphocytes (Lee & Norppa, 1995).

Indomethacin at 100 μmol/l inhibited DMBAinduced forward mutation to 8-azaguanine resistance in *S. typhimurium* strain TM677 by 69% (Shamon *et al.*, 1994). Transformed foci can be induced in Balbc3T3 cells in a two-stage assay, with initiation by 3-methylcholanthrene (0.5 μg/ml) and promotion by TPA 0.1 μg/ml). Indomethacin inhibited the induction of transformed foci in a dose-dependent manner, causing 81% inhibition at a concentration of 20 μg/ml (Semba & Inui, 1990).

Indomethacin reversed the inhibition of gap-junction intercellular communication in liver cells from male Wistar rats induced by the hepatic tumour promoter phenobarbital, 1,1,1-trichloro-2,2-(*p*-chlorophenyl)ethane (DDT) or γ-hexachlorocyclohexane (lindane). Treatment with 2 mmol/l phenobarbital inhibited intercellular communication by 30%, as measured by microinjection of fluorescent Lucifer Yellow dye after a 5-h incubation. Indomethacin did not reverse the inhibition of communication induced by DDT or lindane (Leibold & Schwartz, 1993)

4.3 Mechanisms of chemoprevention

4.3.1 *Inhibition of carcinogen activation*

In 1971, Vane reported that indomethacin inhibits prostaglandin production by interfering with the cyclooxygenase(COX)[1]-catalysed oxygenation of arachidonic acid. For a full description of this mechanism, see the General Remarks. An additional important mechanism by which indomethacin may prevent cancer is inhibition of carcinogen activation (Szarka *et al.*, 1994).

There is indirect evidence that COX catalyses conversion of the benzene metabolite, hydroquinone, into reactive oxygen products that accumulate in bone marrow and damage DNA. Indomethacin inhibits activation of hydroquinone,. thereby preventing DNA damage and further myelotoxicity (Schlosser *et al.*, 1990). Another mutagen similarly activated and formed during protein pyrolysis is 3-methylimidazo[4,5-*f*]quinoline (IQ). Once formed, IQ and its methylated derivatives are potent mutagens (Wild & Degen, 1987; Petry *et al.*, 1989). Indomethacin and other NSAIDs which prevent bioactivation of these molecules may prevent cancer through this mechanism (Earnest *et al.*, 1992).

4.3.2 *Effects on cell proliferation and apoptosis*

Early investigations demonstrated that indomethacin and other NSAIDs interfere with a diverse range of biological processes related to cell growth including reductions in glycolysis (Cooney & Dawson, 1977), uncoupling of oxidative phosphorylation (Whitehouse, 1964), decreased mucopolysaccharide formation (Kalbhen *et al.*, 1967) and interference with calcium ion uptake and other calcium-mediated processes (Northover, 1977). Enzyme pathways found subsequently to be inhibited by indomethacin include phospholipase A_2 (Kaplan *et al.*, 1978; Lobo & Hoult, 1994), 15-lipoxygenase (Siegel *et al.*, 1980), myeloperoxidase (Shacter *et al.*, 1991) and glutathione *S*-transferase (Wu & Mathews, 1983).

A direct inhibitory effect of indomethacin on cellular proliferation is indicated by the results of studies of cell cultures, showing that indomethacin inhibits cell multiplication and progression of the cell cycle from G_1 to S phase (Bayer & Beaven, 1979; Bayer *et al.*, 1979). Several recent reports support the concept that indomethacin prevents tumour cell growth through alterations in the cell cycle and induction of apoptosis leading to cell death (Lu *et al.*, 1995; Shiff *et al*, 1996). In one study, indomethacin reduced the levels of two key cyclin-dependent kinases, p33 and p34, in HT-29 colon cells, both of which are crucial to cell cycle progression. Indomethacin induced apoptosis in these cells and increased the proportion of cells in the G_0/G_1 phases, which correlated with its ability to suppress cell proliferation (Shiff *et al.*, 1996). A similar mechanism has not yet been demonstrated in human colon tissue.

Indomethacin may also protect against cancer by altering the activity of enzymes other than cyclooxygenase. These effects include inhibition of enzymes such as cyclic AMP protein kinase and phosphodiesterase, both of which may be critical to cancer initiation and promotion (Kantor & Hampton, 1978;

[1] Used as a synonym for prostaglandin endoperoxide synthase (PGH synthase)

Abramson & Weissmann, 1989). Likewise, indomethacin may exert cancer preventive effects in the colon by preventing induction of ornithine decarboxylase, the first and rate-limiting enzyme in polyamine biosynthesis (Narisawa *et al.*, 1985, 1987). Data showing an association between ornithine decarboxylase activity, tumour promotion and cell proliferation in various organs including the colon and skin have been reported (Pegg & McCann, 1982; Slaga, 1983). More importantly, in patients with adenomatous polyps, ornithine decarboxylase activity is markedly elevated (Luk & Baylin, 1984). Suppression of ornithine decarboxylase by indomethacin in mouse skin with concomitant prevention of skin carcinogenesis by the tumour promoter TPA (Furstenberger & Marks, 1978, 1980; Verma *et al.*, 1980) further supports the theory that this enzyme is involved in carcinogenesis.

Although not direct chemopreventive mechanisms, other growth regulatory effects exerted by indomethacin, such as inhibition of angiogenesis (Gullino, 1981; Ziche *et al.*, 1982; Peterson, 1986), may be relevant. The results of a recent study conducted in murine 3T3 fibroblasts suggest that the ability of indomethacin to block angiogenesis may be related to its inhibitory effect on hyaluronic acid production (August *et al.*, 1994). Hyaluronic acid has been recovered from the stroma of several malignant tumours (Knudson *et al.*, 1984), including human breast tumours (Bertrand *et al.*, 1992; Shuster *et al.*, 1993), and is believed to play a role in cell migration. Interruption of hyaluronic acid synthesis, therefore, may be yet another potential target of the action of indomethacin.

Nitric oxide stimulates COX-2 activity in a concentration-dependent manner (Salvemini *et al.*, 1993). At pharmacological, micromolar doses, indomethacin had no effect on nitric oxide synthase activity or mRNA expression, in contrast to aspirin, which inhibited the enzyme through a post-translational modification mechanism at millimolar concentrations (Amin *et al.*, 1995).

4.3.3 Immune surveillance

Another mechanism that may be relevant to the chemopreventive action of indomethacin is immunomodulation (Honn *et al.*, 1981; Plescia, 1982; Goodwin & Ceuppens, 1985; Hwang, 1989). Of the arachidonic acid metabolites, only prostaglandin E_2 has a defined function in regulating both humoral and cellular immune responses (Goodwin, 1981, 1984). Elevated tissue levels of prostaglandin E_2 have been shown to suppress immune surveillance by inhibiting T-cell proliferation, natural killer cell cytotoxicity and lymphokine production (Taffet & Russell, 1981; Goodwin & Ceuppens, 1983). Apparently, prostaglandin E_2 exerts its activity by interfering with the production and activity of interleukin 2, a potent lymphokine needed for T-cell proliferation. Drugs such as indomethacin, which block cyclooxygenase activity and prostaglandin production, stimulate the immune response both *in vitro* and *in vivo* (Han *et al.*, 1983; Goodwin, 1984).

An alternative mechanism implicating the immune system involves the ability of indomethacin to restore expression of major histocompatability antigens (HLA) in human colon cancer cells (Arvind *et al.*, 1996). In human colon tumours and in histologically normal mucosa distant from colon adenomas, expression of class I and II HLA antigens is either reduced or lost. Loss of HLA antigen expression allows cancer cells to escape immune surveillance (McDougall *et al.*, 1990; Tsioulias *et al.*, 1992, 1993). Indomethacin induced expression of the class II antigen, HLA-DR, in a time- and dose-dependent manner and increased the steady-state levels of HLA-DR mRNA (Arvind *et al.*, 1996).

The capacity of indomethacin to stimulate the lipoxygenase pathway, particularly 15-lipoxygenase (Vanderhoek *et al.*, 1984), and influence the immune system through production of leukotrienes and other metabolites is an alternative mechanism worthy of further study. Additional experiments conducted with specific COX-2 inhibitors will assist in delineating the role of this enzyme in chemoprevention.

5. Other Beneficial Effects

Rogers *et al.* (1993) randomized 44 patients with a clinical diagnosis of Alzeimer's disease to indomethacin (100–150 mg/day) or a matching placebo in a double-blind study of six months' duration. Only 14 of 24 (58%) of those randomized to indomethacin and 14 of 20 (70%) of those randomized to placebo completed the trial and were available for measurements. More subjects on indomethacin stopped their treatment, primarily because of gastrointestinal side-effects. Patients on indomethacin who completed the study showed a 1.3% improvement in standardized tests, whereas those on placebo showed a 8.4% decline; at least a 4% decline was seen in only 2 of 14 patients on indomethacin and 12 of 14 patients on placebo.

The remainder of the studies on Alzheimer disease (McGeer *et al.*, 1996), which were observational in nature, were based on a variety of conditions used as surrogates for NSAID use, including history of arthritis and analgesic use. These are summarized in the chapter on aspirin.

6. Carcinogenicity

6.1 Humans

No data were available to the Working Group.

6. 2 Experimental animals

Groups of six-week-old female Sprague-Dawley rats received indomethacin in the drinking-water at a concentration of 10 mg/l [10 ppm] for 92 weeks. The cumulative dose of indomethacin was estimated to be 200 mg per rat. Control rats were given drinking-water only. A significant increase in moderate (10/48; $p < 0.05$) and severe (5/48; $p < 0.05$) hyperplasia of the urinary tract was observed in comparison with the control group (5/49 and 1/49, respectively). The number of rats with mammary adenocarcinomas was higher in those given indomethacin (5/48) than in the untreated control group (1/49; $p < 0.01$). The authors reported a higher incidence of benign mammary gland tumours in the treated (19/48) than in untreated rats (11/49; $p < 0.01$) (Holmäng *et al.*, 1995).

Groups of 50 28-day-old (adolescent) and 97-day-old (adult) male Sprague-Dawley rats received a single application of 25 mg/kg bw indomethacin dissolved in 1 ml acetone on the shaved dorsal skin. A second group received the same dose of indomethacin and acetone plus 21.3 mg/animal of a carrier substance (Amuno®). Control groups received either 1 ml acetone or the carrier only. The animals were observed until natural death. Adolescent rats treated with indomethacin with or without carrier had a lower average body weight than control rats ($p < 0.05$). More malignant tumours were observed in the indomethacin-treated adolescent rats with or without carrier than in their respective controls ($p < 0.02$). In contrast, treatment of adult rats with indomethacin with or without carrier had no effect of the incidence of malignant tumours. Leydig-cell tumours of the testis (distinguished from hyperplasia) were more frequent in indomethacin-treated rats than in either adolescent ($p = 0.005$) or adult ($p = 0.03$) controls. Malignant intestinal tumours were observed in 3/50 adolescent rats treated with indomethacin and in 8/50 also given the carrier but not in control rats (0/50). The increase was not significant. No difference was seen in the adult treated rats (Goerttler *et al.*, 1992). [The Working Group noted that a single dose of indomethacin was used and that the route of administration was inappropriate for carcinogen evaluation.]

Two groups of six-week-old female Sprague-Dawley rats were fed a semisynthetic pelleted diet containing 0.2% *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide for seven weeks. They were then fed the basal diet up to week 92. One group was given indomethacin in the drinking-water at a concentration of 1 mg/l for 92 weeks starting one week before the carcinogen treatment, for an estimated cumulative dose of 200 mg per rat. The controls were fed synthetic diet for 92 weeks. There was no significant difference in body weight between treated and untreated rats. Indomethacin treatment increased the incidence of urothelial tumours from 4/50 to 10/48 ($p < 0.05$) (Holmäng *et al.*, 1995).

Weanling female Fischer 344 rats were fed standard diet and water *ad libitum* [type of diet and consumption not specified] and given

subcutaneous injections of 1,2-dimethylhydrazine [purity unspecified] at 40 mg/rat once a week for 10 weeks. After laparotomy, 42 rats were found to be free of visible metastases and 16 rats had grossly apparent metastases in regional nodes, the peritoneum, the omentum and the liver. One-half of the animals with no metastases were given indomethacin in drinking-water at a concentration of 20 µg/ml [20 ppm] until they died or became moribund; the remaining 21 rats were given drug-free water. Indomethacin treatment reduced the median survival from 69 to 29 days ($p < 0.01$) and increased the number of rats with grossly evident metastases from three to nine ($p = 0.035$). When eight rats with metastases were treated with indomethacin as described above, there was no significant effect on mean survival (Danzi *et al.*, 1984). [The Working Group noted that assignment of rats with and without metastases on the basis of staging laparotomy could be subject to considerable variation.]

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

See also General Remarks and the chapter on sulindac.

(a) Gastrointestinal tract toxicity

In the meta-analysis of Henry *et al.* (1996), described in the chapter on aspirin, the risks for serious upper gastrointestinal complications after use of indomethacin were statistically indistinguishable from those of most other NSAIDs. Within individual studies, however, indomethacin was consistently associated with a higher risk for such complications, and in a ranking analysis it ranked 5 (with 1 the most and 12 the least toxic) of 12 NSAIDs analysed. The pooled relative risk estimate from a meta-analysis of five studies that included indomethacin was 3.0 (95% CI, 2.2–4.2) for low-dose indomethacin users and 7.0 (95% C1, 4.4–11) for high-dose users in comparison with non-users. Given baseline risks for ulcer disease of 0.5 per 1000 per year in younger adults (Garcia Rodriguez *et al.*, 1992) and 4 per 1000 in older adults (Smalley *et al.*, 1996), the rates of serious ulcer complications among indomethacin users can be estimated to be 2 per 1000 in younger adults and 10–20 per 1000 in those 65 years and older.

(b) Reproductive and developmental effects

In non-randomized trials of short courses (one to three days) of indomethacin at doses of 100–400 mg/day for the prevention of pre-term labour, no increase in the risk for congenital anomalies, premature closure of the ductus arteriosus or pulmonary hypertension was observed (Ostensen, 1994). Of 57 infants delivered at or before 30 weeks who had been exposed to indomethacin prenatally, 62% had persistent ductus arteriosus, compared with 44% of unexposed infants matched with exposed infants on gestational age and sex (Norton *et al.*, 1993). This difference was statistically significant. More of the indomethacin-exposed infants with persistent ductus arteriosus required surgical ligation than affected infants who had not been exposed.

There have been case reports of perinatal death associated with severe oligohydramnios in the infants of mothers who used indomethacin (Itskovitz *et al.*, 1980; Veersema *et al.*, 1983). Of 37 fetuses exposed to indomethacin for treatment of pre-term labour, 70% were diagnosed with oligohydramnios, in comparison with 3% of fetuses whose mothers had been treated with agents other than NSAIDs (Hendricks *et al.*, 1990).

In two studies, indomethacin treatment of mothers for pre-term labour was associated with necrotizing enterocolitis in the neonate (Norton *et al.*, 1993; Major *et al.*, 1994).

An unspecified defect was identified from hospital discharge records in one infant among the offspring of 50 women who were members of a health care cooperative in Seattle (USA) and who filled a prescription for indomethacin during the first trimester (Aselton *et al.*, 1985).

(c) Other toxic manifestations

Indomethacin users report a variety of signs and symptoms more frequently than users of other NSAIDs These include vertigo and headache (Fries *et al.*, 1991).

7.1.2 Experimental animals

Many of the toxic effects of indomethacin in non-human species may result from its action as a prostaglandin synthesis inhibitor. As in humans, the primary site of acute toxicity in various species is the gastrointestinal tract. Drug-induced adverse effects have also been reported in the tissues of the kidney, liver, heart and bone, and significant adverse reproductive and developmental effects have been found.

(a) Acute and short-term toxicity

Indomethacin was toxic to several species when administered at low levels. The LD_{50} values were as follows: mouse, 5.7 mg/kg bw day orally for five days (Julou *et al.*, 1969); and rat, 2.4 mg/kg bw per day orally for 14 days (Awouters *et al.*, 1975) and 13 mg/kg bw intraperitoneally for seven days (Klaassen, 1976). No defined symptoms of toxicity were reported (Klaassen, 1976). When slightly higher levels of 5–10 mg/kg bw per day orally were administered to male and female rats for up to 21 days, 77–100% of the animals died within four to 10 days. Surviving female rats had a lower weight gain (Gaetani *et al.*, 1972).

Marked growth inhibition, measured as decreased body-weight gain, and decreased food and water consumption were seen in rats treated with indomethacin at 2.5–5 mg/kg per day orally on five days per week for up to 26 weeks. All female and 40% of male rats treated at 5 mg/kg bw per day died within 24 weeks. Overt signs of toxicity included bloody faeces, emaciation, hypoactivity, piloerection, urinary incontinence, loss of grooming activity and anorexia. None of the surviving rats exhibited any abnormal appearance or behaviour, and no significant changes were seen on haematology, blood chemistry, urinalysis, organ weight analysis or pathology (Nomura *et al.*, 1978).

All male and female rats given indomethacin 1–3 mg/kg bw per day orally on six days per week for 30 days or 3 mg/kg bw per day orally for six to 12 weeks survived and remained in good health. Two of six rats at 6 mg/kg bw per day died after 10 and 13 days (Nomura *et al.*, 1978; Anthony *et al.*, 1994).

(b) Gastrointestinal tract toxicity

Instillation of indomethacin at 12 mg/kg per day directly into the stomach of male and female marmosets resulted in the death of all animals within 20 days. Diarrhoea was observed in animals of each sex treated with the drug at this level of 6 mg/kg per day orally for up to four weeks (Oberto *et al.*, 1990).

Gastrointestinal damage induced by indomethacin has been reported in rats, mice, rabbits, guinea-pigs, dogs and marmosets. Typical lesions include gastric inflammation, mucosal and submucosal haemorrhages, ulceration, perforations and adhesions of the small bowel and peritonitis. The extent of toxicity appears to vary with the excretion of intact drug into the bile and the length of its contact in the small intestine, due to enterohepatic circulation of the drug (Hucker *et al.*, 1966; Yesair *et al.*, 1970a; Duggan *et al.*, 1973, 1975; Klaassen, 1976; Cronen *et al.*, 1982).

In rats, a species considered to be highly susceptible to the ulcerogenic effect of indomethacin (Wilhelmi, 1974), low oral doses rapidly induced marked gastric and duodenal damage when administered as either a single acute dose or over several weeks. Unspecified gastrointestinal lesions were observed within 24–48 h of a single oral dose of 16 mg/kg bw in female rats (Fracasso *et al.*, 1987), while oral doses as low as 6–10 mg/kg bw produced small haemorrhages, small to large (> 2 mm) ulcers and slight to marked hyperaemia (30–100% incidence) within two to five days of treatment (Shriver *et al.*, 1977; Laufer *et al.*, 1994). Multiple oral doses of 6–9 mg/kg bw per day for up to four days or 3 mg/kg per day for up to 11 days resulted in similar lesions, small bowel perforation and adhesions in male rats (Shriver *et al.*, 1977; Laufer *et al.*, 1994). Oral doses of 5 mg/kg bw per day on five days per week for up to 26 weeks produced fibrinous peritonitis, due to perforated ulcers, in the ileum in 40% of male and 100% of female rats that died during the experimental period (Nomura *et al.*, 1978). Few lesions were reported in rats after single or multiple daily oral doses of 1–3 mg/kg for up to four days (Shriver *et al.*, 1977).

Non-ulcerated lesions in the rat caecum, consisting of prominent mucosal folds showing submucosal fibrosis with fibrous obliteration and thickening of the muscularis mucosae, were induced by indomethacin administered in a regimen designed to mimic a course of human treatment. Anthony *et al.* (1994) treated rats for 30 weeks with consecutive doses of 3 mg/kg bw per day for 12 weeks, 4.5 mg/kg bw per day for one week, 6 mg/kg bw per day for one week, no drug for six weeks, 4.5 mg/kg bw per day for two weeks and control diet for eight weeks. These diaphragm-like caecal lesions, similar to lesions observed in the ileum of some patients treated for long periods with NSAIDs (Lang *et al.*, 1988), appear to arise from healed caecal ulcers.

Gastrointestinal ulceration was also reported in mice and guinea-pigs treated twice with indomethacin at doses of 2.5–10 mg/kg bw and 50–100 mg/kg bw orally, respectively (Wilhelmi, 1974). Regions of focal necrosis and subepithelial oedema were noted in rabbits treated intraluminally into the stomach with a solution delivering 100 mg/kg bw of drug for 15 min (Wallace *et al.*, 1991). In dogs, ulceration and inflammation were reported in the small and large intestines after treatment with 2.5 mg/kg bw per day indomethacin orally for up to three years (Stewart *et al.*, 1980). Subacute diffuse inflammation of the gastrointestinal submucosa and serosa with peritoneal involvement, necrosis or ulceration of the mucosa and haemorrhages were also reported in male and female marmosets (*Callithrix jacchus*) treated with indomethacin at 6–12 mg/kg bw per day for up to four weeks (Oberto *et al.*, 1990).

Gastrointestinal damage seems to stem from the ability of indomethacin to suppress prostaglandin synthesis. In this region, prostaglandins are involved in modulation of gastric acid and mucus production, intestinal bicarbonate secretion, regulation of mucosal blood flow and the inflammatory process. Thus, development of indomethacin-induced gastrointestinal lesions may be prevented until weaning in suckling rat pups by prostaglandins, which are known to be present in milk (Bedrick & Holtzapple, 1986). Lesions may develop as a result of drug-induced ischaemia in the mesenteric tissues, an effect demonstrated in dogs, in regions corresponding to drug-induced ulceration (Cronen *et al.*, 1982).

Exacerbation of indomethacin-induced gastrointestinal damage was reported in rats and dogs by an experimentally induced increase in gastric acid production or a decrease in duodenal alkaline secretion. Duodenal lesions (up to a 100% incidence), some penetrating to the muscularis mucosae, and a few lesions in the stomach were precipitated in dogs subsequently injected with gastric acid-inducing histamine (40–80 µg/kg bw intramuscularly, four times per hour for 6 h) beginning 12 h after a single oral dose of 70 mg indomethacin. Alone, this dose produced no ulcers in either the stomach or duodenum within 18 h (Takeuchi *et al.*, 1988). A similar increase in gastric damage was produced in rats (Elliot *et al.*, 1996).

In 630 Wistar rats receiving 12–14 mg/kg bw indomethacin by gavage, there was significantly more intestinal ulceration (two- to fourfold) in those receiving a regular diet than in those on fat-free diets, independent of feeding schedule, sex or castration. Fasting also reduced the intestinal toxicity observed in animals fed a regular diet by two- to fivefold (Del Soldato & Meli, 1977).

(c) Nephrotoxicity

The primary renal lesions induced by indomethacin in animals and frequently in human patients are papillary necrosis and interstitial inflammation (Jackson & Lawrence, 1978). Drug-induced alterations in renal function and architecture were observed in rats, dogs and marmosets, which may be the result of either tissue phospholipid accumulation, inhibition of prostaglandin synthesis or a combination of the two. Renal damage induced by other agents may also be enhanced by indomethacin.

Papillary necrosis was observed in rats after a single dose of 75 mg/kg bw (Arnold *et al.*, 1974). Renal papillary necrosis associated with either short- or long-term treatment with indomethacin may be the result of selective phospholipid accumulation in renal tissue, the papillae being the most sensitive. In rats treated subcutaneously with 10 or 50 mg/kg bw per day for three days, indomethacin caused a marked increase in all papillary phospholipids

(sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine), with an increase in sphingomyelin and phosphatidylethanolamine in the cortex and no observed effect in the medulla. Alterations in renal papillary phospholipid concentrations were observed even at doses as low as 1 mg/kg per day for up to four weeks, but no significant changes were observed in the medulla or cortex (Fernández-Tomé & Sterin Speziale, 1994). This result agrees with other reports of phospholipid accumulation preceding cellular necrosis (Mingeot-Leclercq *et al.*, 1988).

Other renal effects of indomethacin include degenerative changes in the renal parenchyma after oral administration of 5–10 mg/kg bw per day to male and female rats for up to 21 consecutive days (Gaetani *et al.*, 1972), and dose-related subacute interstitial inflammation of the renal cortex in female and male marmosets treated with 2–12 mg/kg bw per day for up to four weeks (Oberto *et al.*, 1990). Although no renal lesions were seen on histological examination of rats treated orally with 2 mg/kg bw for up to four months (Kleinknecht *et al.*, 1983), indomethacin accelerated the destruction of renal glomeruli in puromycin aminonucleoside-induced nephrosis.

Intravenous administration of 4 mg/kg bw indomethacin to anaesthetized dogs produced a very rapid, sharp decrease in renal blood flow and a decrease in water and sodium excretion (Tost *et al.*, 1995). These effects may be related to inhibition of prostaglandin synthesis, as prostaglandins are involved in the regulation of renal blood pressure (Flower *et al.*, 1985); however, this effect was not observed in alert dogs or rats, suggesting an interaction with anaesthesia (Swain *et al.*, 1975).

(d) Cardiovascular toxicity

Indomethacin caused premature constriction of the ductus arteriosus in the offspring of rats (Momma & Takao, 1989), rabbits (Sharpe *et al.*, 1975) and sheep (Levin *et al.*, 1979) when given near the end of gestation. In pregnant rats treated orally with 2.5–10 mg/kg bw on gestational day 21 or 22, fetal ductal constriction appeared within 6 h of maternal treatment and lasted for up to 36 h. Hampering of fetal cardiac function by retardation of significant blood flow through the ductus arteriosus can result in fetal acidaemia, hypoxaemia, pulmonary arterial hypertension, altered morphological development of the pulmonary vascular bed, right ventricular hypertrophy, diminished ventricular cavity, left ventricular dilatation, degenerative changes in the papillary muscles of the tricuspid valve, fetal death within 24 h of maternal treatment and respiratory difficulties in the neonate (Momma & Takao, 1989).

Intraventricular injection of 4 mg/kg bw indomethacin to cats diminished the coronary circulation and, to a lesser degree, reduced myocardial oxygen consumption (Stepaniuk & Stoliarchuk, 1985).

Indomethacin impairs scar formation after experimental myocardial infarct in dogs and enhances murine myocarditis due to coxsackie virus B_4 (Hammerman *et al.*, 1983; Khatib *et al.*, 1992).

(e) Hepatotoxicity

Degenerative changes in the liver, including vacuolar changes and fatty liver, were reported in male and female rats treated with 5–10 mg/kg bw per day for up to 21 days (Gaetani *et al.*, 1972). Alterations in a variety of hepatic microsomal drug-metabolizing enzymes were observed in rats dosed with indomethacin (Burke *et al.*, 1983), as noted above.

(f) Effects on bone formation

Bone formation during the healing of fractures or induction of heterotopic bone is retarded or completely inhibited by indomethacin. This effect, observed experimentally in rats and rabbits, may be elicited in the early phase of bone induction by a reduction in the inflammatory response, causing less favourable circumstances for bone formation (Rö *et al.*, 1976; Sudmann & Bang, 1979; Allen *et al.*, 1980).

(g) Haematological effects

Indomethacin induced changes in blood and blood chemistry, including iron-deficiency (microcytic) anaemia, hypoalbuminaemia, leukocytosis, thrombocytosis and decreased total serum proteins, in rats treated with 3–6 mg/kg bw per

day in the diet for up to 12 weeks or 5 mg/kg bw per day orally for up to 21 days (Gaetani *et al.*, 1972; Anthony *et al.*, 1994). No drug-induced alterations in haematological or blood chemical parameters were observed after treatment of male and female rats orally with 1–3 mg/kg bw per day on six days per week for 30 days (Nomura *et al.*, 1978).

Slight hypotrophy of the bone marrow, thymus and testis and slight thyroid hypertrophy were seen after oral administration of 5–10 mg/kg bw per day indomethacin to male and female rats for up to 21 days (Gaetani *et al.*, 1972).

(h) Reproductive and development effects

Indomethacin induced reductions in fetal and decidual tissue weights, fetal malformations, fetal death and prolonged gestation and parturition. The developmental toxicity of indomethacin was recently reviewed (Lione & Scialli, 1995).

Effects on fertility. Indomethacin reduced fertility in male mice treated with 146 µg/day for seven days and rats treated with 0.8 mg/kg bw orally daily for 28 days or 2 mg/kg bw intraperitoneally for seven days. It had anti-mating effects in female rats treated with 0.8-4 mg/kg bw orally, daily from pro-estrous for a period of six cycles before mating (Marley & Smith, 1974; Yegnanarayan & Joglekar, 1978; Löscher & Blazaki, 1986). Significant increases in the number of abnormal sperm were observed in mice treated with 12–36 mg/kg bw per day orally or 12–24 mg/kg bw per day intraperitoneally for 2–30 days (Shobha Devi & Polasa, 1987).

Effects on ovulation. Indomethacin has been reported to suppress ovulation in rats, mice, rabbits, cows, and two species of monkey. The doses sufficient to completely block induced ovulation were reported to be 7 mg/kg bw subcutaneously or 7.5–8 mg per animal intraperitoneally in rats, (Armstrong & Grinwich, 1972; Orczyk & Behrman, 1972; Yegnanarayan & Joglekar, 1978), 200 µg subcutaneously in mice (Saksena *et al.*, 1974), 3 mg/kg bw orally for two days before induction of ovulation in rabbits (O'Grady *et al.*, 1972; Yegnanarayan & Joglekar, 1978) or two injections of 15 mg/kg bw intramuscularly in cynomolgus monkeys (*Macaca fascicularis*) (Jaszczak, 1975). Induced ovulation was also suppressed in rhesus monkeys (*Macaca mulatta*) (Wallach *et al.*, 1975). In cows, injection of 20 mg of the drug directly into the preovulatory ovarian follicle completely blocked ovulation; however, it was ineffective when given as 5 g intramuscularly five times over 24 h or by intrauterine infusion of 1440 mg total dose per uterine horn (De Silva & Reeves, 1985).

Effects on gestation. Pregnancy disruption resulting from indomethacin treatment, reported in many species may result from reductions in implantation, suppressed placental development or adverse fetal effects. Prostaglandin inhibition may prevent implantation, resulting in lowered ova retention due to tubal disturbances or reduced uterine vascular permeability. Inhibition or delay of implantation was reported after treatment early in gestation in mice (150 µg/day subcutaneously on gestation days 1–4 or a single injection of 225 µg on day 2; Lau *et al.*, 1973), in rats (4 mg/kg bw orally daily on days 1–7, 3 mg/kg subcutaneously on days 3 and 4, or 400 µg into the uterine horns on day 4; Yegnanarayan & Joglekar, 1978; Gupta *et al.*, 1981; Phillips & Poyser, 1981), in rabbits (8–20 mg/kg bw intravenously every 12 h from 2 days before mating to nine days after mating, and similar doses administered on gestation days 4–7; El-Banna *et al.*, 1976; Hoffman, 1978), and in hamsters (0.1 mg/kg bw subcutaneously to pregnant females on gestation day 4; Evans & Kennedy, 1978). Other implantation-related effects include reductions in litter size in hamsters and marked reductions in the number of decidual implantation sites, fetal, placental and decidual tissue weights, and fetal viability. Increased fetal resorptions in rabbits (an apparent anti-implantation effect if it occurs early in gestation) were also observed (Hoos & Hoffman, 1983).

Inhibition of placental development was suggested to be the basis of pregnancy failure in mature gilts given 10 mg/kg bw per day indomethacin in the diet on days 10–25 of gestation (Kraeling *et al.*, 1985).

Post-implantation effects. Maternal treatment with indomethacin in the post-implantation

period (mid-gestation) resulted in increased numbers of intrauterine deaths and fetal resorptions in mice (5 mg/kg subcutaneously on gestation days 8–15 or 15 mg/kg bw subcutaneously on days 8–10 or 13–15, Persaud & Moore, 1974); apparent abortion and no successful deliveries at term in rats (4 mg/kg bw orally daily on days 10–16 of pregnancy; Yegnanarayan & Joglekar, 1978) and a reduction in the viability of implanted fetuses in rabbits (8 mg/kg bw subcutaneously twice daily on gestation days 9–12; Hoffman, 1978).

Maternal treatment near term resulted in increased numbers of fetal deaths in rats (0.1 and 1.0 mg/kg bw twice daily on days 18–21 of gestation; Aiken, 1972), in rabbits (2.5–10 mg/kg bw per day subcutaneously on gestation days 26–29; Harris, 1980) and in ewes (0.5–1 mg/kg bw intravenously or 75 mg orally three times daily for four days on gestation days 123–139; Levin *et al.*, 1979). Some of the stillbirths in rats were attributed to placental separation (Aiken, 1972).

Teratogenicity. Indomethacin appears to be teratogenic in mice but not rats. Mouse fetuses were born with eventration of the abdominal viscera, meromelia and defective limb posture when pregnant mice were treated subcutaneously with either 5 mg/kg bw on days 8–15 or 15 mg/kg bw on gestation days 13–15 (Persaud & Moore, 1974). Evidence that indomethacin induces cleft palate in mice was provided both *in vitro* and *in vivo* (Montenegro & Palomino, 1989, 1990). Indomethacin was not teratogenic in rats when administered to dams on gestation days 10–11 at 4 mg/kg bw orally three times (Klein *et al.*, 1981).

Prolongation of gestation. Delay in the onset of parturition, previously a therapeutic indication for use of indomethacin in humans, has also been reported in laboratory animals, including hamsters, rabbits and rhesus monkeys. Parturition onset was delayed in pregnant and pseudopregnant hamsters (300 or 600 µg twice daily on days 14–16 of pregnancy or 1 mg daily from day 5 of pseudopregnancy), although the treatment did not affect the duration of parturition (Lau *et al.*, 1975). In rabbits, prolongation of the gestation period was route- and time-dependent: 8–10 mg/kg per day in the drinking water from day 20 until delivery prolonged gestation, while subcutaneous administration of similar levels near the end of gestation (days 29–31) did not suppress plasma prostaglandin levels and did not prolong gestation (Challis *et al.*, 1975). With doses similar to those used in human therapy, Novy *et al.* (1974) reported up to 20 days' prolongation of gestation in rhesus monkeys treated with 100 mg/day on days 150–165 of gestation and 200 mg/day on days 166–187 of gestation. These results were confirmed at lower doses (10–15 mg/kg bw per day on days 150–165 of gestation and 21–28 mg/kg bw per day from day 166 of gestation until delivery) (Manaugh & Novy, 1976).

Administration of indomethacin at the end of gestation appeared to provoke both maternal and fetal adverse events at parturition in rats, rabbits and rhesus monkeys. The maternal events included protracted parturition, haemorrhage and gastric ulcers in rats treated with 0.1 or 1.0 mg/kg bw twice daily on days 18–21 of gestation (Aiken, 1972). Fetal events including signs of prolonged intrauterine stress (staining of the fetal skin, umbilical cord and placental membranes with meconium and a virtual absence of amniotic fluid) were noted in rhesus monkey fetuses at delivery. Four of eight fetuses died, two probably from prolonged labour-induced stress or hypoxia. No gross morphological abnormalities were seen in either the fetuses or the placentas (Manaugh & Novy, 1976). Fetal lung maturation was inhibited at birth after treatment of pregnant rabbits at 10 mg/kg bw per day intramuscularly for three days before delivery (Bustos *et al.*, 1978). Persistent fetal circulation syndrome was reported in neonatal rats in a study in which pregnant rats were treated with 2–4 mg/kg bw per day orally from gestation day 17 to delivery (Harker *et al.*, 1981). The appearance of medial hypertrophy and newly muscularized arterioles, combined with immature, thick saccular walls, produced a decreased surface for oxygen exchange and increased pulmonary vascular resistance.

Prolongation of the length of the oestrus cycle was reportedly induced by indomethacin

in guinea-pigs injected subcutaneously with 10 mg/kg bw twice daily for 12 days beginning on day 7 of the cycle or implanted with 33 mg per uterine horn, providing a slow-release dose of 0.2–0.6 mg/day. Oestrus cycles were lengthened by three days in the animals treated with 20 mg/kg bw per day, while cycles of up to 75 days were observed in the implanted animals. Two animals treated with 20 mg/kg bw per day died with gastrointestinal perforations after 11 days of treatment. The authors suggested that the drug blocked the formation of luteolysin in the uterus, prolonging the life of the corpus lutea (Horton & Poyser, 1973). No effect on the length of the oestrus cycle was noted in female rats treated with 0.8–4 mg/kg bw per day orally from pro-oestrus for six cycles before mating (Yegnanarayan & Joglekar, 1978). The authors suggested that the doses used in the studies could not result in the high intrauterine concentrations required to inhibit corpus luteum regression.

7.2 Genetic and related effects

7.2.1 Humans

No data were available to the Working Group.

7.2.2 Experimental models

The genetic and related effects of indomethacin are listed in Table 6.

Indomethacin induced DNA damage in *Bacillus subtilis* (Kuboyama & Fujii, 1992). It did not induce mutation in *Escherichia coli* or *Drosophila melanogaster* (King *et al.*, 1979), but it induced mutation in *Salmonella typhimurium* tester strain TA100 in the presence of exogenous metabolic activation (Kuboyama & Fujii, 1992). It did not induce chromosomal aberrations or aneuploidy (Ishidate *et al.*, 1988) in hamster cells *in vitro*. Indomethacin induced chromosomal aberrations and sperm abnormalities in mice *in vivo* (Shobha Devi & Polasa, 1987). Conflicting results were reported for micronucleus induction in mice (Shobha Devi & Polasa, 1987; King *et al.*, 1979). It did not induce sister chromatid exchange in human lymphocytes *in vivo* (Kullich & Klein, 1986).

Table 6. Genetic and related effects of indomethacin

End-point	Test code	Test system	Results[a]		Dose[b] (LED or HID)	Reference
			–	+		
D	BSD	*B. subtilis rec*, differential toxicity	+	0	100	Kuboyama & Fujii (1992)
G	SA5	*S. typhimurium* TA1535, reverse mutation	–	–	1790	King *et al.* (1979)
G	SA7	*S. typhimurium* TA 1537, reverse mutation	–	–	1790	King *et al.* (1979)
G	SA8	*S. typhimurium* TA 1538, reverse mutation	–	–	1790	King *et al.* (1979)
G	SA9	*S. typhimurium* TA 98, reverse mutation	–	–	1790	King *et al.* (1979)
G	SA9	*S. typhimurium* TA98, reverse mutation	–	–	27	Kuboyama & Fujii (1992)
G	SA0	*S. typhimurium* TA100, reverse mutation	–		1790	King *et al.* (1979)
G	SA0	*S. typhimurium* TA100, reverse mutation	–	+	27	Kuboyama & Fujii (1992)
G	ECK	*E. coli* K12, forward or reverse mutation	–	0	10 740	King *et al.* (1979)
G	DMX	*D. melanogaster*, sex-linked receessive recessive lethal mutation	–	0	895	King *et al.* (1979)
C	CIC	Chromosomal aberration, Chinese hamste r cells *in vitro*	–	0	250	Ishidate *et al.* (1988)
C	SLH	Sister chromatid exchange, human lymphocytes *in vitro*	–	0	1.1	Kullich & Klein (1986)
A	AIA	Aneuploidy, human cells *in vitro*	–	0	250	Ishidate *et al.* (1988)
C	CVA	Chromosomal aberation, mouse spermatocytes *in vivo*	+	0	12	Shobha Devi & Polasa (1987)
M	MVM	Micronucleus formation, mice *in vivo*	(+)	0	24	Shobha Devi & Polasa (1987)
M	MVM	Micronucleus formation, mice *in vivo*	+	0	179	King *et al.* (1979)
P	SPM	Sperm morphology, mice *in vivo*	+	0	12	Shobha Devi & Polasa (1987)

Definitions of the abbreviations and terms used are given in Appendix 1.

[a] In the absence (–) and presence (+) of an exogenous metabolic activation system; + positive, (+) weakly positive; – negative; 0, not determined

[b] Lowest effective dose (LED) or highest ineffective dose (HID) expressed as µg/ml for in-vitro studies and as mg/kg body weight per day for in-vivo studies

8. Summary of Data

8.1 Chemistry, occurrence and human exposure

Indomethacin has been used for over 30 years as an analgesic and anti-inflammatory agent. It is used in the treatment of a variety of musculoskeletal conditions, notably rheumatoid and osteoarthritis. It is conventionally prescribed at doses of 25–100 mg three or four times daily.

8.2 Metabolism and kinetic properties

Conventional oral administration results in a high level of bioavailability, although foods taken concomitantly may delay and/or reduce absorption. Indomethacin is strongly bound to serum albumin. After its distribution, indomethacin undergoes glucuronide conjugation, *O*-demethylation and *N*-deacylation. Less than 10% of an oral dose is recovered as the unchanged parent compound in urine; indomethacin is also eliminated in bile and undergoes extensive enterohepatic recirculation.

8.3 Cancer-preventive effects

8.3.1 Humans

No studies have been reported that specifically address protection by indomethacin against cancer. Case reports of the use of indomethacin in patients with familial adenomatous polyposis showed regression of polyps in about one-half of the patients.

8.3.2 Experimental animals

The chemopreventive efficacy of indomethacin was assessed in mouse, rat and hamster models. In seven studies in mice, the effects of indomethacin were studied on carcinogenesis in the oesophagus, urinary bladder, cervix and skin. Indomethacin was effective in all of the studies, but appeared to be less effective during late stages of carcinogenesis and in the skin.

The cancer-preventive efficacy of indomethacin was investigated in 20 studies in rats in models of cancers of the oesophagus, colon, urinary bladder, tongue, liver, mammary gland, nervous system and kidney. It was effective in all 12 studies in which the colon was the target organ, but inconsistent results were obtained in models of mammary gland carcinogenesis: it was effective in three studies and ineffective in another three. In single studies in rats, indomethacin had chemopreventive effects in models of cancers of the urinary bladder, liver and tongue.

The efficacy of indomethacin in inhibiting oral cavity tumorigenesis in hamsters remains controversial. No conclusive reduction was seen in pancreatic tumorigenesis in hamsters.

Indomethacin had antimutagenic activity in a variety of test systems.

8.3.3 Mechanism of action

While the anti-inflammatory action of indomethacin is directly linked to its ability to inhibit the cyclooxygenases, the precise molecular mechanism(s) whereby indomethacin and other non-steroidal anti-inflammatory drugs exert their chemopreventive effects remain unclear. Mechanisms secondary to prostaglandin reduction, such as enhanced immune surveillance and stimulation of T-cell proliferation, may be involved. Furthermore, indomethacin may exert a protective effect against cancer by influencing receptors or affecting enzymes other than cyclooxygenases.

8.4 Other beneficial effects

One small clinical trial in which many patients could not be followed up suggests that indomethacin slows the progression of Alzheimer's disease.

8.5 Carcinogenicity

8.5.1 Humans

No data were available to the Working Group.

8.5.2 Experimental animals

Indomethacin given to rats in the drinking-water for life induced hyperplasia of the urinary tract. In a further study in rats, the incidence of urinary bladder tumours induced by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide was enhanced by concomitant treatment with indomethacin.

8.6 Toxic effects

8.6.1 Humans

Indomethacin has a wide variety of adverse effects, the most clinically important of which

are ulcers and bleeding in the upper gastrointestinal tract. Indomethacin increases the risk for these complications in a dose-dependent manner. Indomethacin decreases renal function and increases blood pressure, sometimes causes rash and headache and rarely results in hepatotoxicity and aseptic meningitis.

8.6.2 Experimental animals

Many of the toxic effects of indomethacin in experimental animals may be due to inhibition of prostaglandin synthesis. As in humans, the primary site of acute toxicity is the gastrointestinal tract, although adverse effects have also been reported in kidney, liver, heart and bone.

Toxic effects on male fertility, female mating behaviour, ovulation and gestation, teratogenicity and effects on fetal circulatory development have been reported in isolated studies in experimental animals.

In one study in mice treated *in vivo*, indomethacin induced chromosomal aberrations in spermatocytes and micronuclei in bone marrow.

9. Recommendations for Research

Toxicity would be a major drawback to developing indomethacin as a chemopreventive agent in humans. The preventive efficacy of indomethacin has been assessed extensively in animal models, and no further experimental investigation is needed, unless specific mechanisms of action are addressed.

10. Evaluation[1]

10.1 Cancer-preventive activity

10.1.1 Humans

There is *inadequate evidence* that indomethacin has cancer-preventive activity in humans.

10.1.2 Experimental models

There is *sufficient evidence* that indomethacin has cancer-preventive activity in experimental animals. This evaluation is based on models of cancers of the colon and oesophagus.

10.2 Overall evaluation

Epidemiological studies in humans provide *inadequate evidence* for the cancer-preventive activity of indomethacin, although some data suggest that it prevents the progression of adenomatous polyps in patients with familial adenomatous polyposis. In experimental animals, there is *sufficient evidence* that indomethacin prevents colon cancer. The adverse effects of indomethacin include dose-dependent bleeding and ulceration in the upper gastrointestinal tract and hepatic and renal toxicity.

Despite the availability of extensive data from studies of experimental models, indomethacin cannot be regarded as a chemopreventive agent in humans, because of inadequate epidemiological data.

11. References

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[1] For definitions of the italicized terms, see the Preamble, pp. 12–13

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