Aspirin[1]

1. Chemical and Physical Characteristics

1.1 Name

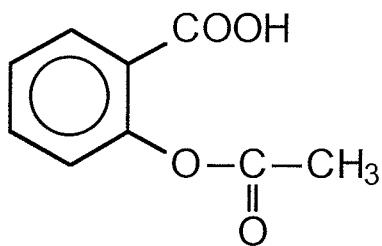*Chemical Abstracts Services Registry Number*
50-78-2

Chemical Abstracts Primary Name
Salicylic acid acetate

IUPAC Systematic Name
Benzoic acid, 2-acetyloxy

Synonyms
2-(Acetyloxy)benzoic acid; 2-acetoxybenzoic acid; o-acetylsalicylic acid; acidum acetylsalicylicum; acetylsalicylic acid

1.2 Structural and molecular formulae and relative molecular mass

$C_9H_8O_4$ Relative molecular mass: 180.15

1.3 Physical and chemical properties

The data presented are taken from Budavari (1989) and Reynolds (1993), unless otherwise specified.

Description
Colourless or white needle-like crystals or white crystalline powder; odourless or almost odourless

Melting-point
135 °C

Solubility
One gram dissolves in 300 ml water at 25 °C, in 100 ml water at 37 °C, in 5 ml ethanol, 17 ml chloroform, 10–15 ml ethyl ether; less soluble in anhydrous ether

Spectroscopy
Ultraviolet, infrared, nuclear magnetic resonance and mass spectral data have been reported.

Stability
Stable in dry air but gradually hydrolyses in contact with moisture to acetic and salicylic acids. Decomposes in boiling water. Also unstable in solutions of alkali hydroxides and carbonates (pK_a 3.49 at 25 °C)

1.4 Technical products

Trade names
Aspirin is marketed throughout the world under many trade names, which include the following: AAS, Acentérine, Acesal, Acetard, Aceticyl, Acetilum Acidulatum, Acetophen, Acetosal, Acetosalic Acid, Acetyl, Acetylin, Acetylo, Acetylsal, Actispirine, Acylpyrin, Adiro, Albyl, Albyl-Selters, Angettes, Apernyl, Arthrisin, Artria, A.S.A., Asadrine, Asaferm, Asalite, Asatard, Aspalox, Aspegic, Aspergum, Aspinfantil, Aspirin, Aspirina, Aspirinetta, Aspirisucre, Aspisol, Aspro, Asrivo, ASS, Asteric, Astrix, Bamycor, Bamyl, Bamyl S, Bebesan, Bonakiddi, Bufferin, Calmantina, Calmo Yer Analgesico, Caprin, Cardiprin, Cartia, Casprium Retard, Catalgine, Cemirit, Chefarine-N, Claradin, Claragine, Codalgina Retard, Colfarit, Contradol, Contrheuma, Cosprin, Delgesic, Dispril, Disprin, Dolean pH 8, Doleron, Dolomega, Domupirina, Dreimal, Dulcipirina, Duramax, Easprin, ECM, Ecotrin, Empirin, Encaprin, Endydol, Enterosarin, Enterosarine, Entrophen, Extra Strength Tri-Buffered Bufferin, Flectadol, Gepan, Globentyl, Godamed, Halgon, Helicon, Helver Sal, Idotyl,

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[1] Aspirin® is the Bayer trade mark for acetylsalicylic acid in more than 70 countries.

Istopirine, Ivépirine, Juvéprine, Kalcatyl, Kilios, Kynosina, Lafena, Levius, Licyl, Longasa, Magnecyl, Magnyl, Measurin, Mejoral Infantil, Melabon, Monobeltin 350, Neuronika, Novasen, Novid, Nu-Seals, Okal Infantil, Orravina, Platet, Premaspin, Primaspan, Primaspin, Protectin-OPT, Pyracyl, Rectosalyl, Resprin, Reumyl, Rhodine, Rhonal, Riane, Sal, Salacetin, Salcetogen, Saletin, Salicilina, Sanocapt, Santasal, Saspryl, Sinaspril, Solprin, Solpyron, Solusprin, Soparine, Spalt, SRA, Supasa, Superaspidin, Temagin ASS, Togal ASS, Trineral, Trombyl, Winsprin, Xaxa, ZORprin.

Aspirin is also marketed in many fixed combinations with other compounds, and especially with ascorbate, codeine and caffeine.

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

2.1 Occurrence

Aspirin is not known to occur as a natural product.

2.2 Production

Aspirin, the acetyl derivative of salicylic acid, is synthesized from the acid with acetic anhydride using sulfuric acid as catalyst (Roberts & Caserio, 1965). The basis of commercial production, which is approximately 40 000 t/year worldwide, was not known to the Working Group.

2.3 Use

Aspirin and its salicylate metabolite have analgesic, anti-inflammatory and antipyretic properties. Aspirin was first marketed in 1899 (Vane et al., 1990). It is used for the relief of mild-to-moderate pain such as headache, dysmenorrhoea, myalgia and dental pain. It is also used in acute and chronic inflammatory disorders such as rheumatoid arthritis, juvenile rheumatoid arthritis and osteoarthritis. Aspirin inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis.

Aspirin is usually taken by mouth. Various dosage forms are available, including plain uncoated, buffered, dispersible, enteric-coated and modified-release tablets. Aspirin may be administered rectally or intravenously as a complex with lycin.

When aspirin is used as an analgesic and antipyretic, the conventional dose is 0.3–0.9 g, which may be repeated every 4–6 h according to clinical needs, up to a maximum of 4 g daily (Reynolds & Prasad, 1982). Generally, 4–8 g daily in divided doses are used for acute musculoskeletal and joint disorders such as rheumatoid arthritis and osteoarthritis.

Use of aspirin in children has been dramatically decreased after reports of a relationship between its use and the development of Reye syndrome, a very rare but possibly fatal combination of hepatic insufficiency and encephalopathy. One of the few indications in which aspirin therapy is still considered for children is juvenile rheumatoid arthritis. Suggested doses for this condition are 80 and 100 mg/kg bw daily in five or six divided doses, although up to 130 mg/kg daily are employed for some children.

Aspirin is used for the secondary prevention of myocardial infarct and stroke in patients with a history of such disorders. Large clinical studies have shown that doses of more than 300–325 mg daily are unnecessary, and some authorities recommend doses of about 75–100 mg daily.

2.4 Analysis

Accepted standard procedures for the assay of aspirin are given in the national pharmacopoeias of Argentina, Australia, Brazil, China, the Czech Republic, Egypt, France, Germany, Hungary, India, Italy, Japan, Mexico, the Netherlands, Poland, Portugal, Romania, the Russian Federation, Spain, Switzerland, Turkey, the United Kingdom and the United States, and in the European, Nordic and international pharmacopoeias.

Aspirin as its metabolite salicylic acid can be analysed in urine, plasma and saliva by colorimetry, thin-layer chromatography and high-performance liquid chromatography (Legaz et al., 1992). In pharmaceutical preparations, it can be determined by high-performance liquid chromatography (Menouer et al., 1982) gas-liquid chromatography (Galante et al., 1981) and differential spectrophotometric analysis (Amer et al., 1978) using proton magnetic resonance spectrometry (Vinson & Kozak, 1978; Al-Badr & Ibrahim, 1981).

2.5 Human exposure

Use of aspirin in the general population has been estimated from studies on use of non-steroidal anti-inflammatory drugs (NSAIDs) and cancer risk with data on the consumption of aspirin by study cohorts and by community control groups.

In a study by Kune et al. (1988) in Australia, of 727 community controls (average age, 65 years), 67 of the 398 men (17%) and 80 of the 329 women (24%) reported using aspirin. No data were provided on the frequency or duration of use. Paginini-Hill et al. (1989) reported aspirin use in a California retirement community (Table 1). The average age at the time of responding to the questionnaire was 73 years. Schreinemachers and Everson (1994) found that 59% of 12 668 subjects (age range, 25–74 years) had reported aspirin use within 30 days of interview in the US National Health and Examination Study.

In a study by Giovannucci et al. (1994), 47 900 US male health professionals aged 40–75 years were surveyed by questionnaire. Regular aspirin use was defined as more than twice weekly. A total of 33 806 (70%) did not use aspirin (average age, 56 years) and 14 094 (30%) did (average age, 59 years). The reasons for taking aspirin were surveyed in 185 men, who reported one or more of the following: cardiovascular disease, 25%; decrease in risk for cardiovascular disease, 58%; joint or musculoskeletal pain, 33%; headache, 25% and other reasons, 7.0%.

Giovannucci et al. (1995a) also questioned 121 701 female participants in the Nurses' Health Study (age range, 30–55 years) on four occasions over eight years; 15% reported regular aspirin use (defined as two or more tablets

Table 1. Aspirin use in a California retirement community

Aspirin use	Men (5051)		Women (8818)	
	No.	%	No.	%
None	3490	69	6021	68
Less than daily	685	14	1417	16
Daily	876	17	1380	16
Total use	1561	31	2797	32

From Paganini-Hill et al. (1989)

per week) in each questionnaire and 15% reported no aspirin use in any of the periods.

Greenberg et al. (1993) questioned 793 patients involved in a clinical trial of nutrient supplements on two occasions and categorized them as using aspirin not at all, intermittently or consistently, depending on whether they listed aspirin as one of their medications on zero, one or two questionnaires, respectively. The results are shown by age in Table 2.

Aspirin use increased by 4% among men with coronary heart disease or at high risk for coronary heart disease following publication of the results of trials on the cardiovascular prevention effects of aspirin. Nearly 50% of participants who reported a history of myocardial infarct, however, apparently did not take aspirin regularly (Shahar et al., 1996).

Although the population-based data on aspirin use are limited, particularly with respect to dose, two general observations are warranted:
• Aspirin consumption is high, in keeping with its ready availability, low cost and value in a wide range of conditions.
• Because the incidence of musculoskeletal and cardiovascular disease increases with age, aspirin use rises concomitantly.

Table 2. Aspirin use in a clinical trial of nutrient supplements, by age

Age (years)	No use		Intermittent use		Consistent use	
	No.	%	No.	%	No.	%
< 50	54	82	8	12	4	6
50–59	170	79	26	12	19	9
60–69	277	72	51	13	59	15
≥ 70	92	74	13	10	20	16

From Greenberg et al. (1993)

## 3. Metabolism, Kinetics and Genetic Variation

3.1 Human studies

3.1.1 Metabolism

The metabolism of acetylsalicylic acid (aspirin) is summarized in Figure 1.

Aspirin is rapidly hydrolysed to salicylic acid (2-hydroxybenzoic acid) in the intestinal wall (Spenney, 1978), liver (Ali & Kaur, 1983) and erythrocytes (Costello *et al.*, 1984). Salicylic acid is further metabolized in the liver and kidneys into its glycine conjugate salicyluric acid and its glucuronic acid conjugates, salicyl phenolic glucuronide and salicyl acyl glucuronide. Ring hydroxylation products of salicylic acid are also formed, albeit in much smaller amounts; these include gentisic acid

Figure 1. Main metabolic pathways of aspirin
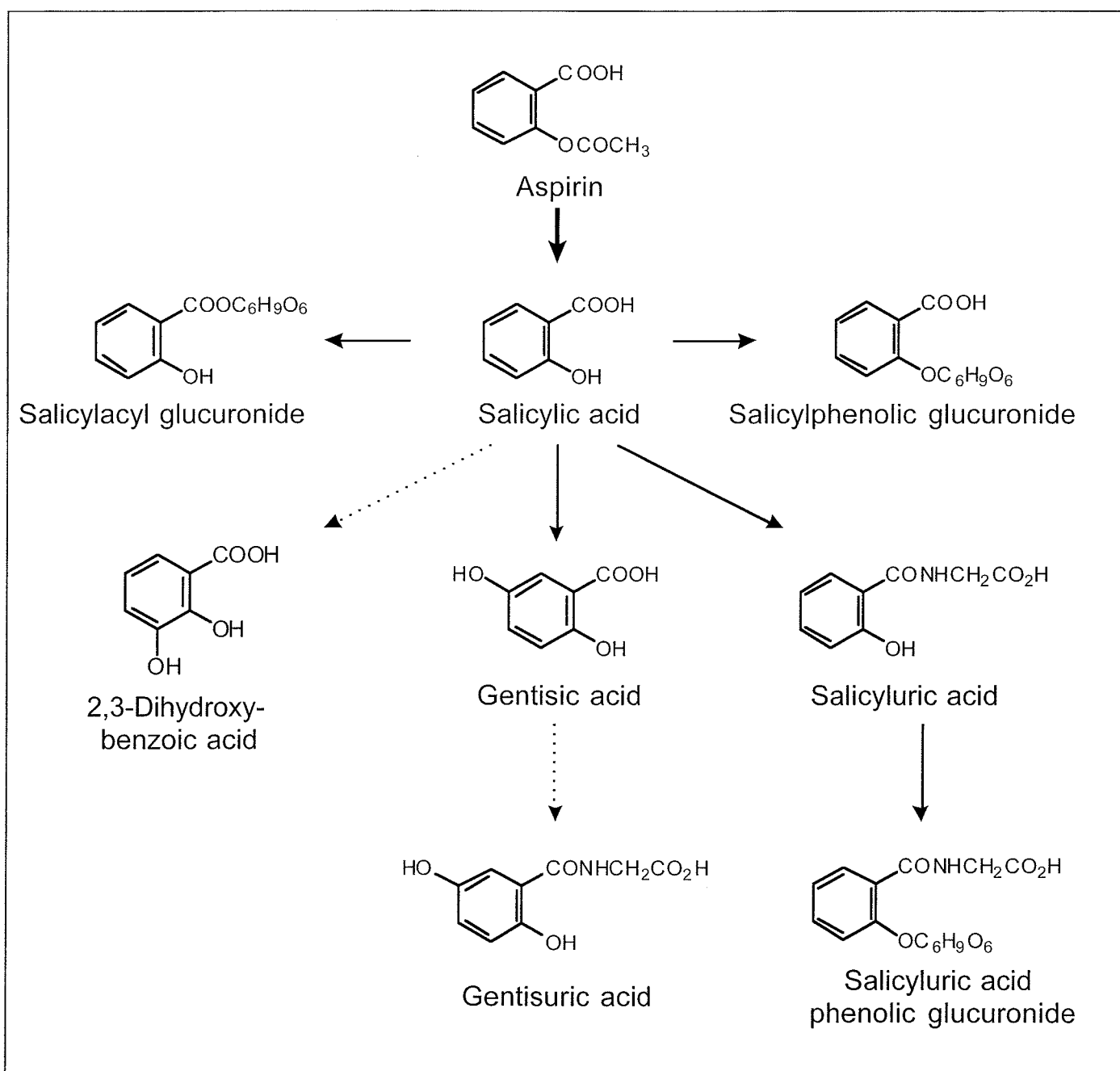
From Patel *et al.* (1990). Dotted lines indicate minor pathways

(2,5-hydroxybenzoic acid) and 2,3-dihydroxy-benzoic acid. Additional metabolites, also formed in minor amounts, include gentisuric acid and salicyluric acid phenolic glucuronide (Ali & Kaur, 1983; Costello et al., 1984; Hutt et al., 1986; Grooteveld & Halliwell, 1988). Small amounts of salicylic acid remain unchanged and are excreted.

The first step in the metabolism of aspirin, its hydrolysis to salicylic acid, is catalysed by a family of enzymes that are all serine esterases (Inoue et al., 1979a,b, 1980; White & Hope, 1984). The esterases in the intestinal wall and liver perform most of the hydrolysis of aspirin (Rowland et al., 1972); however, the erythro-cyte esterases also contribute significantly to this process (Costello et al., 1984). Thus, the haematocrit can be an important determinant of the half-life of aspirin. The rate of hydrolysis of aspirin to salicylic acid can vary considerably with age (Windorfer et al., 1974), sex (Gupta & Gupta, 1977) and concomitant disease (Needs & Brooks, 1985).

After conversion of aspirin to salicylic acid, several fairly well characterized metabolic and elimination pathways affect the ultimate dispo-sition of aspirin (Needs & Brooks, 1985). The major pathways for the metabolism of salicylic acid are conjugation with glycine to form sali-cyluric acid and conjugation with glucuronic acid to form salicyl phenolic glucuronide. Both follow Michaelis–Menten kinetics and are sat-urable. In contrast, the remaining pathways are minor and follow first-order kinetics (Levy & Tsuchiya, 1972).

Two properties of these pathways are critical to the metabolism and elimination of aspirin: the saturability of the 'primary' pathways and the self-induction of the metabolism. These phenomena are summarized below.

Salicylic acid, formed after a low, 325-mg, dose of aspirin, is metabolized by the pathways leading to salicyluric acid and salicyl phenolic glucuronide. As the amount of aspirin ingested is increased, these two pathways become satu-rated and the minor pathways are used to a greater extent, leading to increased amounts of their products. Thus, when increasing doses of aspirin saturate the two main metabolic path-ways, the serum salicylate levels increase, the

amounts of salicyluric acid and salicyl phenolic glucuronide do not increase further and larger amounts of gentisic acid, salicyl acyl glu-curonide and other compounds are produced. As would be expected, the percentage of each metabolic product in the total pool of aspirin metabolites varies with dose (Patel et al., 1990).

These findings, consistent with those of oth-ers (Levy et al., 1969; Levy, 1979; Bochner et al., 1981; Hutt et al., 1986), demonstrate clearly the dose-dependent saturability of the primary pathways and the contribution of salicylic acid to the total elimination of salicylate after toxic doses, when the other major metabolic path-ways are saturated.

Auto-induction of aspirin metabolism was suggested by observations in patients treated with aspirin for long periods. In these patients, the serum salicylate levels decreased with the length of treatment (Furst et al., 1977; Muller et al., 1977; Rumble et al., 1980). The amount of salicyluric acid in urine was increased, indicat-ing self-induction of aspirin metabolism.

3.1.2 Pharmacokinetics
The pharmacokinetic properties of aspirin have been reviewed by Needs and Brooks (1985).

(a) Absorption
After oral administration, aspirin is rapidly and extensively absorbed from the stomach but mostly from the upper small intestine (Flower et al., 1985). Absorption occurs rapidly, by pas-sive diffusion of the non-ionized lipophilic molecules; for example, the absorption half-life of aqueous aspirin ranges from 4.5 to 16 min (Rowland et al., 1972). In this study, 68% of the dose reached the systemic circulation unhy-drolysed.

Many factors affect the rate of absorption of aspirin, including the pH at the mucosal sur-faces, the rate of gastric emptying, conditions that affect intestinal transit time and, if tablets are given, their dissolution rate. Of these fac-tors, the most important is the last. Liquid preparations are absorbed most rapidly: Serum levels peak 15–20 min after intake of liquid for-mulations, 2–4 h after ingestion of regular tablets, and 4–6 h or more after intake of enteric–coated aspirin. The latter, which resists

dissolution in the acidic stomach, dissolves mainly after passing into the alkaline environment of the small intestine (Liberman & Wood, 1964; Briggs *et al.,* 1977; Ross-Lee *et al.,* 1982).

It is of interest that, in patients on long-term treatment, the bioavailability of enteric-coated aspirin is similar to that of regular preparations, as the two formulations provide comparable steady-state concentrations of salicylate (Orozco-Alcala & Baum, 1979). When absorption is delayed by prolonged gastric emptying, metaclopramide can accelerate it (Ross-Lee *et al.,* 1983).

The effect of pH is of particular interest (reviewed by Gugler & Allgayer, 1990; Brouwers & De Smet, 1994). Aspirin, with a pK_a of 3.5, is a weak acid and is 99% non-ionized at pH 1; it can therefore diffuse through lipid membranes. If the pH of the stomach is increased, more aspirin is ionized, and this decreases its rate of absorption (Flower *et al.,* 1985). In the case of tablets, however, a rise in pH increases the solubility and thus tablet dissolution; the overall effect is to enhance absorption. High doses of antacids increase urinary pH, thus increasing urinary excretion of salicylic acid, leading to decreased plasma levels (Clissold, 1986).

In patients prescribed long-term aspirin use, the rate of absorption is not very important: accumulation is controlled entirely by oral bioavailability and the rate of plasma clearance (Verbeeck, 1990). A high absorption rate reduces the lag time between drug intake and the pharmacological effect, such as pain relief. This type of effect is useful in the treatment of acute conditions such as dysmenorrhoea and headaches (Chan, 1983; Diamond & Freitag, 1989).

(b) Distribution

After absorption, aspirin is distributed throughout the body tissues and fluids, mainly by pH-dependent passive processes, and binds to plasma proteins, especially albumin. The apparent volume of distribution of salicylate ranges from 9.6 to 13 litres in adults (Graham *et al.,* 1977) and children (Wilson *et al.,* 1982).

Aspirin has unusual accumulation characteristics. The plateau level of salicylate in the body attained by repetitive administration of fixed doses of aspirin at constant intervals increases more than proportionally with increasing doses. The time required to attain the plateau also increases with dose. It was observed clinically that a 50% increase in the daily dose of aspirin produced about a 300% rise in the concentration of salicylate in the serum (Paulus *et al.,* 1971). This rise was attributed to the fact that the saturable processes (formation of salicyluric acid and salicyl phenolic glucoronide) contribute less to the elimination of salicylate from the body as the amount of salicylate in the body increases. These observations account for the pronounced effects that relatively small changes in the maintenance dosage of aspirin have on salicylate concentrations in body fluids and the pharmacological effects of aspirin.

A compartmental model has been used to evaluate the absorption, metabolism and excretion of aspirin and salicylic acid given in low (30 and 100 mg) or moderate (400 mg) doses (Dubovska *et al.,* 1995). The model confirmed the linearity of the kinetics of aspirin, showed that the apparent volume of distribution and clearance of aspirin are independent of dose and showed that the metabolic kinetics of salicylic acid at 400 mg are dose-dependent.

Both aspirin and salicylic acid are partially bound to proteins, especially albumin; 80–95% of salicylic acid is bound to plasma albumin (Murray & Brater, 1993). The binding of salicylic acid is reversible, but aspirin acetylates human serum albumin (Hawkins *et al.,* 1968; Pinckard *et al.,* 1968). Salicylic acid reaches: (i) synovial fluid, where its concentration is lower than that in plasma (Rabinowitz *et al.,* 1982); (ii) cerebrospinal fluid, where both aspirin and salicylic acid diffuse slowly because of the high degree of ionization at plasma pH (Flower *et al.,* 1985); (iii) saliva, where its concentration is proportional to that in plasma (Roberts *et al.,* 1978) and (iv) breast milk (Findlay *et al.,* 1981).

Salicylates administered to the mother are transferred readily to the fetus (Schoenfeld *et al.,* 1992). As aspirin has a short half-life, only a small amount of unmetabolized compound reaches the fetus. The fetus binds less salicylates in plasma than adults and has reduced

metabolic activity, in particular glucuronidation, and less effective elimination. Therefore, fetuses and newborns whose mothers took salicylates before delivery may have plasma concentrations up to four times higher than those of their mothers.

(c) Elimination

After oral intake, the plasma levels of aspirin rise sharply, reaching a maximum at 15–20 min. They then decline rapidly, and only small amounts remain after 2 h (Rowland et al., 1972). Although the half-life in the declining phase is short, it is always longer than the half-life of aspirin administered intravenously, due to continued absorption of aspirin during the declining phase. The salicylic acid levels rise rapidly and eventually exceed those of aspirin, because of slower elimination rather than differences in distribution (Rowland et al., 1967).

Hydrolysis of aspirin to salicylic acid in the plasma is rapid, with a half-life of 15–20 min (Rowland et al., 1972). Salicylic acid is removed from the body by parallel, competing pathways of renal elimination and formation of metabolites. Renal excretion of salicylic acid is saturable (Dubovska et al., 1995), occurs by first-order kinetics and is extremely sensitive to urinary pH, urinary organic acids and urinary flow rate.

No differences in pharmacokinetics have been demonstrated between old and young adults (Roberts et al., 1983; Silagy, 1993). Diseases in which the serum albumin concentration is altered are characterized by changes in the free salicylate fraction. For example, hypoalbuminaemic cirrhotic patients had increased unbound salicylic acid concentrations, although the kinetics of aspirin and salicyclic acid were not altered (Roberts et al., 1983). Zapadnyuk et al. (1987) reported, however, that the pharmacokinetics of aspirin were not the same in people aged 20–30, 60–74 and 75–89 years after a single oral administration of 14 mg/kg body weight (bw). The constants of absorption and elimination and the total clearance value decreased with age; and the half-life declined from 4 to 12 h across the age range. In the older patients, the area under the concentration–time curve increased.

The pharmacokinetics of a low dose of aspirin (60 mg/day) were studied during the second and third trimesters in pregnant women at high risk for placental insufficiency (Asymbekova et al., 1995). In the 16 women with uncomplicated pregnancies, the changes in the kinetics of aspirin were a lower concentration–time index, higher total clearance and a larger distribution volume. Similar changes were found in the four women with advanced placental insufficiency.

(d) Drug interactions

The pharmacokinetic interactions of aspirin and various salicylates have been reviewed extensively (Miners, 1989; Verbeeck, 1990). Metabolic drug interactions involving aspirin are theoretically possible, but no studies have shown conclusively that hydrolysis of aspirin is altered by co-administered drugs. A number of treatments, however, affect the rate or extent of absorption of aspirin, including activated charcoal, antacids, cholestyramine and metoclopramide. Caffeine and metoprolol have been reported to increase the peak salicylic acid concentration after administration of aspirin, and co-administration of dipyridamole and aspirin results in higher plasma aspirin concentrations. The mechanism(s) responsible for the latter observation remains unknown.

Many of the drug interactions involve displacement of the co-administered drug from plasma protein, as, for example, in the case of interactions with diclofenac, flurbiprofen, ibuprofen, isoxicam, ketoprofen, naproxen, phenytoin and tolmetin. After displacement of these agents, the clearance of total drug increases and, consequently, the plasma concentration of total drug decreases. Although generally not measured, the unbound concentration of the interacting drug should not be markedly altered. Salicylic acid also increases the total plasma clearance of fenoprofen, but, unlike the interactions with other propionic acid non-steroidal compounds, plasma protein binding displacement does not appear to be involved.

There is no firm evidence that salicylic acid induces the metabolism of co-administered

drugs; however, it can inhibit their metabolism. Such an effect has been reported for salicyl-amide, valproic acid and zomepirac. Certain co-administered drugs may alter the metabolism of salicylic acid; its metabolism is inhibited after treatment with benzoic acid, salicylamide, zomepirac and possibly cimetidine. Salicylic acid elimination is enhanced by oral contraceptives and by corticosteroids.

3.2 Experimental models

The pharmacokinetics of aspirin have been studied in many species, including rats and dogs (Sechserova et al., 1979; Aonuma et al., 1982; Rabinowitz et al., 1982; Laznicek & Laznickova, 1994). The fate of aspirin has been followed by using radiolabelled salicylate or by simply measuring its plasma concentrations or those of its metabolites (Iwamoto et al., 1982; Hatori et al., 1984).

After intravenous or oral administration of aspirin at 10 mg/kg bw to male Wistar rats, 88 and 86% of the dose, respectively, was excreted in urine, mostly as salicylic acid and its conjugated forms. This finding suggests that the gastrointestinal absorption of aspirin in rats is essentially complete. Orally administered aspirin is subject to first-pass metabolism in both the gut and liver of rats (Iwamoto et al., 1982).

The kinetics of sodium salicylate are dose-dependent, and its plasma concentration declines by a first-order process (Yue & Varma, 1982). The metabolism of orally administered ^{14}C-aspirin in rats over a 10-fold dose range (10–100 mg/kg bw) resulted in excretion of 81–91% of the dose in urine during the first 24 h; salicylic acid was the major urinary metabolite (43–51%) (Patel et al., 1990). The excretion of salicyluric acid decreased with increasing dose, whereas that of gentisic acid and salicyl phenolic and acyl glucuronides increased. The profile of aspirin metabolites was qualitatively similar in humans and rats, but there were quantitative differences. A limited capacity to form salicyluric acid was observed in both species. In rats, the dependence on this pathway was low and was compensated by increased use of other routes. In contrast, in humans, the dependence on salicy-

luric acid formation was high and, in cases of overdose, compensation by other routes was incomplete.

In another study in rats, the concentration in major tissues and organs of aspirin labelled with ^{14}C on both the acetyl and carboxyl groups was determined by measuring the distribution of ^{14}C tracers in tissue sections (Hatori et al., 1984). During the first 10–30 min after oral administration, the degradation to salicylate was 38% in the stomach wall, 64% in the liver and 86% in the lung.

The age-dependence of aspirin metabolism was demonstrated in a study in calves (Sechserova et al., 1979). The older the animals, the lower were the total salicylate and salicylic acid levels and the higher the salicyluric acid level.

Two serine esterases involved in the hydrolysis of aspirin to salicylic acid have been purified to homogeneity from rat liver (Kim et al., 1990). Both have a low K_m for aspirin and a wide substrate spectrum. At present, these enzymes are categorized as arylesterases (EC 3.1.12) and carboxyesterases (EC 3.1.1.1).

3.3 Genetic variation

No data were available to the Working Group.

4. Cancer-preventive Effects

4.1 Human studies

4.1.1 Studies of colorectal cancer

(a) Methodological considerations

Most of the data on aspirin and colorectal cancer or adenomas come from epidemiological (observational) studies of sporadic disease in the general population. It was not possible in these studies to completely separate use of aspirin from that of other NSAIDs; however, as use of non-aspirin NSAIDs became widespread relatively recently, long-term use in studies of the general population involved predominately aspirin.

Intervention studies are generally considered to be more reliable than observational studies in the investigation of causal relationships. This may not be the case, however, if long-term use is a necessary parameter, and clinical trials may be limited by the fact that the randomized

treatment is too brief. In such instances, observational studies may be informative because individuals who report longer use can be identified and studied.

Several potential problems should be considered in interpreting the epidemiological studies: (i) bleeding and other aspirin-induced symptoms can prompt closer medical surveillance earlier than would otherwise occur; (ii) changes in aspirin use may be precipitated by the early symptoms of neoplasia; and (iii) other behaviour that affects colorectal cancer risk may be associated with use of aspirin. An overall perspective of these issues is presented on p. 63.

Throughout this section, the term 'relative risk' is used in a generic sense to refer to measures of association such as odds ratios, risk ratios and 'rate ratios'.

(b)   Cohort studies

Nine published cohort studies provide information on aspirin-containing drugs and the risk for colorectal cancer. The studies are discussed chronologically; those completed before 1991 address NSAIDs as a side issue, whereas later studies focus directly on aspirin and other NSAIDs. The relevant studies are summarized in Table 3.

Studies of cancer occurrence in patients with rheumatoid arthritis have been conducted in Finland and Sweden. Such studies were considered to be relevant by the Working Group because of the prolonged, intense use of aspirin and to a lesser extent NSAIDs by such patients; however, it was recognized that the effects of aspirin could not be separated from those of other drugs used to treat rheumatoid arthritis or from those of the disease itself.

Isomäki et al. (1978) identified 46 101 patients (11 483 men, 34 618 women) in Finland who were reimbursed for treatment of rheumatoid arthritis by the national health insurance between 1967 and 1973. Using computer linkage with the national cancer registry for that period, the authors found approximately the same numbers of newly diagnosed cases as expected among the arthritis patients for cancers of the oesophagus (males: 5 observed, 7.5 expected; females: 21/20), and colon (11/9.9 and 33/39, respectively) and for

cancers of the stomach (51/54) and rectum (7/11) in males. Women had fewer than expected cancers of the stomach (80/100) and rectum (20/35); the reductions were statistically significant ($p < 0.05$).

Laakso et al. (1986) conducted a much smaller study of 500 men and 500 women treated for rheumatoid arthritis at a National Rheumatism Foundation hospital in Finland between 1970 and 1980. The cohort largely overlapped with that of Isomäki et al. (1978) and is omitted from Table 3. The numbers of deaths from cancer were: one from oesophageal cancer (2 expected), three from stomach cancer (11 expected), none from colon cancer (3 expected) and one from rectal cancer (none expected). None of these differences was significant.

A larger study in Sweden by Gridley et al. (1993) comprised 11 683 men and women with a diagnosis of rheumatoid arthritis recorded in a population-based registry between 1965 and 1983. These patients were followed from the date of discharge from hospital through 1984 in the national cancer and mortality registries. For men and women combined, the numbers of cases observed and expected, the standardized incidence ratio (SIR) and the 95% confidence intervals (CIs) for cancers of the digestive tract were as follows: oesophagus, 11/8.3, SIR=1.32, 0.7–2.4; stomach, 39/62, SIR=0.63, 0.5–0.9; colon, 44/70, SIR=0.63, 0.5–0.9; rectum, 28/39, SIR=0.72, 0.5–1.1.

[The Working Group noted the potential for confounding by underlying disease in these studies.]

Stemmermann et al. (1989) wrote a brief description of the Japan–Hawaii Cancer Study conducted in 1971–75 on 137 Japanese men residing in Hawaii who reported use of aspirin, an aspirin and caffeine combination, or dextropropoxyphene for at least one week during the previous month. Among analgesic users, three colorectal cancers were observed, with 4.3 expected on the basis of the incidence among 652 non-users in the study.

Paganini-Hill et al. (1989) identified newly diagnosed cases of various chronic diseases from hospital records for 13 987 residents of a California retirement community between

Table 3. Cohort studies of use of non-steroidal anti-inflammatory drugs and the risk for colorectal cancer in the general population

Reference	Population	Study size	End-point	Drug	Frequency	Results (RR)[a]	Comments
Isomäki et al. (1978)	Rheumatoid arthritis, Finland; 34 618 women and 11 483 men, 1967–73	**Women** 33 cases 20 cases **Men** 11 cases 7 cases	Colon cancer Rectal cancer Colon cancer Rectal cancer (incidence)	Therapy for arthritis	Heavy use	0.84 (NS) 0.58 ($p < 0.05$) 1.1 (NS) 0.64 (NS)	
Gridley et al. (1993)	Rheumatoid arthritis, Sweden: 7933 women and 3750 men, 1965–84	44 cases 28 cases	Colon cancer Rectal cancer (incidence)	Therapy for arthritis	Heavy use	0.63 (0.5–0.9) 0.72 (0.5–1.1)	Risk for stomach cancer also reduced
Paganini-Hill et al. (1989, 1991); Paganini-Hill (1995)	US retirement community; 13 987 elderly men, and women, 1981–87	181 cases	Colon cancer (incidence)	Aspirin	Daily	1.5 (1.1–2.2)	Median age, 73 No data on aspirin after baseline
Thun et al. (1991, 1992, 1993)	American Cancer Society, 662 424 US adults, 1982–88	950 deaths 138 deaths	Colon cancer (fatal) Rectal cancer (fatal)	Aspirin Aspirin	≥16 times/ month	0.58 (0.45–0.74) 0.66 (0.37–1.2)	Multivariate estimates No data on aspirin after baseline Decreased risk mostly confined to users of ≥10 years
Schreinemachers & Everson (1994)	12 688 US adults, 1971–87	169 cases	Colorectal cancer (incidence)	Aspirin	Last 30 days	0.74 (0.49–1.1)	RR reduced under age 65
Giovannucci et al. (1994)	Harvard Health Professionals: 47 900 US men, 1986–91	251 cases	Colorectal cancer (incidence)	Aspirin	≥ 2 tablets/ week	0.68 (0.52–0.92)	All cancers and metastatic or fatal cancers
Giovannucci et al. (1995)	US Nurses Health Study, 89 446 women, 1984–92	297 cases	Colorectal cancer (incidence)	Aspirin	≥ 2 tablets /week ≥ 20 years	0.56 (0.36–0.90)	Risk decreased with duration but not with dose > 2–4 months

RR, relative risk; NS, not significant

[a] In parentheses, 95% confidence interval

1981 and 1987. The median age of the participants at the time of enrolment was 73 years. The study population consisted mostly of white, moderately affluent, well-educated people, about two-thirds of whom were women. People who reported on a mailed questionnaire in 1989 that they used aspirin at least daily had a 50% higher incidence of colon cancer than did those who used aspirin less than monthly (40 cases; RR, 1.5; 95% CI, 1.1–2.2). Two subsequent reports on the same study reported follow-up of the cohort through May 1991 (Paganini-Hill et al., 1991; Paganini-Hill, 1995). A statistically nonsignificant 38% higher incidence of colon cancer was found in men but not women. This study differs from those that show an inverse relation between aspirin use and the risk for colorectal cancer: the participants were older, no data were available on the duration of aspirin use, and the participants lived in a single retirement community. The finding that ischaemic heart disease was significantly more common in daily aspirin users than in non-users (men: RR, 1.9; 95% CI, 1.1–3.1; women: RR, 1.7; 95% CI, 1.1–2.7) raises the possibility that patients with coronary symptoms or risk factors for vascular disease may have begun taking aspirin shortly before enrolment in the study because of local medical practices. [The Working Group noted that changing local medical practices disproportionately affect small regional studies, increasing misclassification of long-term exposures.]

Thun et al. (1991, 1992, 1993) measured death rates from colonic and other cancers according to aspirin use at enrolment in the American Cancer Society study of 662 424 US adults, who were followed from 1982 to 1988. People who reported using aspirin or an aspirin–caffeine combination 16 or more times per month for at least one year had 40% lower death rates from colon cancer than those who reported no aspirin use (men: RR, 0.60; CI, 0.40–0.89; women: RR, 0.58, CI, 0.37–0.90). Adjustment in the analysis for obesity, dietary vegetable and fat consumption and physical activity did not attenuate the reduction in risk. The results were also not changed by exclusion from the analysis of people whose underlying disease might have affected both aspirin use

and colon cancer risk; the diseases included prevalent cancer, heart disease, stroke or reporting being 'sick' at the time of enrolment. Use of acetaminophen was not associated with a lower risk for colon cancer (Thun et al., 1991).

The trend of decreasing colorectal cancer risk with more frequent aspirin use was stronger among people who had used aspirin for ≥ 10 years than in those with a shorter duration of use. Specifically, the RR for fatal colon cancer among people who reported using aspirin 16 or more times per month for ≥ 10 years was 0.36 in comparison with people who reported no aspirin use, whereas the RR for the group who reported this level of use for 1–9 years was 0.71. For both men and women, aspirin use 16 or more times per month for at least one year reduced the risk for fatal colon cancer (RR, 0.58; CI, 0.45–0.74) and fatal rectal cancer (RR, 0.66, CI, 0.37–1.2) (Thun et al., 1993).

Because of its size, prospective design, dose–response trends and internal consistency, the American Cancer Society study gave support to the NSAID hypothesis. Its limitations are the use of a single, brief, self-administered questionnaire, the lack of data on dose (as opposed to frequency or duration) and on use of NSAIDs other than aspirin and the reliance on death from cancer rather than incidence to define the presence of disease.

Schreinemachers and Everson (1994) reported on 12 668 people aged 25–74 who provided information on aspirin use when enrolled in the First National Health and Nutrition Examination Survey between 1971 and 1987. Over the average follow-up of 12 years (through 1987), the incidence of colorectal cancer was 26% lower among participants who had used any aspirin in the 30 days before interview than among those who had used no aspirin (RR, 0.74; Cl, 0.49–1.1). The analyses controlled for obesity but not for physical activity or diet. The question on aspirin did not differentiate continuing use from brief or occasional use.

Giovannucci et al. (1994) assessed the incidence of colorectal adenoma and carcinoma among 47 900 men aged 40–75 in the Harvard Health Professionals study. Regular users of aspirin (two or more tablets per week in 1986) had a lower risk for colorectal cancer

at all stages (RR, 0.68; CI, 0.52–0.92) and at advanced (metastatic or fatal) stages (RR, 0.51; CI, 0.32–0.84) than non-users. Age, history of polyps or previous endoscopy, parental history of colorectal cancer, smoking, body mass, leisure time physical activity and intakes of red meat, vitamin E and alcohol were controlled for in the analyses. The inverse association became progressively stronger with more consistent use of aspirin, i.e. regular use reported on more than one questionnaire. Colorectal adenomas were less common in aspirin users than in non-users whether or not the patients had been found to have occult blood in the faeces, suggesting that bleeding and early diagnosis of polyps did not account for the lower cancer risk in aspirin users. The inverse association was strongest with metastatic or fatal colorectal cancer.

Giovannucci et al. (1995) reported the incidence of colorectal cancer in relation to the dose and duration of use of aspirin among 89 446 US female nurses from 1984 through 1992. Data on aspirin use were obtained by questionnaire in 1980, 1982, 1984 and 1988,

and deaths and newly diagnosed cases of colorectal cancer were ascertained over eight years. The duration of aspirin use correlated most strongly with the reduced risk for colorectal cancer. The RR for colorectal cancer decreased progressively with years of use (Figure 2; p for trend = 0.008). Nurses who reported taking two or more aspirin tablets weekly for 20 or more years had a significantly lower risk than non-users (RR, 0.56; CI, 0.36–0.90). Although the RR appeared to be decreasing after five years of regular use, the decrement did not become statistically significant until more prolonged use. Controlling for several dietary factors, physical activity, alcohol and smoking did not alter these results. [The Working Group noted that one strength of this study is the repeated measures of aspirin use, which allow more detailed analysis of dose and duration than is possible in other studies.]

(c)   Case–control studies

Six published case–control studies examined the risk for colorectal cancer in relation to use of aspirin-containing drugs (Table 4). A further two studies addressed the risk for colorectal

Figure 2. Age-adjusted relative risks for colorectal cancer and 95% confidence intervals according to the number of consecutive years of regular use of aspirin as compared with non-users of aspirin
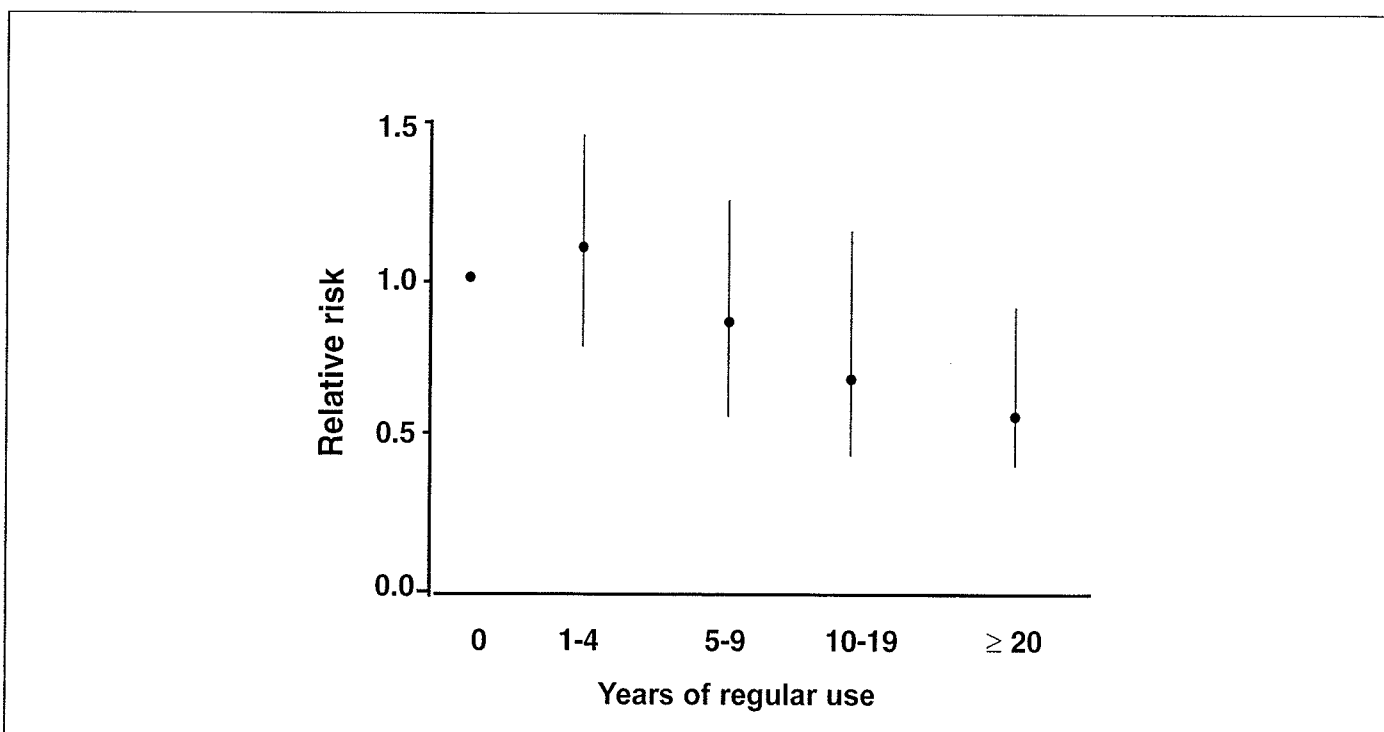
From Giovannucci et al. (1995). Regular aspirin use defined as consumption of two or more tablets per week

cancer in relation to medical conditions that served as a proxy for aspirin use.

Kune *et al.* (1988) assessed whether the risk for newly diagnosed colorectal cancer was associated with illnesses, operations and medications, using a population-based tumour registry in Melbourne, Australia. Personal interviews were conducted to determine whether 715 histologically confirmed cases and 727 randomly selected controls had used any of eight medications, two of which were aspirin-containing drugs or other NSAIDs. People who responded 'yes'; were asked to characterize their use as 'daily', 'weekly' or 'don't know'. Those who used aspirin [exact usage unspecified] had a 40% lower incidence of colorectal cancer than persons who reported no aspirin use (RR, 0.60; CI, 0.0.82). Persons who used other NSAIDs had a 23% lower incidence of colorectal cancer (RR, 0.77; CI, 0.60-1.0). Having chronic arthritis was also inversely associated with the risk for colorectal cancer (RR, 0.66, CI, 0.53–0.83). The association with aspirin use was similar in 392 cases of colon cancer and in 323 cases of rectal cancer, and the inverse association remained after adjustment in the analysis for diet and vitamin supplementation. Information on how long people in the study had used NSAIDs was collected but not presented. [The Working Group noted that controls were not selected contemporaneously and that the analyses of use of non-aspirin NSAIDs did not adjust for simultaneous use of aspirin.]

Rosenberg *et al.* (1991) examined the relationship between use of NSAIDs and newly diagnosed colorectal cancers in a large, hospital-based, case–control study in four northeastern US cities. Nurses interviewed 1326 patients with colorectal cancer, aged 30–69, and 4891 hospitalized controls (1011 with other cancers, 3880 with trauma or acute infection) about the use of salicylates (aspirin), indoles (indomethacin), fenamates (mefanamic acid), pyrazolones (phenylbutazone) and oxicams (piroxicam). Regular use was defined as use on at least four days a week for at least three months. Virtually all of the NSAID use was of aspirin. Regular NSAID users had a 50% lower risk for colorectal cancer than those who had never used NSAIDs (RR, 0.5; CI, 0.4–0.8). The risk

decreased with duration of use and increased after cessation of NSAID use, although neither trend was statistically significant. The hospital-based design of the study did not allow complete exclusion of bias from the selection of controls or confounding by diet or physical activity, which were not measured.

Suh *et al.* (1993) compared aspirin use among 830 patients hospitalized for colorectal cancer (490 colon, 340 rectum) at the Roswell Park Memorial Institute, USA, with that in two control groups: 1138 healthy visitors from a screening clinic and 524 hospital patients who had neither cancer nor digestive diseases. The cases were all diagnosed in 1982–91. Patients who took at least one aspirin daily had a lower risk for colorectal cancer than did non-users in analyses including the screening clinic controls (RR in men, 0.24; CI, 0.12–0.50; RR in women, 0.54; CI, 0.26–1.13). Similar estimates with wider confidence intervals were seen in analyses that included the hospitalized controls. No clear gradient of increasing risk was seen with the frequency of aspirin use, nor were analyses by duration of aspirin use presented.

Peleg *et al.* (1994) compared hospital pharmacy records for 97 newly diagnosed cases of colorectal cancer and 388 controls, who were followed for at least four years at a municipal hospital in Atlanta, Georgia, USA. Medications were supplied free or at low cost to poor patients, thus providing monthly records of the issuance of prescription and non-prescription aspirin, non-aspirin NSAIDs and acetaminophen. The risk for colorectal cancer decreased with the use of both aspirin and non-aspirin NSAIDs but not with acetaminophen. As shown in Table 4, The inverse trend was more strongly associated with duration of use (estimated as the number of days for which NSAIDs were dispensed during the four-year risk period) than with the dose of NSAIDs. Diet and physical activity could not be controlled for. In a second report, Peleg *et al.* (1996) described 93 cases of colorectal cancer that were included in the above study. The paper is discussed separately in relation only to sporadic adenomatous polyps (Table 5).

Muscat *et al.* (1994), at the American Health Foundation, interviewed 511 patients with

Table 4. Case–control studies of non-steroidal anti-inflammatory drugs (NSAIDs) and the risk for colorectal cancer in the general population

Reference	Population	Study size	End-point	Drug	Frequency	Results (RR)[a]	Comments
Kune et al. (1988)	Population-based, Melbourne, Australia, 1980–81	715 cases 727 controls	Colorectal cancer (incidence)	Aspirin NSAIDs	Daily (?) Daily (?)	0.60 (0.44–0.82) 0.77 (0.60–1.01)	Adjusted for diet Unadjusted for aspirin use
Rosenberg et al. (1991)	Hospital-based, four cities in eastern USA, 1977–88	1326 cases 4891 controls	Colorectal cancer (incidence)	Mostly aspirin	≥ 4 days/week ≥ 3 months	0.5 (0.4–0.8)	Trend with duration NS; risk increased after cessation
Suh et al. (1993)	Hospital-based, Roswell Park, USA, 1982–91	830 cases 1138 clinic controls 524 hospital controls	Colorectal cancer (incidence)	Aspirin	≥ 1 per day in 4 years before study	0.24 (0.12–0.50) 0.54 (0.26–1.1)	Men Women
Peleg et al. (1994)	Hospital-based, Atlanta, USA, 1988–90	97 cases 388 controls	Colorectal cancer (incidence)	Aspirin and non-aspirin	Used aspirin > 624 days in 4 years before study Used NSAIDs > 313 days in 4 years before study	0.08 (0.01–0.59) 0.25 (0.09–0.73)	Urban poor
Muscat et al. (1994)	Hospital-based, American Health Foundation, 1989–92	511 cases 500 controls	Colorectal cancer (incidence)	NSAIDs	≥ 3 times/week for ≥ 1 year	0.64 (0.42–0.97) 0.32 (0.18–0.57)	Men Women
Müller et al. (1994)	Hospital-based, US veterans, 1988–92	12 304 cases 49 216 controls	Colon cancer (incidence)	NSAIDs Other anticoagulants	Not stated	0.52–0.91 1.2–1.3	For 6 diseases treated with NSAIDs For diseases treated with other anticoagulants
Reeves et al. (1996)	Population-based, women in Wisconsin, 1991–92	184 cases 293 controls	Colorectal cancer (incidence)	Aspirin and non-aspirin	≥ 1 tablet at least twice weekly for ≥ 1 uear	0.65 (0.40–1.0)	Non-aspirin NSAIDs more strongly associated than aspirin
Bansal & Sonnenberg (1996)	Hospital-based, US veterans, 1981–93	371 cases among 11 446 veterans with and 52 243 without inflammatory bowel disease	Colorectal cancer (incidence)	NSAIDs	Not stated	0.68 (0.65–0.72)	

RR, relative risk; NS, not significant

[a] In parentheses, 95% confidence interval

colorectal cancer and 500 hospitalized controls about use of NSAIDs and acetaminophen. Use was defined as consumption at least three times a week for at least one year before hospitalization of the patient. By this definition, NSAID use was associated with a significantly lower risk for colorectal cancer in both men (RR, 0.64; CI, 0.42–0.97) and women (RR, 0.32; CI, 0.18–0.57). There were too few cancers for a reliable assessment of the trend in risk with duration of NSAID use or of differences among subgroups of people taking NSAIDs for different indications. Acetaminophen was not associated with a lower risk for colorectal cancer.

Müller et al. (1994) tested whether gastrointestinal bleeding induced by aspirin might lead to colonoscopy, early diagnosis and surgical removal of adenomatous polyps, rather than aspirin truly inhibiting tumorigenesis. By comparing 12 304 patients first treated for colon cancer at a Veterans Administration hospital in the USA between 1988 and 1992 with 49 216 controls matched to the cases for age, sex and race, the researchers assessed whether the incidence of colon cancer was increased or decreased among veterans with conditions treated by aspirin or NSAIDs (such as ischaemic heart disease, peripheral vascular disease, arterial embolism and thrombosis, osteoarthrosis and spondylosis), in comparison with patients with conditions treated with non-aspirin anticoagulants (atrial fibrillation, phlebitis and thrombophlebitis). The incidence of colon cancer was significantly lower in veterans with the six conditions generally treated with aspirin (RR, 0.52–0.91) and was significantly higher in those with the diseases treated with non-aspirin anticoagulants (1.2–1.3).

Reeves et al. (1996) conducted a population-based study of 184 women in Wisconsin, USA, whose invasive colorectal cancer was first reported to the State tumour registry between 1991 and 1992, and 293 population-based controls. All of the women were aged 40–74 years. NSAID use was ascertained by telephone interview; regular use was defined as taking at least one tablet at least twice weekly for at least one month. Regular use was associated significantly with a lower incidence of colorectal cancer (RR, 0.65; CI, 0.40–1.0) in analyses in which

adjustment was made for age, previous sigmoidoscopy, family history and body mass index. A statistically significant trend of decreasing risk was seen with increasing duration but not with the frequency of NSAID use. Non-aspirin NSAIDs were associated with a stronger reduction in colorectal cancer risk (RR, 0.43; CI, 0.20–0.89) than aspirin (RR, 0.79; CI, 0.46–1.4).

Bansal and Sonnenberg (1996) examined whether diseases potentially associated with use of NSAIDs were associated with the risk for colorectal cancer among 11 446 veterans hospitalized for inflammatory bowel disease at Veterans Administration hospitals between 1981 and 1993. In a comparison of 371 patients with both colorectal cancer and bowel disease and 52 243 with colorectal cancers only, the researchers found a lower risk for death from colorectal cancer in patients with other conditions usually associated with use of NSAIDs than in patients not likely to take these drugs (RR, 0.68; CI, 0.65-0.72). [The Working Group noted that the cases of colon cancer first diagnosed between 1988 and 1992 at a Veterans Administration hospital would overlap with those in the study of Müller et al. (1994); however, the designs of the two studies are sufficiently different to be reported separately.]

(d) Randomized clinical trial of colorectal cancer and adenomatous polyps

One randomized clinical trial provides some information on aspirin use in relation to colorectal cancer. The US Physicians' Health Study (Gann et al., 1993) was a double-blind, placebo-controlled trial of 22 071 male physicians, which was designed to evaluate the effects of alternate daily doses of aspirin (325 mg) and β-carotene on the risks for cardiovascular disease and cancer. The men taking aspirin were removed from the trial after a mean follow-up of five years because of a significant reduction in the incidence of non-fatal myocardial infarct. In a subsequent analysis of the 33 cases of colorectal cancer, an end-point that the trial had not been designed to evaluate, it was found that the RR for invasive cancer associated with use of aspirin compared with the placebo was 1.2 (95% CI, 0.80–1.7) over the five years of

treatment. An additional analysis of colorectal cancer incidence by year of treatment showed RRs of 2.0 (0.75–5.3) for year 1, 1.2 (0.53–2.7) for year 2, 1.3 (0.58–2.8) for year 3, 1.0 (0.48–2.1) for year 4 and 0.77 (0.34–1.8) for year 5 or later, which may be compatible with an emerging protective effect of aspirin with increasing duration of treatment.

In a subsequent analysis of 13 years of follow-up, after most of the patients on placebo had begun taking aspirin, the findings for colorectal cancer were re-examined in two ways: by classifying participants according to their initial randomization [RR, 1.1; CI, 0.85–1.3 (Sturmer et al., 1996)] and by actual aspirin use (RR, 0.88; 0.59–1.3). [The Working Group noted that, even with the longer follow-up, use of aspirin could not have exceeded 13 years and the study still had limited statistical power for addressing the risk for colorectal cancer.]

4.1.2 Studies of sporadic adenomatous polyps in the colon

The relationship between the presence of adenomatous polyps and subsequent development of colorectal cancer is discussed in the General Remarks. The studies described below are summarized in Table 5.

(a) Cohort studies

Greenberg et al. (1993) assessed whether self-reported use of aspirin modified the recurrence of adenomatous polyps among 793 patients participating in a randomized trial of nutrient supplements and antioxidants. All participants had a histologically confirmed colorectal adenoma removed within three months before enrolment in the study, leaving no known polyps in the colon. Participants reported their use of medications at six and 12 months after enrolment, and had a second colonoscopy after one year. 'Consistent' aspirin users (people who reported taking aspirin on both questionnaires) had a lower recurrence of adenomatous polyps than did non-users (25% vs 34%, RR, 0.52; CI, 0.31–0.89). Similar results were found after four years of observation (RR, 0.58; CI, 0.36–0.91) (Tosteson et al., 1995). An important strength of this study, despite the self-reported non-randomized aspirin use, was

that all participants underwent full colonoscopy at predefined intervals, minimizing the possibility that bleeding induced by aspirin might bias detection of polyps.

Giovannucci et al. (1994) examined colorectal adenomas as well as carcinomas in the Harvard Health Professionals study. The analysis was based on 472 distal adenomas among 10 521 men who reported having had a colonoscopy or sigmoidoscopy between 1982 and 1986 for reasons other than bleeding. The diagnosis of adenoma was verified from medical records. The RR, adjusted for other measured risk factors for the occurrence of adenomas of the descending and sigmoid colon and rectum, in men who reported aspirin use in 1986 was 0.65 (CI, 0.42–1.0) in comparison with non-users. Adenomas were less common among aspirin users than non-users whether or not the patients had faecal occult blood.

Giovannucci et al. (1995) also assessed colorectal adenoma incidence in relation to aspirin use among 89 446 US nurses from 1980 through 1990. The analyses included 564 cases of adenomatous polyps of the descending and sigmoid colon (371) or rectum (193), confirmed by a histopathological report. Having had an endoscopy between 1980 and 1990 was reported slightly more often by women who took 14 or more aspirin tablets per week in 1980 than in non-users (21.5% vs. 17.6%); however, large adenomas (> 1 cm in diameter) were no more common in women who used aspirin at this level (0.38%) than in non-users (0.40%). [The Working Group noted that these findings could be compatible either with inhibition of the growth by aspirin or early detection and removal of smaller adenomas in nurses taking aspirin.]

(b) Case–control studies

Logan et al. (1993) examined the risk for colorectal adenomas in relation to aspirin use among subjects in a randomized trial of faecal occult blood screening in Nottingham, United Kingdom. A total of 147 patients who had faecal occult blood and an adenomatous polyp were compared with two control groups: 176 with blood in the faeces but no adenoma or carcinoma detected by colonoscopy, sigmoidoscopy or barium enema and 153 people with

Table 5. Studies of non-steroidal anti-inflammatory drugs (NSAIDs) and risk for sporadic adenomatous polyps

Reference	Population	Study size	Drug	Frequency	Results (RR)[a]	Comments
Cohort studies						
Greenberg et al. (1993)	US polyp prevention trial; 793 adults with previous adenomas	259 recurrent polyps	Aspirin	Any use at start and end of 1 year	0.52 (0.31–0.89)	Colonoscopy minimized detection bias
Giovannucci et al. (1994b)	Harvard Health Professionals: 10 521 US men, 1986–91	472 distal adenomas	Aspirin	≥ 2 tablets/week	0.65 (0.42–1.0)	Inverse association stronger with regular use reported on more than one questionnaire
Giovannucci et al. (1995)	Harvard Health Professionals, 89 446 US female nurses, 1980–90	564 distal adenomas	Aspirin	≥ 14 tablets	Not given	No effect on incidence of adenomas > 1 cm
Case–control studies						
Logan et al. (1993)	Within randomized trial of FOB testing; Nottingham, United Kingdom	147 cases Controls: 176 negative FOB 153 positive FOB	NSAIDs	Any use	0.49 (0.3–0.8) 0.66 (0.4–1.1)	vs. controls with no FOB vs. controls with FOB
Suh et al. (1993)	Hospital-based, Roswell Park, USA	212 cases Controls: 1138 screening clinic 524 hospital	Aspirin	≥ 2 times/day	0.61 (0.26–1.4) 0.53 (0.19–1.5)	vs. screening clinic controls vs. hospital controls
Martinez et al. (1995)	Endoscopy patients at gastrointestinal clinics, Texas, USA	157 cases 480 controls	NSAIDs	≥ 1 daily ≥ weekly	0.36 (0.20–0.63) 0.77 (0.39–1.6)	
Peleg et al. (1996)	Hospital-based, Atlanta, USA, 1990–93	113 cases with adenoma 226 controls	NSAIDs (aspirin and non-aspirin)	< 320 CCDs 320–700 CCDs ≥ 700 CCDs	0.59 (0.23–1.5) 0.56 (0.20–1.5) 0.31 (0.11–0.84)	CCD ≥ 700 equals 'standard dose' of NSAIDs on 42% of days of observation

RR, relative risk; FOB, faecal occult blood; CCD, calculated cumulative dose

[a] In parentheses, 95% confidence interval

no blood in the faeces and who were not examined further. When the latter were used as the referent, the RR for colorectal adenoma in persons ever having used aspirin was 0.49 (CI, 0.3–0.8). This inverse association was also present, but weaker, when the cases were compared with the controls who had faecal occult blood (RR, 0.66; CI, 0.4–1.1). There were no clear trends with frequency of use, but use for longer than five years resulted in a lower risk than use for shorter periods. NSAIDs other than aspirin, but not acetaminophen, were also associated with a lower risk for adenomas.

Suh *et al.* (1993), at the Roswell Park Memorial Institute, compared aspirin use among 212 hospitalized patients found to have adenomatous polyps of the colon or rectum with that of two control groups: 1138 healthy visitors from a screening clinic and 524 hospital patients who had neither cancer nor digestive diseases. All of the cases were diagnosed between 1982 and 1991. Patients who took aspirin two or more times daily had a lower risk for colorectal cancer than did non-users. The RR for adenomas in comparison with the screening clinic controls was 0.61 (CI, 0.26–1.4) and that in comparison with the hospital controls was 0.53 (0.19–1.5).

Martinez *et al.* (1995) conducted a retrospective, clinic-based study of 157 patients with adenomatous polyps and 480 controls, all of whom had undergone endoscopy at collaborating gastroenterology clinics in Houston, Texas, USA. The RR for adenomatous polyps among persons who took aspirin or other NSAIDs at least daily when compared with those who never used NSAIDs was 0.36 (CI, 0.20–0.63); the estimate for use one to six times per week was 0.77 (CI, 0.39–1.6). An advantage of this study was that all of the patients underwent endoscopy, reducing the potential for screening bias.

Peleg *et al.* (1996) compared the hospital pharmacy records for 113 cases of colorectal adenoma diagnosed between 1 August 1990 and 1 March 1993 with those of 226 controls from the same municipal hospital in Atlanta, Georgia, USA. Using these records, the researchers computed calculated cumulative doses of all NSAIDs over the 55-month study: This corresponds roughly to the number of

days a patient was prescribed a 'standard daily dose' of any NSAID during the study. Patients prescribed a cumulative dose of ≥ 700 (equivalent to receiving a 'standard dose' of NSAIDs for 400 days during the two-year-and-seven-month study), had a significantly reduced risk for adenoma than controls (RR, 0.31; CI, 0.11–0.84). Data for cumulative doses of < 320 and 320–700 are given in Table 5. [The Working Group noted that the 'standard daily dose' defined in this study is intermediate between an anti-inflammatory dose and an analgesic dose and approximately one-half of the anti-inflammatory level. The researchers did not define their interpretation of a 'standard dose' of aspirin.]

(c) Randomized clinical trial

The efficacy of aspirin in inhibiting sporadic adenomatous polyps was examined in one randomized trial (Gann *et al.*, 1993). In the US Physicians' Health Study, men randomized to 325 mg aspirin every other day for five years had a slightly lower risk for cancer *in situ* or self-reported polyps than did men receiving the placebo (122 vs. 142 cases; RR, 0.86; CI, 0.68–1.1). [The Working Group noted that it was impossible to exclude the possibility that polyps existed at the time of enrolment. This would have tended to dilute any beneficial effect of aspirin on colorectal cancer incidence. It was also impossible to distinguish between adenomatous and hyperplastic polyps.]

4.1.3 Studies of oesophageal and gastric cancers

These studies are summarized in Table 6.

Isomäki *et al.* (1978) found five incident cases of cancer of the oesophagus (7.4 expected) and 51 cases of cancer of the stomach (54 expected) in patients with rheumatoid arthritis in Finland in 1967–73. The number of cases expected was calculated from general population rates. Gridley *et al.* (1993) likewise found fewer cases of gastric cancer than expected (observed/expected, 39/62; RR, 0.63; CI, 0.5–0.9) but not of oesophageal cancer (11/8.3; RR, 1.3; CI, 0.7–2.4) among patients with rheumatoid arthritis in Sweden. In neither of these studies were covariates other than age, sex and time period controlled for.

Table 6. Cohort studies of use of non-steroidal anti-inflammatory drugs and risks for oesophageal and gastric cancers

Reference	Population	Study size	End-point	Drug	Frequency	Results (RR)[a]	Comments
Isomäki et al. (1978)	Rheumatoid arthritis, Finland; 34 618 women and 11 483 men, 1967–73	5 cases	Oesophageal cancer	Therapy for arthritis	Heavy use	0.67 (NS)	
		51 cases	Gastric cancer (incidence)			1.1 (NS)	
Gridley et al. (1993)	Rheumatoid arthritis, Sweden; 8787 women and 3750 men, 1965–84	11 cases	Oesophageal cancer	Therapy for arthritis	Heavy use	1.3 (0.7–2.4)	
		39 cases	Gastric cancer (incidence)			0.63 (0.5–0.9)	
Thun et al. (1993)	American Cancer Society, 635 031 US adults, 1982–88	176 deaths	Oesophageal cancer (fatal)	Aspirin	≥ 16 times/month	0.78 (0.42–1.4)	Multivariate estimates
		308 deaths	Gastric cancer (fatal)	Aspirin		0.49 (0.22–1.12)	No data on aspirin after baseline Decreased risk mostly confined to users of ≥ 10 years
Schreinemachers & Everson (1994)	12 688 US adults, 1971–87	20 cases	Gastric cancer (incidence)	Aspirin	Last 30 days	0.93 (0.49–1.7)	
Funkhouser & Sharp (1995)	12 688 US adults, 1971–87	15 cases	Oesophageal cancer	Aspirin	Last 30 days	0.10 (0.01–0.76)	Multivariate estimate, also for alcohol use and smoking

RR, relative risk; NS, not significant

[a] In parentheses, 95% confidence interval

Thun *et al.* (1993), in a study described on p. 52, found lower death rates from cancers of the oesophagus and stomach among regular aspirin users (16 or more times per month in the past year) than in people who had never used aspirin. The trend of decreasing risk with more frequent aspirin use was statistically significant for gastric cancer (p for trend = 0.002) and of borderline significance for oesophageal cancer (p = 0.054). Smoking, alcohol consumption, dietary consumption of fat and fruit, vegetables and grains and obesity were adjusted for in the analysis.

Schreinemachers and Everson (1994) found no difference in the number of newly diagnosed cases of gastric cancer among participants in the National Health and Nutrition Examination Survey who were followed from 1971 to 1987 (RR, 0.93; CI, 0.49–1.7; 30 cases). These authors did not consider oesophageal cancer, but in another report of the same study, Funkhouser and Sharp (1995) found significantly fewer oesophageal cancers among people who used aspirin occasionally than in non-users (RR, 0.10; CI, 0.01–0.76), although this is based on only 15 cases. No cases of oesophageal cancer were observed among regular aspirin users.

Garidou *et al.* (1996) conducted a small, hospital-based case–control study in Greece. They found that chronic intake of any analgesic was non-significantly inversely associated with both squamous-cell carcinoma (five cases; RR, 0.6; CI, 0.2–1.9) and adenocarcinoma (four cases; RR, 0.5; CI, 0.2–1.6) of the oesophagus. [The Working Group noted that chronic analgesic use was not defined. Some controls may have used analgesics for long periods because of chronic or recurrent injuries.]

4.1.4 Studies of cancers other than in the digestive tract

Aspirin was included in a hypothesis-generating cohort study designed to screen 215 drugs for possible carcinogenicity, which covered more than 140 000 subscribers enrolled between July 1969 and August 1973 in a prepaid medical care programme in northern California (USA). Computer records of persons to whom at least one drug prescription was

dispensed were linked to cancer records from hospitals and the local cancer registry. The observed numbers of cancers were compared with the expected numbers, standardized for age and sex, derived from the entire cohort. Three publications have summarized the screening 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 2393 persons who received aspirin–phenacetin–caffeine–butalbital in combination, there was a significant ($p < 0.01$) deficit of breast cancer (two observed, 9.6 expected) in the seven-year follow-up. No negative or positive association with use of this combination was reported in the 15-year follow-up.

The incidences of cancers of the lung, bladder, prostate and breast were similar among daily aspirin users and non-users in the study of Paganini-Hill *et al.* (1989).

In the previously described study of the incidence of cancer among Swedish patients with rheumatoid arthritis (Gridley *et al.*, 1993), women with this condition, who probably had prolonged exposure to NSAIDs, had reduced rates of breast cancer (SIR, 0.79; CI, 0.6–1.0). There was no evidence of protection from cancers other than of the breast, colon and rectum and oesophagus.

Thun *et al.* (1993) found no consistent differences in the death rates of aspirin users and non-users from cancers outside the digestive tract among over 600 000 US adults in the American Cancer Society study. The death rates were either not statistically significantly different or were seen in one sex only, with no dose–response trend. Cancer sites that were examined included buccal cavity and pharynx, respiratory system, breast (female), genital system, urinary system, lymphatic and haematopoietic systems and other or unspecified. The RR for breast cancer among women who reported taking aspirin 16 or more times monthly was 0.88 (CI, 0.62–1.2) in comparison with non-users.

Schreinemachers and Everson (1994) reported significantly lower incidences among users of aspirin than non-users for cancers of the trachea, bronchus and lung (RR, 0.68; CI,

0.49-0.94; 72 cases in aspirin users, 91 cases in non-users) and breast cancer in women (RR, 0.70; CI, 0.50–0.96; 79 cases in aspirin users, and 68 cases in non-users).

In a large, international, population–based, case–control study, salicylates were not associated with renal-cell cancer (McCredie et al., 1995). No dose–response relationship was found. Moreover, the lack of association was not altered by restricting analgesic use to that five or 10 years before the year of diagnosis.

Egan et al. (1996) found no association between regular aspirin use and the incidence of breast cancer in a cohort study of 89 528 registered US nurses in 1990. The analyses were based on 2414 cases identified over 12 years of follow-up (RR, 1.0; CI,. 0.95–1.1). Rosenberg et al. (1991) reported no associations between regular use of aspirin and cancers of the breast, lung, endometrium, ovary, testis or urinary bladder or leukaemia, lymphoma or malignant melanoma in preliminary analyses of a case–control study of drug use in Boston, USA.

Harris et al. (1996) described a case–control study of breast cancer and NSAID use in the USA. They interviewed 511 women with newly diagnosed breast cancer at one centre and 1534 women who had undergone screening mammography. Use of any NSAID three or more times weekly for at least one year was associated with a reduced risk for breast cancer (RR, 0.66; CI, 0.52–0.83). The risks were similar for users of aspirin alone, ibuprofen alone and all NSAIDs combined. The most heavily exposed women, with regard to dose and duration, had the lowest risk. [The Working Group noted the incomplete descriptions of the study population, the participation rates and exposure categories. An earlier report (Harris et al., 1995) probably covered a subset of this study and was therefore not considered separately.]

4.1.5 Issues in interpreting the evidence for prevention of colorectal cancer

In considering the epidemiological data on use of aspirin and risk for colorectal cancer, the Working Group carefully considered the following sources of potential bias and confounding because of their concern about the implications for colorectal cancer prevention in the general population.

Publication bias. The Working Group was not aware (and did not think it plausible) that informative studies have been excluded from the literature.

Screening/detection. It is possible that bleeding induced by NSAIDs including aspirin may lead to endoscopy and earlier detection and removal of colorectal adenomas, resulting in a lower incidence of and death rates from colorectal cancer. Earlier detection of invasive cancer might also reduce mortality. This hypothesis cannot, however, explain the lower prevalence and incidence of colorectal adenoma among aspirin users observed in several studies, which contradicts the hypothesis of screening bias.

Indications for usage. It is possible that the reasons why aspirin users take aspirin influence the risk for colorectal cancer. In many of the studies, the reasons for aspirin use are not well characterized. This uncertainty is particularly apparent for long-term users, among whom the putative protective effect appears to be most marked. Nevertheless, two considerations reduce this concern. First, the association is observed in a variety of populations with different underlying morbidities. Second, the association is not observed with acetominophen.

Life-style factors potentially associated with aspirin use and colorectal cancer. The Working Group considered possible confounding by physical activity, diet, obesity and other life-style factors that potentially affect the risk for colorectal cancer. In studies in which these factors were addressed, no diminution of the association was found, and no other known risk factor is sufficiently strong to account for the association with aspirin. It is possible but implausible that unknown risk factors could be associated strongly enough with both aspirin use and the risk for colorectal cancer to account for the findings.

Factors associated with aspirin intolerance. The possibility that genetic or life-style factors associated with intolerance to aspirin use could also be associated with risk for colorectal cancer was considered. The Working Group was unaware of any evidence to support this hypothesis.

Also, the small proportion of the general population who are intolerant to aspirin makes this implausible.

Exposure measurement. Information on the effects of prolonged use of aspirin is available in only a few studies; this is an important limitation of the aggregated epidemiological data. In most studies, measurement of aspirin use was based on self-reports relating to use a few years before the onset of the studied end-point (adenomas or cancers). This is likely to be a misclassified surrogate for long-term aspirin use; however, non-differential misclassification would have the effect of minimizing any true effect of aspirin. Differential misclassification leading to a spurious association is conceivable only in the case–control studies, but the Working Group concluded that this was unlikely to explain the observed findings. The Group also considered the biological plausibility of an association between aspirin use and colorectal cancer, given the short interval between reported exposure and outcome. Two interpretations are compatible with a causal association: recent aspirin use is a good surrogate for long-term use, and/or aspirin has a fairly rapid effect on cancer prevention. In view of the evidence that duration of exposure is important — and that a reduced risk for colorectal cancer may indeed be achieved, at least in part, through prevention of adenomas — the Working Group recognized that the interpretation of a causal association depends on the assumption that reported recent aspirin use is a good surrogate for long-term use. There was no independent evidence to support or refute this assumption.

4.2 Experimental models[1]
4.2.1 Experimental animals

(a) Colon
Short-term studies. Aspirin was evaluated for its ability to reduce the incidence and growth of aberrant crypt foci in the rat colon (Mereto *et al.*, 1994). Forty-eight male Sprague-Dawley rats were treated with either aspirin (10 mg/kg bw per day) by intragastric administration for 12 consecutive days, then given saline or 1,2-dimethylhydrazine (25 mg/kg bw) on days 4 and 9 or were treated with 1,2-dimethylhydrazine only. About half of the animals in all groups were killed four weeks after the first administration of 1,2-dimethylhydrazine; the rest were killed after eight weeks, and colonic aberrant crypt foci were quantified after methylene blue staining. When given both four and eight weeks after the beginning of treatment with 1,2-dimethylhydrazine, aspirin caused a significant (60%) decrease in the number of foci ($p < 0.01–0.001$). In addition, the numbers of larger foci (with three or more aberrant crypts per focus) were significantly lower (70% reduction; $p < 0.01$) at both times in aspirin-treated rats.

Groups of 14 seven-week-old male Fischer 344 rats were given subcutaneous injections of aspirin at doses of 0.2 and 0.4 mg/kg diet [200 and 400 ppm] for 35 days. Azoxymethane in saline (15 mg/kg bw) was given on days 7 and 14 of the experiment. Control rats were injected with saline only. All rats were killed on day 35, and aberrant crypt foci were quantified after methylene blue staining. At the doses used, aspirin did not inhibit either the number of aberrant crypt foci or the number of crypts per focus (Pereira *et al.*, 1994).

In a similar experiment, 20 adult male Fischer 344 rats received subcutaneous injections of azoxymethane at a dose of 15 mg/kg bw once per week for two weeks. Rats were then randomized to diets containing 0, 0.2 or 0.4 g/kg [200 or 400 ppm] aspirin; these doses represented 40 and 80% of the maximum tolerated dose (MTD; see General Remarks) of aspirin determined in the same laboratory. Azoxymethane had induced about 170 aberrant crypt foci by eight weeks. Aspirin at either dose suppressed the formation and progression of foci to foci of multiple aberrant crypts ($p < 0.05$), although the degree of inhibition was greater at 400 than 200 ppm (Wargovich *et al.*, 1995).

Long-term studies. These studies are summarized in Table 7.

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[1] In this section, the concentrations of aspirin in the diet are given in the units used by the authors of the study and in square brackets as ppm, to allow ready comparison of studies.

Table 7. Results of experiments on the chemopreventive activity of aspirin on colon carcinogenesis in male rats

Strain	Carcinogen, dose and route of administration	Aspirin, dose, route and duration of administration	Tumour incidence (% animals with tumours)		Tumour multiplicity (tumours/tumour-bearing animal)		Reference
			Control	Aspirin	Control	Aspirin	
Sprague-Dawley	DMH, 30 mg/kg bw intragastrically, once	10 mg/kg bw per day, subcutaneously, − 1 and + 1 week	50	17	1. 0	1.0	Craven & DeRubertis (1992)
	DMH, 30 mg/kg bw intragastrically, once	10 mg/kg bw per day, subcutaneously, 2 + 36 weeks	42	42	1.2	1.0	
Wistar	DMH 30 mg/kg bw, subcutaneously 18 times, weekly	5 mg/kg bw per day 30 mg/kg bw per day 60 mg/kg bw per day intragastrically, 18 weeks	100 100 100	100 50 25	NR	NR	Davis & Patterson (1994)
Fischer 344	AOM, 15 mg/kg bw, subcutaneously, twice, weekly	200 ppm 400 ppm − 2 to + 50 weeks in the diet	78 78	53 47	1.7 1.7	0.80 0.70	Reddy et al. (1993)

DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; NR, not reported

Groups of 12–20 male Sprague-Dawley rats, 21 days of age, were given subcutaneous injections of aspirin at 10 mg/kg bw per day one week before and one week after a single dose of 1,2-dimethylhydrazine at 30 mg/kg bw by intragastric administration or were given aspirin four weeks after administration of 1,2-dimethylhydrazine up to the end of the experiment at 36 weeks. A 66% reduction in the incidence of 1,2-dimethylhydrazine-induced adenocarcinomas was seen in rats receiving aspirin for one week before and after the carcinogen, but aspirin had no effect on the tumour response when given four weeks after the carcinogen (Craven & DeRubertis, 1992). [The Working Group noted the small numbers of animals per group.]

In a similar study, groups of 16 male Wistar rats, two months old, received daily doses of aspirin at 0, 5, 30 or 60 mg/kg bw by intragastric administration for 18 weeks. One-half of each group also received weekly injections of 30 mg/kg bw 1,2-dimethylhydrazine for 18 weeks. Aspirin at all doses progressively reduced the number of tumours ($p < 0.03$) and the percentage of tumours ≥ 5 mm in diameter ($p < 0.03$). At the two higher doses, aspirin significantly reduced the incidence of tumours ($p < 0.03–0.01$) (Davis & Patterson, 1994). [The Working Group noted the small number of animals per group.]

In a further study, groups of 48 male Fischer 344 rats, five weeks old, were fed diets containing 0, 200 ppm (40% MTD) or 400 ppm (80% MTD) aspirin. Two weeks later, 36 rats per group were given subcutaneous injections of 15 mg/kg bw azoxymethane once weekly for two weeks; the other 12 rats per group were treated with the vehicle only. After 52 weeks on their respective dietary regimens, all rats were necropsied, and all tumours were subjected to histopathological examination. At both dietary concentrations, aspirin reduced the incidence, multiplicity and size of azoxymethane-induced colon adenocarcinomas ($p < 0.5–0.01$) (Reddy et al., 1993).

(b)   Liver

The chemopreventive effect of aspirin on hepatocarcinogenesis induced by a choline-deficient semisynthetic diet containing 1.75 g/kg methionine, 0 mg/kg choline and 0.11 mmol/kg phosphatidylcholine, was examined in male Fischer 344 rats. The levels of aspirin in the diet were 0.1, 0.2, 0.4 or 0.8% [1000, 2000, 4000 or 8000 ppm]. The duration of the study was 30 weeks. Administration of aspirin at the two higher concentrations reduced the development of preneoplastic and neoplastic nodules in the liver ($p < 0.05–0.001$). In the group fed 0.4% aspirin the number of γ-glutamyl transpeptidase-positive nodules per square centimetre was reduced from 1.7 in the group on control diet to 0.20, and the size of the nodules was decreased from 4.82 to 0.39 mm^2. No nodules were seen in the group given the diet containing 0.8% aspirin (Denda et al., 1994).

Male Fischer 344 rats, six to eight weeks of age, were fed a commercial diet and hepatocellular carcinomas were induced in all animals by initiation with an intraperitoneal injection of 200 mg/kg bw N-nitrosodiethylamine and selection by feeding 0.002% 2-acetylaminofluorene for two weeks and giving a single intragastric intubation of 1 mg/kg bw carbon tetrachloride. Aspirin, mixed in the diet and administered at a concentration of 0.75% [7500 ppm] one week later, decreased the multiplicity of hepatocellular carcinomas from 4.08 to 2.21 ($p < 0.01$). No significant differences in incidence were observed (Tang et al., 1993).

In an initiation–promotion model of hepatocarcinogenesis, nine male Fischer 344 rats were given a single intraperitoneal injection of 200 mg/kg bw N-nitrosodiethylamine; after two weeks of recovery, the animals received the basal diet supplemented with 0.05% phenobarbital for 10 weeks and were then killed. Another group also received aspirin in the diet at a concentration of 0.75 or 1.0% [7500 or 10 000 ppm]. Aspirin treatment significantly retarded the body-weight gain and reduced the average food intake throughout the experiment; it also reduced the number and percent of areas of the liver occupied by γ-glutamyl transpeptidase-positive foci in a dose-dependent manner ($p < 0.01$) but did not appreciably influence the average size of foci (Denda et al., 1989).

Groups of 14–16 male Fischer 344 rats received a choline-deficient, L-amino acid-defined

diet or the same diet containing 0.1 or 0.2% [1000 or 2000 ppm] aspirin for 12 and 30 weeks, at which time the surviving animals were killed. By 12 weeks, the number and areas of the liver staining for γ-glutamyl transferase- positive foci were decreased in a dose-dependent manner by aspirin ($p < 0.05$–0.01), and by 30 weeks, the numbers and percent area of the liver occupied by nodules staining for glutathione S-transferase placental form were also decreased dose-dependently ($p < 0.05$–0.01) (Endoh et al., 1996).

(c) Urinary bladder

In a study in B6D2F$_1$ mice, the MTD for aspirin was first determined in a preliminary experiment for dose selection and dietary tolerance and found to be 1000 mg/kg diet [1000 ppm]. Groups of 75 male mice, five to six weeks of age, then received weekly oral doses of 7.5 mg N-nitrosobutyl(4-hydroxybutyl)amine in ethanol:water (ratio not given) or vehicle only for eight weeks (total dose, 60 mg/mouse). Aspirin was administered in the diet at doses of 400 and 800 ppm (representing 40 and 80% of the MTD) beginning one week before the first dose of nitrosamine and continuing until termination of the study at 24 weeks. The terminal body weights and survival were not reduced by inclusion of aspirin in the diet. The incidence of transitional-cell carcinomas in the urinary bladders of nitrosamine-treated mice (22/75) was similar to that in mice treated with the carcinogen plus aspirin (22/73) (Rao et al., 1996).

Groups of 32 Fischer 344 rats received 2% N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide in the diet with 0.5% aspirin [5000 ppm] for 12 weeks; aspirin was continued for one week, and then the animals were given control diet for 56 weeks. Aspirin significantly reduced the incidence of urinary bladder carcinomas: in only 10 of 27 (37%) rats treated with aspirin but in 18 of 21 (87%) rats treated with the carcinogen only. Forestomach tumours, however, were not seen in rats fed the carcinogen alone but developed in 7 of 27 rats fed carcinogen plus aspirin (Murasaki et al., 1984).

Groups of 32 five-week-old male Wistar rats received basal diet for 32 weeks; basal diet for 32 weeks with water containing 0.05%

N-nitrosobutyl(4-hydroxybutyl)amine in weeks 2–10; a diet containing aspirin at 1 g/kg [1000 ppm] in weeks 1–20 and water ad libitum for 32 weeks; or a diet containing aspirin at 1 g/kg [1000 ppm] in weeks 1–20, drinking-water containing the nitrosamine at 0.05% during weeks 2–10 and then basal diet and water for the remainder of the 32 weeks. Aspirin significantly reduced ($p < 0.05$) the incidence of carcinogen-induced urinary bladder tumours: 8 of 32 rats treated with the nitrosamine developed bladder tumours, whereas only 1 of 32 rats treated with carcinogen plus aspirin developed a bladder tumour (Klän et al., 1993).

(d) Pancreas

Groups of 20 outbred female Syrian golden hamsters, five weeks old, received five weekly doses of 10 mg/kg bw N-nitrosobis(2-oxopropyl)amine by subcutaneous injection; one group of 19 hamsters was also given aspirin (purity, 99.5%) from weeks 6 to 32 (time of terminal kill). A control group of 30 hamsters was given tap-water. Aspirin treatment had no effect on body-weight gain. Neither the reduction in the incidence of pancreatic tumours, from 20/28 to 10/19, nor the reduction in the multiplicity of pancreatic adenocarcinomas, from 1.3 to 0.84, was significant (Takahashi et al., 1990).

4.2.2 In-vitro models

No data were available to the Working Group.
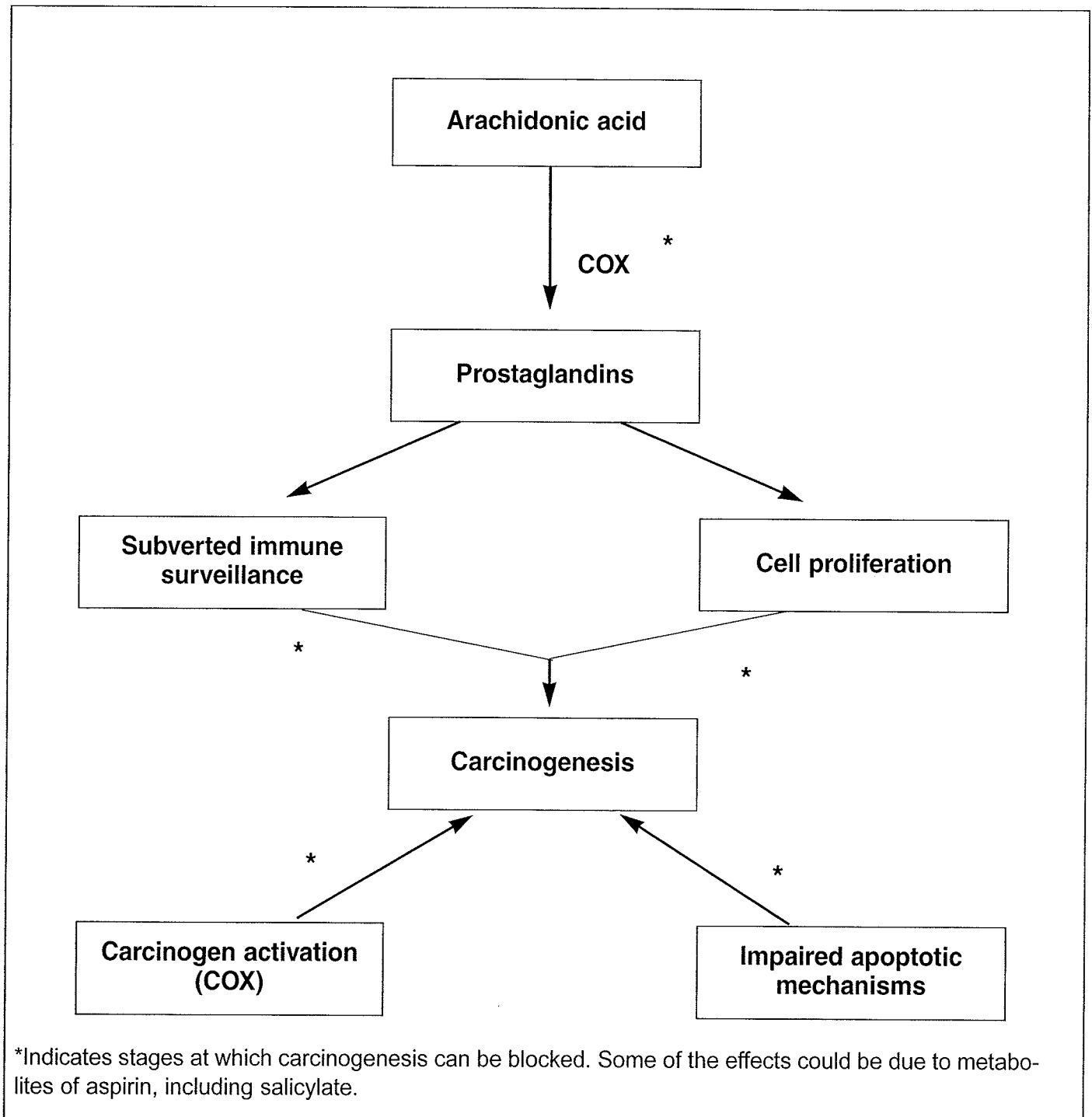
4.3 Mechanisms of chemoprevention

Most research into the mechanisms of the chemopreventive action of aspirin has been centred around four areas that are thought to be related to the development of colorectal cancer: (i) activation of carcinogens, (ii) cell proliferation, (iii) apoptosis and (iv) immune surveillance. These hypotheses are summarized in Figure 3.

4.3.1 Inhibition of carcinogen activation

Cyclooxygenases (COX-1 and COX-2)[1] may be involved in the initiation of carcinogenesis in three general ways: activation of carcinogens to DNA-binding forms, production of malondialdehyde and formation of peroxyl radicals.

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[1] Used as synonyms for prostaglandin endoperoxide synthases (PGH synthases)

Figure 3. Proposed mechanisms of prevention of colon cancer by aspirin

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                        ┌─────────────────────────┐
                        │     Arachidonic acid     │
                        └─────────────────────────┘
                                    │
                                    │  COX  *
                                    ▼
                        ┌─────────────────────────┐
                        │      Prostaglandins      │
                        └─────────────────────────┘
                          ╱                     ╲
                        ╱                         ╲
        ┌──────────────────────┐        ┌──────────────────────┐
        │  Subverted immune    │        │   Cell proliferation  │
        │    surveillance      │        │                       │
        └──────────────────────┘        └──────────────────────┘
                    *                ╲   ╱
                                      ▼     *
                        ┌─────────────────────────┐
                        │      Carcinogenesis      │
                        └─────────────────────────┘
                          ╱                     ╲
                  *     ╱                         ╲     *
        ┌──────────────────────┐        ┌──────────────────────┐
        │ Carcinogen activation │        │  Impaired apoptotic   │
        │        (COX)          │        │     mechanisms        │
        └──────────────────────┘        └──────────────────────┘
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*Indicates stages at which carcinogenesis can be blocked. Some of the effects could be due to metabolites of aspirin, including salicylate.

Activation of carcinogens by COX is well documented (reviewed by Eling et al., 1990), and it has also been demonstrated that aspirin can inhbit such processes (Krauss & Eling, 1985; Levy & Weber, 1992; Liu et al., 1995). An illustrative example is the conversion of 2-aminofluorene to a DNA-binding product by both COX isozymes. This conversion is inhibited by aspirin.

Malondialdehyde is produced by the enzymic and nonenzymic breakdown of prostaglandin H and during lipid peroxidation (reviewed by Marnett, 1992). It is a directly acting mutagen in bacterial and mammalian systems and it is also carcinogenic in rats. Aspirin and other NSAIDs inhibit the formation of malondialdehyde only via the breakdown of prostaglandin H_2.

Minchin *et al.* (1992) demonstrated in an enzyme-free system *in vitro* that aspirin directly activates several N-hydroxyarylamines by O-acetylation.

4.3.2 Inhibition of cell proliferation

An indirect pathway by which aspirin could inhibit the proliferation of colonocytes is inhibition of the proliferative effect of prostaglandins. This effect, which requires acetylation of COX by aspirin, has been documented only in cultured colon cancer cells. The plausibility of this idea rests on the already mentioned observations that colon tumours produce increased amounts of prostaglandin E_2, colon adenomas and carcinomas overexpress COX and mice lacking both the *Apc* and *Cox-2* genes have 90% fewer intestinal tumours than those lacking only *Apc*.

Aspirin induced a concentration-dependent reduction in the proliferation rate of HT-29 human colon cancer cells. This effect was accompanied by distinctive morphological changes in these cells. Of greater interest, aspirin altered the distribution of HT-29 cells in the various phases of the cell cycle in a non-liear, concentration-dependent fashion at concentrations starting at 1 mmol/litre (Shiff *et al.*, 1996).

Salicyclic acid, the metabolite of aspirin, also inhibited the growth of human colorectal tumour cell lines at concentrations of 1–5 mmol/litre (Elder *et al.*, 1996). The inhibitory effect was greater against carcinoma and adenoma cell lines transformed *in vitro* than against adenoma cell lines. Accumulation of many cells in the G_0/G_1 cell cycle phase and apoptosis were also noted. It is doubtful that the inhibition of cell proliferation in these studies was due to a reduction in prostaglandin production (Hanif *et al.*, 1996). [The Working Group noted that as the typical concentrations in humans *in vivo* would be less than 1 μmol/litre, the physiological significance of these in-vitro observations remains in doubt.]

4.3.3 Apoptosis

Most NSAIDs can induce apoptosis in colon cancer cell lines *in vitro*, but conflicting findings are reported for aspirin. Shiff *et al.* (1996) found that although indomethacin, naproxen and piroxicam induced apoptosis in HT-29 cells, aspirin at 1500 μmol/litre did not. Three methods were used to detect apoptosis: DNA laddering on agarose gel electrophoresis, fluorescent-activated cell sorting to detect sub-diploid peaks based on DNA content and acridine orange staining to highlight cellular morphological changes such as DNA condensation. Elder *et al.* (1996), however, demonstrated a convincing, dose-dependent apoptotic response of HT-29 colon cancer cells to salicylate and a similar response in transformed adenoma cells but not in all adenoma cell lines. Acridine orange staining of floating cells was used to quantify apoptosis. Elder *et al.* used concentrations up to 5 mmol/litre but did detect an effect at 1500 μmol/litre, equivalent to the concentration used by Shiff *et al.* The latter group tested aspirin, whereas Elder *et al.* tested salicylate, which is more appropriate, since it is the predominant pharmacological metabolite in patients on long-term aspirin treatment.

4.3.4 Immune surveillance

The role of aspirin in immune surveillance is uncertain, as is the central idea that immune surveillance is important in carcinogenesis. The role of aspirin in immune surveillance has been addressed both directly and indirectly in cell culture systems. The finding that colon cancers contain elevated levels of prostaglandin E_2 (Rigas *et al.*, 1993) prompted studies of the role of this compound in regulation of the expression of classes I and II HLA antigens in SW1116 colon cancer cells. Prostaglandin E_2 down-regulated the expression of the class II antigen HLA-DR in SW1116 cells in a concentration- and time-dependent manner. The effect of aspirin at 100–200 μmol/litre was reversible and specific (Arvind *et al.*, 1995). Other eicosanoids such as prostaglandin F_{2a} and leukotriene B_4 had no such effect. The reduction of HLA-DR by prostaglandin E_2 was accompanied by reduced mRNA levels of *HLA-DRa* and reduced transcription of the

corresponding gene. (*HLA-DRa* is one of the two genes that code for the heterodimeric HLA-DR protein). In contrast, the expression of HLA class I genes was not affected. An additional study by the same group confirmed that prostaglandin, E_2, prostaglandin $F_{2\alpha}$ and leukotriene B_4 did not affect the expression of MHC class I antigens in SW1116 and HT-29 human colon adenocarcinoma cells. Furthermore, 16,16-dimethyl prostaglandin E_2, a stable analogue of prostaglandin E_2, did not affect their expression in mice, even when treated with a colon carcinogen (Feng et al., 1996).

The effect of aspirin on the expression of class II antigens was also assessed from a different viewpoint (Arvind et al., 1996). Aspirin induced a several-fold increase in the expression of HLA-DR in HT-29 human colon adenocarcinoma cells, which do not normally express these antigens. These effect was accompanied by increased steady-state mRNA levels of *HLA-DRa* and an increased transcription rate of the gene. The study clearly established a transcriptional effect of aspirin on an HLA class II gene, suggesting a potentially important immunological effect of this versatile compound. This finding is consistent with results showing up-regulation of MHC expression in rats after treatment with piroxicam (Rigas et al., 1994).

Several studies (reviewed by Rumore et al., 1987) have demonstrated that aspirin induces interferon, decreases the antibody response, inhibits antigen–antibody interactions, alters T-lymphocyte functions and alters leukocyte migration.

Nitric oxide synthesized by inducible nitric oxide synthase has been implicated as a mediator of inflammation. Aspirin and salicylate at millimolar concentrations have been reported to inhibit induction of inducible nitric oxide synthase in murine macrophages activated by lipopolysaccharide (Amin et al., 1995; Brouet & Ohshima, 1995) and in neonatal rat cardiac fibroblasts treated with interferon-γ and tumour necrosis factor-α (Farivar & Brecher, 1996).

5. Other Beneficial Effects

5.1 Antiplatelet effects
5.1.1 Background
Aspirin is widely used to prevent myocardial infarct, thrombotic stroke and death from vascular events in populations at high risk for vascular conditions. Randomized trials have confirmed the efficacy of aspirin in secondary prevention in patients with documented coronary, cerebral or peripheral vascular disease. The absolute benefits are smaller for healthy people, however, and the net improvement less certain. This section covers the mechanism whereby aspirin inhibits platelet aggregation, how aspirin differs mechanistically from other NSAIDs and from non-NSAID antiplatelet drugs and what is known currently about the lowest effective dose.

5.1.2 Mechanism
Aspirin inhibits platelet aggregation and thrombosis by permanently inactivating COX-1 in platelets (Moncada & Vane, 1979). Irreversible acetylation of the hydroxyl group of a single serine residue essentially stops platelet thromboxane A_2 production for the 7–10-day life of the platelet. Unlike nucleated cells, platelets cannot resynthesize COX-1. Aspirin is a more potent inhibitor of platelet thromboxane production than other NSAIDs because it binds covalently, rather than reversibly, to COX-1 (Patrono, 1994). The mechanism by which aspirin inhibits platelet aggregation and prevents clot propagation differs fundamentally from that of various recently developed antiplatelet drugs, which block the binding of fibrinogen to receptors on the platelet membrane but do not inhibit COX or the production of platelet thromboxane (CAPRIE Steering Committee, 1996; Cohen, 1996).

5.1.3 Secondary prevention in populations at high risk for cardiovascular events
Prophylactic aspirin therapy is known to reduce the risk for vascular thrombosis in high-risk populations (Antiplatelet Trialists' Collaboration, 1988, 1994a,b). A comprehensive

overview of 145 randomized trials, 50 involving aspirin alone, found aspirin to be beneficial in high-risk settings: patients with acute myocardial infarct, those with a past history of myocardial infarct or clinical cerebrovascular disease, those with a past history of stroke or transient ischaemic attack and those with various other vascular problems (including unstable angina, stable angina, a history of vascular surgery, angioplasty, atrial fibrillation, valvular disease and peripheral vascular disease). Most of the trials involved daily doses of 75–325 mg aspirin (Antiplatelet Trialists' Collaboration, 1994b).

In absolute terms, the benefit of aspirin is greatest in patients with acute myocardial infarct, among whom 38 vascular events were prevented per 1000 patients treated for only about one month. Similar benefits were seen for patients with prior myocardial infarct, prior stroke, transient ischaemic atack or other 'high risk' cardiovascular conditions, although longer treatment (16–33 months) was required to obtain them. Much smaller benefits were observed in primary prevention trials, with four vascular events prevented in 1000 patients over an average of 62 months of therapy (Antiplatelet Trialists' Collaboration, 1994; Hirsh et al., 1995).

5.1.4 Primary prevention in populations at average risk for cardiovascular events

Among men in the general population, prophylactic aspirin administration reduces the incidence of non-fatal myocardial infarct but has not yet been demonstrated to reduce overall mortality from cardiovascular disease (Hirsh et al., 1995).

5.1.5 Lowest effective dose for prevention of cardiovascular events in high-risk populations

The optimal dose of aspirin for long-term prophylactic cardiovascular therapy is unknown. In the randomized trials reviewed by the Antiplatelet Trialists' Collaboration (1994b), daily doses of 75–150 mg seemed to be as effective as higher doses in preventing vascular events, although the statistical power to examine this issue was limited. Several well-designed

randomized trials of secondary prevention have shown aspirin to be effective in preventing thrombosis at doses of 75 mg/day (RISC Group, 1990; SALT Collaborative Group, 1991; Juul-Moller et al., 1992; Lindblad et al., 1993). One study indicated that a dose as low as 30 mg/day may be protective (Dutch TIA Trial Study Group, 1991). The theory that very low doses of aspirin might be as effective and less toxic than higher doses is biologically plausible because aspirin inhibits platelet thromboxane A2 production almost completely (Patrono, 1994). The efficacy of doses lower than 325 mg every other day has not yet been tested in randomized trials in the context of primary prevention.

5.2 Alzheimer disease

McGeer et al. (1996) recently reviewed 17 epidemiological studies of the use of NSAIDs and steroids as possible protective factors against Alzheimer disease. Five of six case–control studies in which 'arthritis' was used as a surrogate for use of NSAIDs or steroids and one of two in which rheumatoid arthritis was examined reported a reduced risk for Alzheimer disease associated with those diagnoses (combined data for arthritis, RR, 0.56; CI, 0.44–0.70). In three other studies of patients with rheumatoid arthritis, Alzheimer disease was noted to be uncommon. The summary measure of risk in three additional case–control studies of the use of NSAIDs and risk for Alzheimer disease was 0.50 (0.34–0.72) as a group; similarly, steroids were associated with a summary odds ratio of 0.66 (0.43–0.99) in four case–control studies.

In nearly all of the reviewed studies, the diagnosis of Alzheimer disease was made clinically, usually by a neurologist, so that attempts were made to exclude vascular and other dementias. In all of these studies, information on use of aspirin was obtained retrospectively, so that recall bias or change in use of aspirin precipitated by the disease itself could not be reliably excluded.

5.3 Reproductive outcomes

Combined analyses of all randomized trials of antiplatelet therapy in the possible prevention of pregnancy-induced hypertension have been

published (CLASP Collaborative Group, 1994; ECPPA Collaborative Group, 1996). Most of the trials were of aspirin (50–150 mg/day), but a few were of aspirin with dipyridamole. When the results of all the trials were taken together, antiplatelet therapy was associated with a reduction of 23% in the incidence of pre-eclampsia (ECPPA Collaborative Group, 1996). The results are heterogeneous, however, and when the small trials (including fewer than 200 women) are excluded, the apparent reduction is 17%. All of the trials that found strong effects of antiplatelet therapy on the prevention of pre-eclampsia were very small, and at least as many women are known to have been randomized in other small but unpublished trials (CLASP Collaborative Group, 1994). If some small trials with unpromising results were not published, the available results from the small trials would give a biased estimate of the effects of antiplatelet therapy, but the large trials would not. The findings of the analysis of the data from the large trials are consistent with this assumption.

In five of the larger trials included in these combined analyses, information on pre-term delivery was presented (Hauth et al., 1993; Italian Study of Aspirin in Pregnancy, 1993; Sibai et al., 1993; CLASP Collaborative Group, 1994; ECPPA Collaborative Group, 1996). No noteworthy effect of aspirin (50 or 60 mg/day) on preterm delivery was observed; however, in the large trial of the CLASP Collaborative Group (1994), the absolute risks of preterm delivery differed substantially between the categories of women studied. Among about 8000 women entered for prophylactic reasons, only one-fifth of the placebo group had pre-term delivery, and the absolute benefit appeared to be about two fewer preterm deliveries per 100 women allocated aspirin. Among just over 1000 women entered for therapeutic reasons, two-fifths of the placebo group had preterm delivery, and the absolute benefit appeared to be about 5 per 100.

In some of the trials of the use of low doses of aspirin in the prevention of pregnancy-induced hypertension or pre-eclampsia, data on intrauterine growth retardation were presented (Uzan et al., 1991; Hauth et al., 1993; Italian Study of Aspirin in Pregnancy, 1993;

Sibai et al., 1993; Viinikka et al., 1993; CLASP Collaborative Group, 1994; ECPPA Collaborative Group, 1996). The RRs associated with use of aspirin were in the range 0.5–1.0. A significant effect was found only in the relatively small trial of Uzan et al. (1991).

A combined analysis of antiplatelet therapy in the prevention of perinatal mortality was presented by the CLASP Collaborative Group (1994) and was subsequently updated by the ECPPA Collaborative Group (1996). This analysis suggested a 1% (standard deviation, 10) reduction in the incidence of perinatal mortality associated with antiplatelet therapy. Data from the trials in which at least 200 women were enrolled suggested a 1% (standard deviation, 10) increase in perinatal mortality associated with antiplatelet therapy.

Shapiro et al. (1976) examined the relationship between perinatal mortality and reported aspirin use at any time during pregnancy in a multicentre cohort study of 41 337 women whose pregnancies lasted at least seven months. Data on drug use were recorded at each antenatal visit and were confirmed for most women by the attending physician or by review of the hospital or clinical record. The women were divided into 1515 who were heavily exposed, 24 866 with intermediate exposure and 14 956 who were not exposed. Heavy exposure was defined as use for at least eight days per month for at least six lunar months. There were 371 (2.5%) perinatal deaths in the unexposed group, 548 (2.2%) in the group with intermediate exposure and 38 (2.5%) in the group with heavy exposure. Thus, there was no association between perinatal mortality and level of exposure to aspirin.

6. Carcinogenicity

6.1 Humans

6.1.1 Renal cancer

Paganini-Hill et al. (1989) reported significantly more hospitalizations from renal cancer among men who used aspirin daily than among non-users in a cohort in a retirement community followed up from 1981 through 1987 (RR, 6.3; CI, 2.2–17; nine exposed cases, six unexposed). Only three cases of renal

cancer were observed among exposed women (RR, 2.1; 0.53–8.5) in the initial report. After 3.5 additional years of follow-up, the statistically significant excess remained in male but not female aspirin users (Paganini-Hill, 1995). No information was available in this study on the duration of aspirin use, past use of phenacetin or current exposure to other analgesics.

Aspirin use was not associated with cancers of the urinary system in the large American Cancer Society cohort (Thun et al., 1993) nor with renal cancer in the two largest trials of aspirin in the primary prevention of heart disease (Hennekens et al., 1990). Steineck et al. (1995) found an increased risk for transitional-cell urothelial cancer among people who reported acetaminophen use (1.6, 1.1–2.3) but not aspirin use (0.7; 0.5–1.0) in a population-based case–control study of 325 cases and 393 controls.

[The Working Group noted that a major limitation of the studies that show aspirin to be associated with urinary tract cancers is the inability to control for past use of phenacetin. A number of the studies did not distinguish between renal-cell and renal pelvic cancer.]

Ross et al. (1989) also found aspirin use to be associated with cancer of the renal pelvis and ureter in a population-based case–control study of 187 cases from the Los Angeles Tumor Registry and 187 neighbourhood controls (RR among nonsmokers, 5.0, 1.7–14); the association with renal pelvis cancer was confined to women. In a large-scale study in Minnesota, USA, no relationship was found between renal-cell carcinoma and regular use or duration of use of aspirin (Chow et al., 1994).

6.1.2 Haematopoietic malignancies

In the studies of rheumatoid arthritis patients in Finland and Sweden (see p. 51), the incidence of haematopoietic malignancies was about twice that of the general population (Isomäki et al., 1978; Gridley et al., 1993). In the study of Gridley et al. (1993), described on p. 51, there was an increased risk for lymphomas (SIR, 2.0; CI, 1.5–2.6). [The Working Group noted that the underlying rheumatoid arthritis and other treatment could account for differences in lymphoma risk in this study.]

6.1.3 Childhood cancer

In some case–control studies of childhood cancer, data on recalled aspirin use during pregnancy was obtained by interviewing the mothers. In a study of 188 cases of leukaemia and 93 controls with other cancers and a second control group of people hospitalized for reasons other than cancer (Manning & Carroll, 1957), the RR for leukaemia associated with reported use of aspirin was 1.5 (CI, 0.87–2.5), in comparison with controls with other cancers and 2.3 (CI, 1.1–.4.8) in comparison with the other control group.

In single studies, no significant association was found between reported aspirin use during pregnancy and brain tumours (Preston-Martin et al., 1982), neuroblastoma (Schwartzbaum, 1992) or rhabdomyosarcoma (Grufferman et al., 1982) in the offspring.

6.2 Experimental animals

No data were available to the Working Group.

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

(a) Upper gastrointestinal tract toxicity

All NSAIDs, including aspirin, cause a dose-dependent increase in the incidence of upper gastrointestinal toxic effects, ranging in severity from dyspepsia to gastrointestinal haemorrhage, ulceration and perforation. The severity and frequency of these complications vary greatly with the dose and duration of use. Aspirin is the only NSAID for which substantial data exist on the toxicity of both prolonged use of low doses and exposure to anti-inflammatory doses.

Toxicity of high doses. At high anti-inflammatory doses of aspirin (> 2400 mg daily), gastrointestinal bleeding and ulceration become important individual and public health problems. In several randomized trials of cardiovascular disease and aspirin use, patients receiving 1 g of aspirin per day had an incidence of six episodes of haematemesis per 10 000 person-months of treatment (Aspirin

Myocardial Infarction Study Research Group, 1980). The incidence during the three-year treatment period was two times higher than that of untreated patients. The incidence of upper gastrointestinal tract ulcers (per 10 000 patient-months of treatment) was 9.7 in patients treated with 972–1500 mg aspirin daily (Coronary Drug Project Research Group, 1976; Britton et al., 1987; Ehresmann et al., 1977; Hess et al., 1985; Fields et al., 1978; Lemak et al., 1986) and 2.1 in the group receiving placebo in the US Physicians' Health Study (Steering Committee of the Physicians' Health Study Research Group, 1989). The absolute rates in these trials may not be generalizable to the general population because people with ulcers were excluded from the study, but they indicate that gastric toxicity due to anti-inflammatory doses of aspirin is a serious problem. Gastric toxicity due to use of NSAIDs, including aspirin, by rheumatoid arthritis patients has been estimated to cause 240 000 hospitalizations and 2600 deaths per year in the USA (Fries et al., 1991).

Toxicity of low doses. Among men in the US Physicians' Health Study who received either placebo or 325 mg aspirin every other day for five years, the incidence of haematemesis or melaena was approximately 1.5 times higher than that in the controls. This corresponds to about 19 episodes of major gastrointestinal bleeding attributable to 100 000 person-months of aspirin treatment. In a trial in the United Kingdom in which some 2400 persons were randomized to receive 300 or 1200 mg of aspirin daily or placebo, the RR for upper gastrointestinal tract haemorrhage in people at 300 mg daily was 3.3 (1.2–9.0) in comparison with those on placebo (Slattery et al., 1995).

(b)   Non-gastrointestinal bleeding, including haemorrhagic stroke

A dose-dependent increase in the risk for serious blood loss requiring transfusion was observed in the combined data from 34 randomized trials (Antiplatelet Trialists' Collaboration, 1994b, Appendix 1; Table 8). Aspirin at doses of 75 mg daily increases the risk for non-fatal extracerebral bleeding requiring transfusion.

Aspirin may also increase the risk for haemorrhagic stroke (Hirsh et al., 1995). In both the British doctors study (Peto et al., 1988) and the US Physicians' Health Study (Steering Committee of the Physicians' Health Study Research Group, 1989), statistically insignificant increases in total stroke incidence were reported in healthy men treated with aspirin. Ten stroke deaths were observed in the US Physicians Study among people treated with aspirin, versus seven in the placebo group (RR, 1.4; CI, 0.54–3.9). The incidence of non-fatal disabling stroke was higher among British doctors treated with aspirin (19 per 10 000 man-years) than in the placebo group (7.4 per 10 000; $p < 0.05$). The total stroke incidence was not increased in an observational study of aspirin use among healthy US nurses (Manson et al., 1991). The net effect of aspirin prophylaxis is consistently beneficial in populations at high risk for ischaemic stroke who have already experienced a cerebrovascular event or symptoms (Antiplatelet Trialists' Collaboration, 1994b). Ishaemic and haemorrhagic strokes cannot be reliably separated in many of the trials of aspirin in secondary prevention.

(c)   Blood pressure

In a meta-analysis of 50 randomized, placebo-controlled trials, short-term treatment with aspirin appeared to have no demonstrable effect on blood pressure, in contrast to many other NSAIDs (Johnson et al., 1994).

(d)   Reye syndrome

Reye syndrome is an acute condition characterized by non-inflammatory encephalopathy and liver injury. This extremely rare syndrome has been reported primarily, although not exclusively, in children (Sullivan-Bolyai & Corey, 1981). A review of six case–control studies from the USA — four published before 1981 and two subsequent studies — designed specifically to address methodological flaws in the earlier studies, supported the role of use of aspirin during a period of antecedent illness (Hurwitz, 1989). A seventh case–control study from the United Kingdom further supports this association (Hall, 1990).

Table 8. Incidence of non-fatal extracerebral bleeding requiring transfusion according to aspirin dose in 34 randomized clinical trials

Aspirin dose[a] (mg/day)	Person-years of treatment	No. of people	Per 100 000 treatment years	
			Incidence	95% CI
Placebo	95 591	75	0.54	0.42–0.67
75–170	62 863	63	0.81	0.63–1.0
300–500	26 134	41	1.1	0.75–1.4
1200–1500	6 590	20	2.2	1.2–3.0

From Antiplatelet Trialists' Collaboration (1994b)

[a] Reflects actual dose used in trials; therefore, scale not continuous

Since 1980, publicity and advice has been followed by a marked decline (at least 50%) in aspirin use among children in the USA. This decline was accompanied by a drastic reduction in the reported number of cases of Reye syndrome in children, notably those over five years old. At these ages, the problems in the differential diagnosis of metabolic disorders and Reye syndrome that may occur with younger children are unlikely (Hurwitz, 1989). Therefore, the consistent positive association between Reye syndrome and aspirin use observed in case–control studies is supported by a temporal association between the use of aspirin in children and the occurrence of the syndrome.

Controlled studies of aspirin use and Reye syndrome are confined to the USA and the United Kingdom. Few children with Reye syndrome who were exposed to aspirin have been reported from other countries. Among 20 children with Reye syndrome reported over a 10-year period in Australia when paediatric aspirin use of aspirin was low, prior exposure to aspirin was found in only one child (Orlowski et al., 1987). In a series of 15 children with Reye syndrome in Germany, three had been exposed to aspirin (Gladtke & Schausiel, 1987). It remains unknown whether aspirin-associated Reye syndrome has occurred outside the USA and the United Kingdom.

(e)   Asthma

Approximately 10% of adults with asthma develop acute, idiosyncratic bronchoconstriction after ingesting aspirin or other NSAIDs (Fischer et al., 1994; Staton & Ingram, 1996). Often called 'aspirin-sensitive' asthma, this condition can be precipitated by any currently available NSAID. Symptoms begin within 15 min to 4 h (usually 1 h) after ingestion, and may include rhinorrhoea, conjunctival irritation, scarlet flushing of the head and neck and severe, even life-threatening asthma. The respiratory symptoms can continue beyond discontinuation of NSAID use (Szczeklik, 1994).

(f)   Nephrotoxicity

The relationship between chronic use of analgesics, most notably phenacetin, and chronic renal failure has been recognized for over 30 years. Although epidemiological studies have consistently found an increased risk for chronic renal failure associated with phenacetin use and prolonged use of mixtures of analgesics, the findings with regard to aspirin have been inconsistent. Two large case–control studies of end-stage renal disease (Pernerger et al., 1994) and early chronic renal failure (Sandler et al., 1989) and one cohort study with a 20-year follow-up (Dubach et al., 1991) reported no excess risk for chronic renal failure in association with aspirin use. In another large case–control study (Pommer et al., 1989), an increased risk for chronic renal disease was associated with aspirin used in combination with other analgesics, but not when used as a single agent. In a further large case-control study, the RR was 2.5 (CI, 1.2–5.2) for chronic renal failure associated with regular aspirin use alone (Morlans et al., 1990); however, in this study, the probability that the association was confounded by prior use of phenacetin-containing compounds could not be excluded

completely. At doses of 1–2 g/day, aspirin may decrease urate excretion, increase plasma urate concentrations and potentially precipitate clinical gout. At higher doses, aspirin either does not affect urate excretion or lowers it (Goodman Gilman *et al.*, 1990). As aspirin inhibits the effects of uricosuric agents, its use in patients treated with these agents is not advised (Editions du Vidal, 1995).

(g) Reproductive and developmental effects

Abruptio placentae. In a randomized controlled trial, Sibai *et al.* (1993) found an increased incidence of abruptio placentae among women who received 60 mg of aspirin per day over that of women who received placebo (Table 9). This was not a primary outcome of the trial, nor indeed of any other trial (Hauth *et al.*, 1995), as no evidence of an increase in the incidence of abruptio placentae was found in randomized trials in which at least 200 women were enrolled. The increased risk found by Sibai *et al.* (1993) may be a chance observation or due to a low incidence of the disorder in the placebo group in that trial (Hauth *et al.*, 1995).

Maternal bleeding around the time of delivery. Hertz-Picciotto *et al.* (1990) reviewed studies of the association between taking aspirin late in pregnancy and maternal haemostatic abnormalities. Clinically observable effects on maternal haemostasis were seen at an average dose of 1500 mg/day or more. Platelet dysfunction was observed at lower doses, such as 60 mg/day. In a randomzed trial of the use of 60 mg of aspirin per day, no noteworthy difference in the incidence of antepartum haemorrhage, other than abruptio placentae or post-partum bleeding of 500 ml or more, was observed in comparison with women given placebo (CLASP Collaborative Group, 1994). A significantly higher percentage (4%) of women allocated to aspirin received blood transfusions after delivery than women allocated placebo (3.2%), independently of differences in the occurrence or degree of post-partum haemorrhage. No effect of aspirin on maternal bleeding complications was observed in other large trials (Italian Study of Aspirin in Pregnancy, 1993; Sibai *et al.*, 1993; Viinikka *et al.*, 1993; ECPPA Collaborative Group, 1996).

Table 9. Use of low doses of aspirin and abruptio placentae: data from randomized trials in which at least 200 women were enrolled

Dose of aspirin (mg per day)	No. of women		Abruptio placentae				RR	95% CI[a]	Reference
	Aspirin	Placebo	Aspirin		Placebo				
			No.	%	No.	%			
60	1485	1500	11	0.7	2	0.1	5.6	1.2–25	Sibai *et al.* (1993)
150	156[b]	74	7[b]	4.5	6	8.1	0.6	0.2–1.6	Uzan *et al.* (1991)
60	302	302	3[c]	1.0	2[c]	0.7	1.5	0.3–8.9	Hauth *et al.* (1993)
50	561	471[d]	7[c]	1.2	9[c]	1.9	0.7	0.2–1.7	Italian Study of Aspirin in Pregnancy (1993)
50	97	100	0[c]		0[c]		Undefined		Viinikka *et al.* (1993)
60	4659	4650	86	1.8	71	1.5	1.2	0.9–1.7	CLASP Collaborative Group (1994)
60	476	494	5	1.1	7	1.4	0.7	0.2–2.3	ECPPA Collaborative Group (1996)

[a] Calculated by the Working Group
[b] Some women received 225 mg/day diprimadole
[c] Data from Hauth *et al.* (1995)
[d] No treatment

Neonatal haemostatic abnormalities. There is a consistent association between bleeding in the newborn and maternal exposure to aspirin at analgesic or antipyretic doses late in pregnancy (Hertz-Picciotto *et al.*, 1990). Such abnormalities may include intracranial haemorrhage, particularly in preterm babies and those with a low birth weight.

In a large randomized trial of 60 mg/day aspirin or placebo, fewer intraventricular haemorrhages were reported among babies born to women allocated aspirin (0.7%) than among those in the placebo group (0.9%); this difference was not statistically significant (CLASP Collaborative Group, 1994). No significant differences in fetal or neonatal deaths attributed to haemorrhage or in the incidence of other neonatal haemorrhages were seen, and no differences in the incidence of fetal bleeding complications were observed in other large trials of low doses of aspirin (Italian Study of Aspirin in Pregnancy, 1993; Sibai *et al.*, 1993; Viinikka *et al.*, 1993; ECPPA Collaborative Group, 1996).

Preterm constriction of the ductus arteriosus. During fetal life, the patency of the ductus arteriosus is maintained by prostaglandins, while COX inhibitors constrict it. As aspirin inhibits COX, it has been postulated that maternal use of aspirin late in pregnancy may lead to preterm constriction of the ductus arteriosus, leading to abnormalities in pulmonary vasculature that would promote pulmonary hypertension in the newborn. Few data are available, however, as abnormalities of pulmonary vasculature are not easy to diagnose, even at routine autopsy (Hertz-Picciotto *et al.*, 1990).

Congenital anomalies. Studies of the association between maternal aspirin use in the first trimester and congenital anomalies are summarized in Table 10. The studies are difficult to compare because of differences in the definition and characterization of anomalies. The only statistically significant increases associated with reported aspirin use were for congenital heart defects (Rothman *et al.*, 1979; Zierler & Rothman, 1985) and for orofacial clefts (Saxén, 1975).

Congenital abnormalities were not found to be associated with aspirin use during the first 14 days of pregnancy (Nelson & Forfar, 1971) or during the first trimester (Weatherall & Greenberg, 1979).

In a comparison of prescriptions for 764 mothers whose children had a defect of the central nervous system with those of an equal number of mothers of control babies, aspirin use for the three months before the last menstrual period and for the first trimester of pregnancy was not associated with increased risk (Winship *et al.*, 1984). This finding was corroborated for all congenital defects in a similar study of prescribed analgesics (Hill *et al.*, 1988), and by McDonald (1994) for use of any analgesic during the first trimester.

The study of Rothman *et al.* (1979) was designed to investigate the relationship between exposure to exogenous sex hormones and congenital heart disease. A total of 460 cases was ascertained among infants born in Massachusetts, USA, during the period 1973–75. Most of the cases were ascertained from a programme designed to provide special care to infants born in New England with serious congenital heart disease, the diagnosis being determined by cardiac catheterization, surgery or, ultimately, autopsy. The remaining cases were ascertained from death certificates. The control series comprised 1500 births selected randomly from all births in Massachusetts during the study period. At the end of each year, questionnaires were sent to the mothers of control infants, and cases were identified from the specialized care programme. Mothers of cases identified from death certificates were interviewed by telephone. Data were obtained on 390 cases (85% of those eligible) and 1254 controls (89%). The RR associated with aspirin consumption at about the time the pregnancy began was 1.3 (one-tailed $p = 0.02$). The authors noted that information about use of non-hormonal drugs was obtained from an open-ended questionnaire and therefore may have been subject to some recall bias. For the cases in which a specific diagnosis was known, reported medication was compared for the major diagnostic

Table 10. Association between maternal aspirin use in the first trimester and congenital malformations

Area and period of study	Cases		Controls or cohort		Aspirin		Prevalence of use in controls	Association with aspirin			Reference
	Type[a]	No.	Type	No.	Source	Method of assessing use[b]		Contrast	RR	95% CI	
UK, South Wales, 1964–66	CNS	279	Population	279	Any	IR, MR	17	Any versus none	1.6[c]	1.1–2.4	Richards (1969)
	CVS	100		100			15		0.8[c]	0.4–1.9	
	Ali	173		173			13		2.2[c]	1.3–3.9	
	MSK	152		152			14		1.8[c]	1.0–3.3	
	All	833		833			14		1.7[c]	1.3–2.2	
UK, Scotland (3 maternity units over 2 years)	Major	175	Hospital	916	Any	Q, Pres	3	Any versus none	1.2	0.6–2.5	Nelson & Forfar (1971)
	Minor	283					2		1.7	1.0–2.9	
	All	458					5		1.5	0.9–2.4	
Finland, 1967–71	ICP	232	Population	226	Any (salicy-lates)	IA, IR	6	Any versus none	1.9	0.9–3.8	Saxén (1975)
	ICL(P)	232		230			5		4.5	2.3–8.6	
	CLP	599		590			6		2.5	1.6–3.7	
USA, multicentre, 1959–65	CNS	266	Cohort	50 282	Any	IA	30[d]	Heavy[e] versus none	0.8[f]		Slone et al. (1976)
	CVS	404							0.9[f]		
	MSK	395							1.0[f]		
	RT	218							1.0[f]		
	GI	301							1.2[f]		
	Major	1393							0.9[f]	0.8–1.1	
USA, Massachusetts, 1973–75	CVS	390	Population	1254	Any	Q	16[g]	Any versus none	1.3	1.0–1.8	Rothman et al. (1979)
UK, England and Wales	Serious[h]	836	General population	836	Prescribed	MR	1.3	Any versus none	1.7	0.7–4.2	Weatherall & Greenberg (1979)
UK, England and Wales, 1971	CNS	764	General population	764	Prescribed	MR	0.4	Any versus none	2.7	0.6–16	Winship et al. (1984)

Table 10 (contd)

Area and period of study	Cases		Controls or cohort		Aspirin		Prevalence of use in controls	Association with aspirin			Reference
	Type[a]	No.	Type	No.	Source	Method of assessing use[b]		Contrast	RR	95% CI	
USA, Massachusetts, 1980–83	CVS	298	Population	738	Any	IR, MR	9	Any versus none	1.4	1.0–2.0	Zierler & Rothman (1985)
UK, England and Wales, 1983–84	CLP LRD	676 115	Population	676 115	Prescribed analgesics	MR	4 4	Any versus none	1.3 0.6	0.8–2.1 0.1–2.5	Hill et al. (1988)
USA and Canada, multicentre, 1976–86	CVS	1381	Other [i]	6966	Any	IR	27	Any versus none	0.9	0.8–1.1	Werler et al. (1989)
Canada, Montreal, 1987–94	Major Minor	787 2386	Hospital	787 2386	Any analgesic	IR	17	Any versus none	1.1 1.3		McDonald (1994)

[a] CNS, central nervous system; CVS, cardiovascular system; Ali, alimentary system (including orofacial clefts): MSK, musculoskeletal system; ICP, cleft palate without other anomaly; ICL(P), cleft lip ≥ palate without other anomaly; CLP, all orofacial clefts, with or without anomalies of other systems; RT, respiratory tract (including orofacial clefts); LRD, limb reduction defects

[b] IR, maternal interview after delivery of index child; MR, review of medical records; Q, questionnaire; Pres, prescription information; IA, maternal interview at antenatal clinic visit

[c] Crude unmatched analysis of matched data

[d] Drug use: prevalence of heavy use, 10%

[e] Defined as taken for at least eight days during at least one of the first four lunar months

[f] Adjusted for range of potentially confounding variables

[g] In periconceptional period

[h] 23% had neural tube defects, 7% limb malformations, 49% oral clefts and 21% other serious malformations

[i] 90% confidence interval

categories. The RR for transposition of the great arteries associated with use of aspirin was 3.3 (90% CI, 1.7–6.6, based on 12 exposed cases).

Zierler and Rothman (1985) carried out a further study of severe congenital heart disease and maternal use of medications in early pregnancy in Massachusetts during the period 1980–83. Congenital heart disease was considered to be severe if the diagnostic and therapeutic procedures included cardiac catheterization or surgery during the first year of life, or if death occurred before the first birthday. Controls were randomly selected from birth certificates filed in Massachusetts. Maternal interviews were completed for 298 cases (68% of those potentially eligible) and 738 controls (79% of those eligible). A positive association was found with reported aspirin use during the first trimester (RR, 1.4; 90% CI, 0.95–2.0). The most frequently reported indications for aspirin use were fever, cold and flu symptoms. The mothers of affected children reported use of aspirin for controlling fever more often than mothers of controls. Confounding by maternal illness did not account for the relationship with aspirin. Because of concern about potential recall bias, the authors also considered information on exposure from obstetric records. The RR associated with use of aspirin was 2.4 (95% CI, 0.6–10). [The Working Group noted that information on aspirin obtained over the counter would be unlikely to be recorded in obstetric records, and that no information was given on the numbers of cases and controls exposed according to these records.] When the data on specific types of cardiac anomalies among the cases were analysed, the RR, adjusted for use of other drugs, reported symptoms and laboratory reports of infection, were 2.4 (90% CI, 0.9–6.4) for aortic stenosis; 3.1 (1.2–8.0) for coarctation of the aorta; 3.2 (1.3–7.5) for hypoplastic left ventricle and 1.7 (0.9–3.7) for transposition of the great arteries.

Werler et al. (1989) analysed data from an on-going surveillance case–control study of birth defects in relation to exposure to drugs and other environmental factors in centres in Greater Boston and Philadelphia, USA, and south-eastern Ontario, Canada, during the period 1976–86 and in five counties in Iowa, USA, in 1983–85. Data on medications taken for various indications were obtained by maternal interview. Cases with identified syndromes that included cardiac abnormalities such as Down syndrome and Holt-Oram syndrome, were excluded. More than 80% of cases and 80% of controls (mothers of infants with non-cardiac malformations) participated. No association was seen between reported aspirin use during the first trimester and total cardiac defects. In addition, no association was apparent for specific defects, comprising aortic stenosis, coarctation of the aorta, hypoplastic left ventricle, transposition of the great arteries or conotruncal defects.

No association between use of aspirin and anomalies of the cardiovascular system was found in a case–control study in South Wales, United Kingdom (Richards, 1969), or in a multicentre cohort study in the USA (Slone et al., 1976). The latter study is the only one in which information relevant to the investigation of a possible dose–response relationship was available. Another analysis of the data collected in the latter study related to specific types of congenital anomaly (Heinonen et al., 1977). The RRs, adjusted for hospital of delivery, were 2.4 (eight exposed cases) for aortic stenosis, 1.5 (seven exposed cases) for transposition of the great arteries, 2.1 (17 exposed cases) for coarctation of the aorta and 3.0 (eight exposed cases) for the tetralogy of Fallot.

A case–control study of orofacial clefts in Finland during the period 1967–71, in which information on exposure was obtained prospectively from prenatal clinic records and retrospectively by interview by the midwife during the mother's first visit to the maternity welfare centre after delivery, suggested a positive association with maternal exposure to salicylates (Saxén, 1975). This was largely accounted for by exposure during the first trimester. [The Working Group noted that, as positive associations were also seen with use of other antipyretic analgesics, opiates (mainly codeine) and penicillins, confounding by pyrexia may have occurred.]

No association between the prevalence of orofacial clefts and aspirin use during the first trimester was found in the multicentre cohort study in the USA (Heinonen et al., 1977). RRs greater than 3 were associated with any exposure to aspirin during the first trimester and the prevalence of rachischisis or cranioschisis, situs inversus or dextrocardia, other adrenal syndromes, abnormal hands and fingers and miscellaneous foot abnormalities. Multivariate analyses were not carried out for specific defects, and these observations may be chance findings. No elevation in risk was found for the broader categories of anomalies considered in the analysis of aspirin categorized by degree of exposure (Slone et al., 1976; Heinonen et al., 1977).

In two of the randomized trials of use of low doses of aspirin during pregnancy, information on follow-up of the offspring was reported (Parazzini et al., 1994; CLASP Collaborative Group, 1995). No difference in the frequency of congenital anomalies was found.

Morbidity later in childhood. In two cohort studies, the relationship between use of aspirin during pregnancy and the IQ of the offspring was investigated (Hertz-Picciotto et al., 1990). In one of these, use of aspirin at least seven times per week was associated with a 10-point lower mean IQ for girls and 1.3-point lower mean IQ for boys at the age of four. Use of aspirin was also related to attention decrement. The credibility of these findings may be strengthened by the lack of association between use of acetaminophen (paracetamol) and either IQ or attention decrement (Streissguth et al., 1987). No association between aspirin use and IQ at the age of four was observed in a multicentre cohort study in the USA (Klebanoff & Berendes, 1988).

An 18-month follow-up of children born to women participating in the Italian Study of Aspirin and Pregnancy (1993) was undertaken by postal questionnaire. Information was obtained on 427 children (72%) of the women given aspirin and 361 (73%) of those who received no treatment. No difference between the groups was apparent in height, weight, respiratory, hearing or vision problems or other disorders. In addition, there was no difference between the groups in terms of the gross or fine motor and language development of the child (Parazzini et al., 1994).

Follow-up of children born to women in the trial of the CLASP Collaborative Group (1994) was restricted initially to children in the United Kingdom and Canada (CLASP Collaborative Group, 1995). Thus, 4168 children in the United Kingdom were assessed at 12 months from information provided by general practitioners and 4365 children in the United Kingdom and Canada were assessed at 18 months from responses to a questionnaire by their parents. The response rate at 12 months was 89% and that at 18 months, 86%. There were no differences between the groups in the frequency of hospital visits during the first 18 months of life for motor deficits, developmental delay, respiratory problems or bleeding. In addition, there were no differences in the proportion of children whose height or weight was below the third centile, in the frequency of abnormal gross motor, fine motor or language development or in the prevalence of abnormal sleep patterns, and there were no differences in feeding problems, mood, behaviour, hearing, vision or respiratory symptoms.

7.1.2 Experimental animals
(a) Gastrointestinal tract toxicity
It has been shown in animal models that aspirin is harmful to the gastric mucosa and is associated with irritation, ulceration and bleeding of the stomach (Kauffman, 1989). These studies have indicated two broad mechanisms by which aspirin causes gastric mucosal damage: one related to inhibition of COX activity and the other independent of the effect of aspirin on COX.

Gastric toxicity due to COX inhibition. Aspirin makes the gastric mucosa more susceptible to injury, inhibits mucus and bicarbonate secretion, alters the physiochemical nature of mucus, stimulates fundic but not antral ^{3}H-thymidine incorporation and makes the epithelial surface less hydrophobic (reviewed by Kauffman, 1989).

Aspirin given intravenously or intragastrically at 60 mg/kg bw per h to rats inhibited COX activity and caused macroscopic mucosal injury; these two effects were well correlated (Konturek et al., 1981). Exogenous prostaglandin E_2 and I_2 completely prevented this mucosal injury. In addition, dose–response studies with lower doses of aspirin showed a significant correlation between COX inhibition and mean ulcer area. Further support for the hypothesis that reduction of endogenous prostaglandins predisposes the mucosa to injury comes from studies in which rabbits were immunized with prostaglandin E_2-thyro-globulin conjugate (Olson et al., 1985; Redfern et al., 1987), which resulted in the production of circulating prostaglandin E_2 antibodies. Gastrointestinal ulcers occurred as early as six weeks after the beginning of immunization. These observations support the notion that endogenous prostaglandins are important for the maintenance of mucosal defence and that their inhibition is an important mechanisms of aspirin-induced mucosal injury.

Gastric toxicity independent of COX inhibition. In most of the studies described below, the similarity of the effects of aspirin and salicylic acid was used to deduce that the mechanism of action of aspirin is not via COX inhibition, since salicylic acid is a less efficient COX inhibitor (Mitchell et al., 1994). As aspirin is rapidly deacetylated to salicylic acid in cells, the half-life of aspirin in interstitial fluid and serum after oral administration is short. Salicylic acid is, however, toxic to cells and affects epithelial function. Salicylates are associated with mucosal barrier injury, resulting in large net cation fluxes, hydrogen back-diffusion and a fall in the transmucosal difference in potential. The mucosal content of ATP is reduced by salicylic acid, affecting ion transport and increasing proton dissipation from surface epithelial cells (reviewed by Kauffman, 1989).

The effects of single and repeated doses of aspirin have been examined by light and electron microscopy. The gastric mucosa of dogs showed dose-dependent epithelial damage and haemorrhage into the lamina propria within 4 h after intraluminal administration of aspirin

(Lev et al., 1972). The damage was more pronounced after one than four weeks, suggesting adaptation to repeated administration of aspirin. Electron microscopic evaluation of the gastric mucosa of mice exposed to 20 mmol/litre aspirin in 1, 10 or 100 mmol/litre HCl for 8 min showed many lysed and exfoliated surface epithelial cells and swelling and disruption of all cells, with enlarged nuclei and clumped nuclear chromatin (Fromm, 1976). Davenport (1967) demonstrated that the gastric mucosal barrier of rats is lost after exposure to either aspirin or salicylic acid. Both drugs increase the proton flux into the mucosa and cause a drop in potential difference, a sensitive measure of epithelial integrity. The back-diffusion of protons leads to cellular acidification and altered cellular metabolism. Studies in rats (Rowe et al., 1987) and rabbits (Fromm & Kolis, 1982) suggest, however, that aspirin and salicylic acid damage the mucosa equally, but only after the gastric luminal pH has been lowered to 1.0. Guinea-pig gastric mucosa perfused with 2 mmol/litre aspirin at pH 4 showed a fall in transmucosal potential, a reduction in ATP content and acid secretion and an increase in proton back-diffusion (Ohe et al., 1980). These observations suggest that one action of aspirin is to damage the energy metabolism of mucosal cells, causing cell death with resultant back-diffusion of protons.

Membrane transport mechanisms are also affected by salicylates. Observations on rabbit antral mucosa exposed to aspirin or salicylic acid at low pH (Fromm, 1976; Kuo & Shanbour, 1976) suggest that salicylates inhibit active ion transport in the gastric mucosa, possibly through decreased ATP production as a result of either enzyme inhibition or their uncoupling effect.

(b) Nephrotoxicity
Aspirin causes a wide spectrum of renal damage including nephrotic syndrome, acute interstitial nephritis, acute tubular necrosis, acute glomerulonephritis and nonspecific renal failure (Clive & Stoff, 1984; Carmichael & Shankel, 1985).

Chronic administration of aspirin at 120–500 mg/kg per day to rats over 18–68 weeks caused renal papillary necrosis and decreased urinary

concentrating ability (Burrell *et al.*, 1991; D'Agati, 1996); however, some investigators have been unable to induce renal papillary necrosis in other species or in rats at lower divided doses, as used in humans (Owen & Heywood, 1986). In a variety of rat strains, administration of aspirin as a single high dose intravenously or by gavage produced acute necrosis of the proximal tubules, rarely accompanied by renal papillary necrosis in susceptible strains like homozygous Gunn rats (Axelsen, 1980; Mittman *et al.*, 1985). This strain of rat, which has an inactivating mutation in one glucuronyl transferase relevant to aspirin, develops renal papillary necrosis after a single oral dose of aspirin (Axelsen, 1976).

A study of administration of 500 mg/kg ^{14}C-aspirin to 3- and 12-month-old male rats showed an age-dependent effect of aspirin on the kidneys (Kyle & Kocsis, 1985). Aspirin induced proximal tubular necrosis in the older animals but only mild, nonspecific cellular changes in the younger group. In addition, the mitochondrial pathway for salicylurate synthesis was significantly inhibited in the older animals. These findings suggest that mitochondrial injury plays an important role in the development of salicylate-induced proximal tubular necrosis.

(c)   Hepatotoxicity

Aspirin-induced liver injury probably has an immunological basis, but neither the detailed mechanism nor the precise incidence rates are known. Studies in mice (Cai *et al.*, 1994, 1995) have demonstrated that long-term treatment with aspirin leads to increased proliferation of hepatic peroxisomes. According to the oxidative stress hypothesis (Reddy & Lalwani, 1983), peroxisome proliferation results in excess formation of hydrogen peroxide, which induces lipid peroxidation. In fact, treatment of mice (Cai *et al.*, 1995) and rats (Goel *et al.*, 1986) with aspirin induced a 1.3-fold increase in basal hydrogen peroxide levels in liver homogenates. It is possible that products of lipid peroxidation interact with DNA, leading to adducts, DNA strand breaks and DNA–protein cross-linking (Vaca *et al.*, 1988).

Long-term treatment of rats with peroxisome proliferators like aspirin decreases the cellular antioxidant defenses (Glauert *et al.*, 1992).

Thus, aspirin may initiate or promote neoplastic change by installing a long-term imbalance between cellular oxidative stress and antioxidant defence.

(d)   Ototoxicity

Aspirin causes mild to moderate, temporary hearing loss in laboratory animals. The deficit is detected behaviourally or electrophysiologically (Boettcher & Salvi, 1991). There appear to be some species differences in susceptibility to salicylate ototoxicity, presumably due in part to differences in the pharmacokinetics of salicylates among species (Myers & Bernstein, 1965; Gold & Wilpizeski, 1966; Eddy *et al.*, 1976). The hearing loss caused by aspirin is accompanied by supra-threshold changes in hearing, including a decrease in temporal integration, poorer frequency selectivity and poorer temporal resolution. The supra-threshold changes are not severe and appear to be quite variable among subjects. Animals exposed to both noise and aspirin have greater hearing loss and cochlear damage than those exposed to noise alone (Eddy *et al.*, 1976; Carson *et al.*, 1989).

(e)   Reproductive and developmental effects

Reproductive effects. Administration of aspirin to adult male rats at a dose of one-tenth of the LD_{50} value for six weeks was accompanied by a decrease in the functional activity of spermatozoids. Repeated inhalation of a concentration of 25 mg/m^3 for four months produced morphological changes in the spermatogenic epithelium and abnormal antenatal development of the progeny (Vasilenko *et al.*, 1979).

Developmental toxicity. Congenital malformations were induced in rats by salicylate poisoning of the mother while the embryos were in early stages of development (Warkany & Takacs, 1959). Among the defects produced were craniorachischisis with well-preserved cerebral and spinal nervous tissues. Other malformations encountered were anencephaly, hydrocephaly, facial clefts, gastroschisis and irregularities of the vertebrae and ribs.

The doses of aspirin most commonly used in animal models are 250–1000 mg/kg of maternal weight. In all species, these doses usually cause

malformations in the surviving fetuses, 25–80% of which are affected. The malformations include cleft lip and palate, hydrocephaly, gastroschisis and skeletal dysplasias (Lubawy & Burriss Garrett, 1977). In mice, aspirin at high doses (500 mg/kg) over a 24-h period on days 8 and 9 or 9 and 10 of gestation induces cleft lip (Trasler, 1965).

When rat embryos were cultured with 100–300 mg/ml salicylic acid, decreases in crown–rump length, somite number and yolk sac diameter are observed (Joschko et al., 1993). A significant increase in the prevalence of malformations is seen, including anomalies of the eye, branchial arch and heart and an absence of forelimb buds. The neural tube is especially vulnerable and frequently fails to close. Cellular and ultrastructural examination reveals extensive cell death in the neuroepithelium, with a lesser effect on mesenchymal cells. It is likely that the extensive cell necrosis and blebbing in the developing neuroepi-thelium at the site of neural tube fusion are involved in failed neurulation, while necrosis at other sites in the cranial neuro-epithelium is linked with intellectual and behavioural abnormalities.

Salicyclic acid, the product of aspirin hydrolysis, is considered to be the causative agent in teratogenicity associated with aspirin (Kimmel et al., 1971; Koshakji & Schulert, 1973). Early studies (Goldman & Yakovac, 1963) showed that the teratogenic action of salicylate compounds occurs through maternally mediated metabolic factors; however, more recent studies with fluorimetric techniques (Kimmel et al., 1971) and culture in vitro (McGarrity et al., 1981) demonstrated that aspirin has a direct effect in the rat embryo in the absence of any maternal influence.

The teratogenicity of aspirin may be mediated via its obvious target, COX, and altered prostaglandin synthesis (Vane, 1971), interference with oxidative phosphorylation (Bostrom et al., 1964) or altered biosynthesis of nucleic acids and proteins (Janakidevi & Smith, 1970). Alternative explanations include a direct effect of salicylic acid on cell membranes, particularly those close to the mesenchyme (Joschko et al., 1993).

Doses of 250–1000 mg/kg of maternal weight cause a high rate of embryo deaths and still-births in all animal species, and fetuses of rats given 125 or 250 mg/kg bw per day of aspirin were shorter and weighed less than those from control rats (Lubawy & Burriss Garrett, 1977).

When aspirin was co-administered with ethanol, as in a study in TO mice (Padmanabhan et al., 1994), it significantly reduced the rate of prenatal ethanol-induced mortality. Pre-administration of a low dose (150 mg/kg bw) of aspirin reduced the ethanol-induced exencephaly, while a higher dose (200 mg/kg bw) increased the incidence of this malformation.

7.2 Genetic and related effects
7.2.1 Humans
No data were available to the Working Group.

7.2.2 Experimental models
The results of tests for genetic and related effects in model systems are summarized in Table 11 and Figure 4. The genetic and related effects of aspirin have been reviewed (Giri, 1993). Aspirin induced DNA damage in Bacillus subtilis (Kawachi et al., 1980; Kuboyama & Fujii, 1992) but not in Escherichia coli (King et al., 1979) or in Salmonella typimurium tester strains (Bruce & Heddle, 1979; King et al., 1979; Bartsch et al., 1980; Kawachi et al., 1980; Oldhham et al., 1986; Jasiewicz & Richardson, 1987; Kuboyama & Fujii, 1992). It did not induce sex-linked recessive lethal mutation in Drosophila melanogaster or mutations in a host-mediated assay (King et al., 1979), and it did not induce mutation, cell transformation (Patierno et al., 1989), aneuploidy (Watanabe, 1982; Ishidate, 1988) or micronucleus formation (Dunn et al., 1987) in mammalian cells in vitro. Mixed results were reported for chromosomal aberrations in mammalian cells in vitro (Meisner & Inhorn, 1972; Kawachi et al., 1980; Watanabe, 1982; Ishidate, 1988; Muller et al., 1991). It induced chromosomal aberrations (Kawachi et al., 1980) but not micronuclei (Bruce & Heddle, 1979; King et al., 1979) in bone-marrow cells of rodents treated in vivo. It did not induce sperm abnormalities in mice in vivo (Bruce & Heddle, 1979).

Table 11. Genetic and related effects of aspirin

End-Test-point code		Test system	Results[a] − +	Dose[b] (LED or HID)	Reference
D	BSD	B. subtilis rec, differential toxicity	+ 0	0.00	Kawachi et al. (1980)
D	BSD	B. subtilis rec, differential toxicity	(+) 0	5000	Kuboyama & Fujii (1992)
G	SA5	S. typhimurium TA1535, reverse mutation	− −	250	Jasiewicz & Richardson (1987)
G	SA5	S. typhimurium TA1535, reverse mutation	− −	1800	King et al. (1979)
G	SA5	S. typhimurium TA1535, reverse mutation	− −	250	Oldham et al. (1986)
G	SA7	S. typhimurium TA1537, reverse mutation	− −	250	Bruce & Heddle (1979)
G	SA7	S. typhimurium TA1537, reverse mutation	− −	250	Jasiewicz & Richardson (1987)
G	SA7	S. typhimurium TA1537, reverse mutation	− −	1800	King et al. (1979)
G	SA7	S. typhimurium TA1537, reverse mutation	− −	250	Oldham et al. (1986)
G	SA8	S. typhimurium TA1538, reverse mutation	− −	250	Jasiewicz & Richardson (1987)
G	SA8	S. typhimurium TA1538, reverse mutation	− −	1800	King et al. (1979)
G	SA8	S. typhimurium TA1538, reverse mutation	− −	250	Oldham et al. (1986)
G	SA9	S. typhimurium TA98, reverse mutation	− −	250	Bruce & Heddle (1979)
G	SA9	S. typhimurium TA98, reverse mutation	− −	250	Jasiewicz & Richardson (1987)
G	SA9	S. typhimurium TA98, reverse mutation	− −	0	Kawachi et al. (1980)
G	SA9	S. typhimurium TA98, reverse mutation	− −	1800	King et al. (1979)
G'	SA9	S. typhimurium TA98, reverse mutation	− −	27	Kuboyama & Fujii (1992)
G	SA9	S. typhimurium TA98, reverse mutation	− −	250	Oldham et al. (1986)
G	SA0	S. typhimurium TA100, reverse mutation	− −	180	Bartsch et al. (1980)
G	SA0	S. typhimurium TA100, reverse mutation	− −	250	Bruce & Heddle (1979)
G	SA0	S. typhimurium TA100, reverse mutation	− −	250	Jasiewicz & Richardson (1987)
G	SA0	S. typhimurium TA100, reverse mutation	− −	0	Kawachi et al. (1980)
G	SA0	S. typhimurium TA100, reverse mutation	− −	1800	King et al. (1979)
G	SA0	S. typhimurium TA100, reverse mutation	− −	27	Kuboyama & Fujii (1992)
G	SA0	S. typhimurium TA100, reverse mutation	− −	250	Oldham et al. (1986)
G	ECK	E. coli K12, forward or reverse mutation	− −	1800	King et al. (1979)
G	DMX	D. melanogaster, sex-linked recessive lethal mutation	− 0	1800	King et al. (1979)
G	GIA	Mutation, other animal cells in vitro	− 0	3000	Patierno et al. (1989)
M	MIA	Micronucleus test, animal cells in vitro	− 0	3600	Dunn et al. (1987)
C	CIC	Chromosomal aberration, Chinese hamster cells in vitro	+ 0	0.00	Ishidate (1988)
C	CIC	Chromosomal aberration, Chinese hamster cells in vitro	+ 0	15.6	Ishidate (1988)
C	CIC	Chromosomal aberration, Chinese hamster cells in vitro	− +	0.00	Kawachi et al. (1980)
C	CIC	Chromosomal aberration, Chinese hamster cells in vitro	− 0	1800	Muller et al. (1991)
A	AIA	Aneuploidy, animal cells in vitro	− −	1500	Ishidate (1988)
T	TCM	Cell transformation, C3H10T1/2 cells in vitro	− 0	3000	Patierno et al. (1989)
C	CHF	Chromosomal aberration, human fibroblasts in vitro	− 0	250	Meisner & Inhorn (1972)
C	CHL	Chromosomal aberration, human lymphocytes in vitro	+ 0	75	Watanabe (1982)
A	AIH	Aneuploidy, human cells in vitro	− 0	300	Watanabe (1982)
H	HMM	Host-mediated assay, microbial cells	− 0	180	King et al. (1979)
M	MVM	Micronucleus formation, mice in vivo	− 0	1000	Bruce & Heddle (1979)
M	MVM	Micronucleus formation, mice in vivo	− 0	360	King et al. (1979)
C	CBA	Chromosomal aberration, animal bone marrow in vivo	+ 0	0.00	Kawachi et al. (1980)
P	SPM	Sperm morphology, mice in vivo	− 0	1000	Bruce & Heddle (1979)

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 done

[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

Figure 4. Profile of genetic and related effects of aspirin
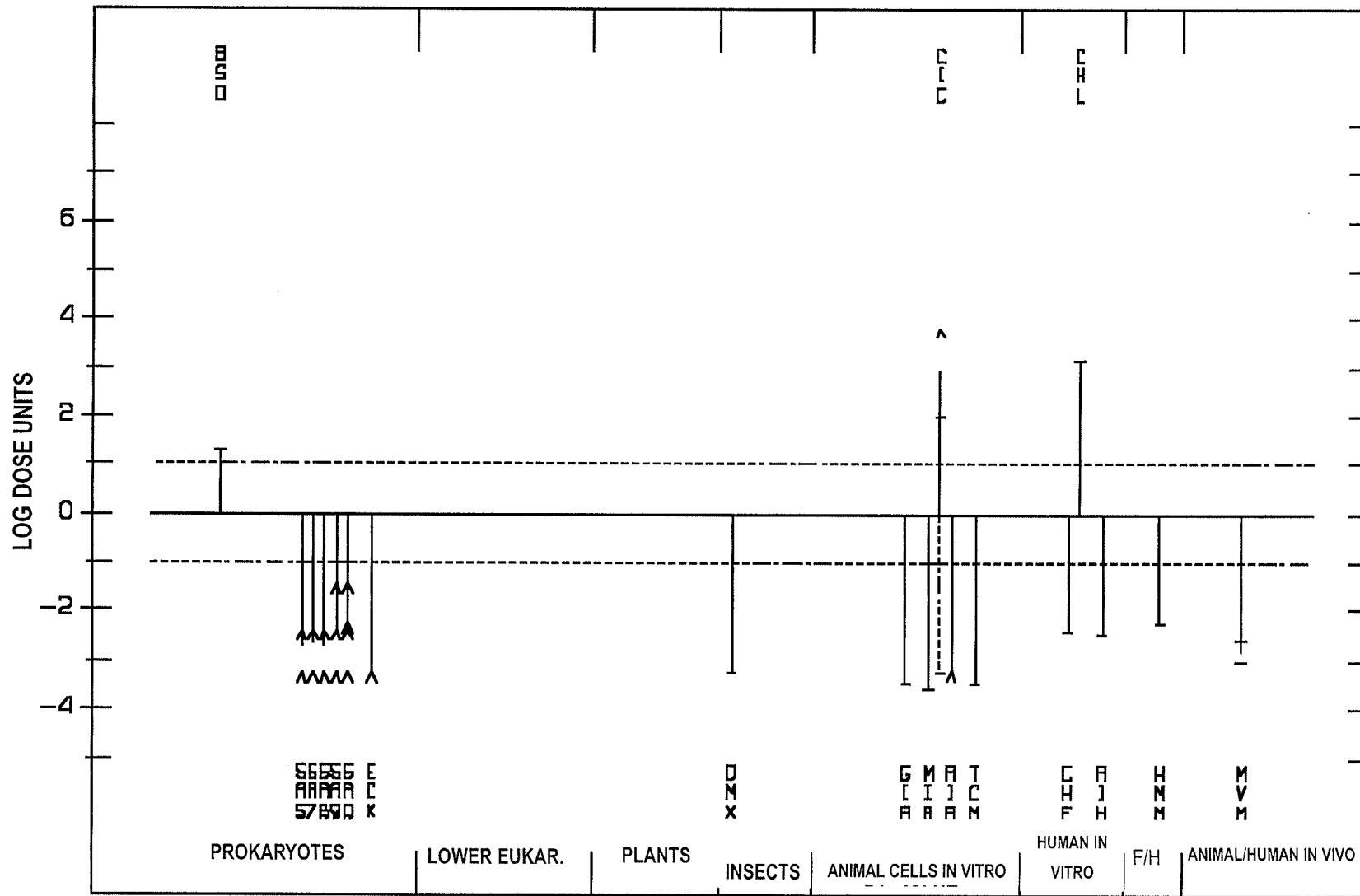
8. Summary of Data

8.1 Chemistry, occurrence and human exposure

Aspirin has been used for nearly a century as an analgesic, anti-inflammatory and antipyretic agent. It is used in the treatment of rheumatism, fever and related conditions; it is also used in the prevention of arterial and venous thrombosis. The doses of aspirin usually used vary according to the therapeutic indication. Low doses (75–300 mg/day) are conventionally used in the prophylaxis of cardiovascular disease; daily doses up to 5 g are used in the treatment of pain and fever. Doses up to 8 g three or four times daily are used in the management of rheumatic complaints. In view of its ready availability, low cost and efficacy in a wide range of conditions, aspirin consumption is common and widespread.

8.2 Metabolism and kinetics

Aspirin is rapidly hydrolysed to salicylic acid in the intestinal wall, liver and erythrocytes. Aspirin is absorbed rapidly after oral administration. Both aspirin and salicylate are partially bound to proteins, especially albumin. Salicylate reaches the synovial fluid, the cerebrospinal fluid, saliva, breast milk and the fetus. Hydrolysis of aspirin to salicylate in the plasma occurs with a half-life of 15–20 min. Salicylate is removed by renal elimination and formation of metabolites. The hydrolysis of aspirin is not altered by concurrent administration of other drugs.

8.3 Cancer-preventive effects
8.3.1 Humans

(a) Colorectal cancer
Most of the data on aspirin and colorectal cancer come from epidemiological studies of the disease in the general population. In several studies, use of aspirin was not separated from use of other non-steroidal anti-inflammatory drugs. Relevant data are also available with respect to adenomatous colorectal polyps, which are thought to be precursor lesions for colorectal cancer. In a few studies, adenomatous and hyperplastic polyps were not distinguished.

Overall, the observational epidemiological studies cover more than 18 000 cases of colorectal cancer and more than 2000 cases of adenomatous polyps. The studies differ in design, location, population and motivating hypothesis. In 12 studies (four cohort and eight case–control) out of 13 that assessed the risk for colorectal cancer, there was a reduced risk associated with sustained aspirin use. In nine of these 12 studies, the reduction in risk was statistically significant, with relative risks of 0.4–0.6 in regular users in comparison with non-users. In all six studies on adenomatous colorectal polyps (two cohort and four case–control), there was a reduction in risk with aspirin use, which was statistically significant in four of them. In addition, two studies on patients with rheumatoid arthritis showed a significant reduction in colorectal cancer incidence in comparison with general population rates.

While information on the duration of treatment and dose of aspirin that are necessary for prevention is limited, the prophylactic effect appears to increase with increasing duration of use.

The observational studies are sufficiently large, taken together, and consistent, despite their diversity, that chance alone cannot explain their results. The Working Group considered several possible problems in the interpretation of the evidence, including publication bias, detection bias, bias due to indications for use of aspirin, genetic predisposition, other confounding factors and problems in the measurement of aspirin use. There is no obvious bias or confounding that could explain the findings. Collectively, the published observational studies provide consistent evidence that aspirin may inhibit one or more stages in the development of colorectal cancer.

The negative findings in the randomized US Physicians' Health Study neither support nor convincingly refute the aspirin–colorectal cancer prevention hypothesis. Factors that could obscure protection by aspirin against colorectal polyps or cancer in this trial include its short duration (randomized aspirin treatment being stopped after a mean of five years) and the small number of cases of cancer expected.

(b) Gastric cancer

Two population-based cohort studies addressed use of aspirin and gastric cancer. In the larger of these, a statistically significant inverse trend for gastric cancer was found with aspirin use. In one of two other population-based cohort studies of similar size and design, in which a diagnosis of rheumatoid arthritis was used as a proxy for exposure to non-steroidal anti-inflammatory drugs, rheumatoid arthritis was associated with a significantly lower risk for gastric cancer. In the other study, no such relationship was found.

(c) Oesophageal cancer

Three studies addressed the relationship between aspirin use and oesophageal cancer. One of these studies demonstrated a statistically significant inverse relationship.

(d) Breast cancer

No consistent association has been observed between use of aspirin and the occurrence of cancer of the breast in women. The larger studies generally found no association, but a reduced risk was reported in two of six cohort studies and in one of two case–control studies.

(e) Cancers at other sites

No reduction in the risks for cancers of the lung, bladder, prostate, buccal cavity, pharynx or genital system or melanoma was found to be associated with aspirin use in the few studies in which results were reported for cancers at these sites.

8.3.2 Experimental animals

Aspirin had chemopreventive activity in two of three studies in rat models in which colonic aberrant crypt foci were used as the end-point. In three studies with rat models of colon cancer, administration of aspirin significantly reduced the incidence and (in one case) the size of colon tumours. Aspirin inhibited hepatocarcinogenesis in four studies in rat models. In a single study in mice, aspirin was ineffective in inhibiting carcinogenesis in a urinary bladder model, but it inhibited bladder carcinogenesis in rats.

8.3.3 Mechanism of action

Aspirin can irreversibly inhibit cyclooxygenases, which may be involved in the initiation of carcinogenesis in three general ways: activation of carcinogens to DNA-binding forms, production of malondialdehyde and formation of peroxyl radicals.

Aspirin at high concentrations reduces the proliferation rate of cultured human colon cancer cells, alters their distribution in the various phases of the cell cycle, increasing the proportion of cells in G_0 or G_1 phase, and can induce apoptosis.

Non-cyclooxygenase-dependent mechanisms have been described that could play a role in its chemopreventive effects.

8.4. Other beneficial effects

Prophylactic aspirin therapy has been proven in large, reliable, randomized trials to reduce by about 25% the risk for myocardial infarct, stroke and vascular death in people with pre-existing cardiovascular conditions. The absolute benefits are greatest for patients with acute myocardial infarct and are smaller in patients with conditions posing a less acute risk. Two randomized trials of healthy people did not show any benefit of aspirin in preventing deaths from circulatory disease.

Although studies of clinically diagnosed Alzheimer disease and non-steroidal anti-inflammatory drugs consistently suggest a 50% reduction in risk, the temporal relationship between such exposure and disease development or progression has not been convincingly demonstrated.

8.5 Carcinogenicity
8.5.1 Humans

Many large-scale epidemiological studies have been conducted of aspirin use and cancer. There is no consistent evidence of an increased risk for cancer at any specific site.

8.5.2 Experimental animals

No data were available to the Working Group.

8.6 Toxic effects
8.6.1 Humans

Aspirin causes a dose-dependent increase, with no known lower threshold, in the incidence of

upper gastrointestinal toxicity, including dyspepsia, gastrointestinal haemorrhage, oesophageal and gastric erosion, perforation and death. Low doses (40 mg) of aspirin inhibit platelet aggregation and can increase the risk for non-fatal bleeding requiring transfusion. The risk for haemorrhagic stroke may also be increased by relatively low doses. A positive association has been observed between use of aspirin in children and Reye syndrome. Aspirin can precipitate or aggravate asthma. Maternal exposure to high but not low doses of aspirin prior to delivery has consistently been associated with bleeding in the mother and the newborn.

8.6.2 Experimental animals

Aspirin is harmful to the gastric mucosa and can result in irritation, ulceration and bleeding in the stomach. Aspirin induces a wide spectrum of renal damage. In rats, it causes papillary necrosis and decreased urinary concentrating ability or acute necrosis of the proximal tubules. Susceptibility to the nephrotoxicity of aspirin appears to be age-dependent. In mice, long-term treatment with aspirin is associated with proliferation of hepatic peroxisomes. High doses of aspirin cause mild to moderate temporary hearing loss, accompanied by changes in suprathreshold hearing, including a decrease in temporal integration, poorer frequency selectivity and poorer temporal resolution.

Congenital malformations can be induced in rats after administration of aspirin at high doses to dams while the embryos are in early stages of development, leading to a variety of anomalies. There is a clear correlation between ingestion of aspirin and congenital malformations, but these adverse effects occur at doses far in excess of those likely to be encountered in the therapeutic setting.

Aspirin induced chromosomal aberrations in Chinese hamster cells and in human lymphocytes *in vitro* in one study. It induced chromosomal aberrations in the bone marrow of rats treated *in vivo* in one study.

9. Recommendations for Research

Definitive evidence for chemopreventive activity nominally requires data from appropriately designed randomized trials. In practice, the duration that is necessary for aspirin to exert the sought-after effect may, for practical purposes, preclude the execution of the necessary randomized trials. Rather, it may be necessary to rely upon observational studies. Such studies can be performed using existing databases, and this possibility merits high priority.

Adenomatous polyps may be perceived as biomarkers for the probable development of colorectal cancer; however, confidence in this perception is progressively increased as the relationship between these lesions and the later stages of colorectal cancer is more fully understood. It is important, therefore, that studies of these biomarkers include, where possible, monitoring of subsequent disease development.

Cohort studies on sporadic cancer are needed, with prospective evaluation of aspirin use over long periods of time — 10 or more years. In addition, the Group identified the need for clinical trials of aspirin use for the prevention of recurrent or primary sproadic adenomas, of colorectal adenomas in patients with familial predisposition and of recurrent colorectal cancer after surgical treatment of a first case.

The Working Group was aware that certain trials are in progress, apart from published data cited in this volume. Obviously, the results of such trials will influence current evaluations of NSAIDs as cancer-preventive agents. The knowledge that trials are in progress mitigates against specific recommendations for future trial design; however, one matter that necessitates attention is determination of the lowest dose of aspirin necessary for effective chemopreventive activity. This parameter should be a specific concern in the design of future trials and/or observational studies.

10. Evaluation[1]

10.1 Cancer-preventive activity

10.1.1 Humans

There is *limited evidence* in humans that aspirin reduces the risk for colorectal cancer, based on the finding of a consistent moderate reduction in risk in observational studies. Individually, none of the potential sources of bias or confounding provides a reasonable explanation for the reduction in risk for colorectal cancer that has been reported; however, the possible cumulative effect of these issues, although not quantifiable, cannot be excluded. Therefore, the Working Group concluded that bias and confounding could not be ruled out with reasonable confidence.

10.1.2 Experimental animals

There is *sufficient evidence* for the cancer-preventive activity of aspirin in experimental animals. This evaluation is based on models of cancers of the colon and liver.

10.2 Overall evaluation

Epidemiological studies in humans provide *limited evidence* for the cancer-preventive activity of aspirin, based on over 20 observational studies (both cohort and case–control), which show a moderately reduced risk for colorectal cancer in people using aspirin regularly, and an indication of greater reduction in risk with prolonged use. In experimental animal models, there is *sufficient evidence* for the prevention of colon cancer by aspirin. Aspirin is toxic, especially at high doses, but beneficial effects for humans have been demonstrated at relatively low doses, especially a reduction in the risk for myocardial infarct and thrombotic stroke in populations with pre-existing disease. These findings indicate the need for more detailed research on cancer prevention, including controlled trials with different regimens of aspirin, including dose, route of administration, frequency and duration, and different cohorts, in an endeavour to determine whether aspirin

can be shown to be of greater benefit in reducing cancer in human populations than its possible off-setting toxicity. Detailed consideration of the total benefits in the prevention of cancer and other diseases in contrast to toxicity will be required before use of aspirin for the prevention of cancer in humans, particularly in asymptomatic populations, can be recommended.

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

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