



SOME DRUGS AND HERBAL PRODUCTS

VOLUME 108

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OF CARCINOGENIC RISKS
TO HUMANS

SULFASALAZINE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 599-79-1 ([O'Neil, 2001](#); [SciFinder, 2013](#))

Chem. Abstr. Serv. Name: Benzoic acid, 2-hydroxy-5-[2-[4-[(2-pyridinylamino)sulfonyl]phenyl]diazenyl] ([O'Neil, 2001](#); [SciFinder, 2013](#))

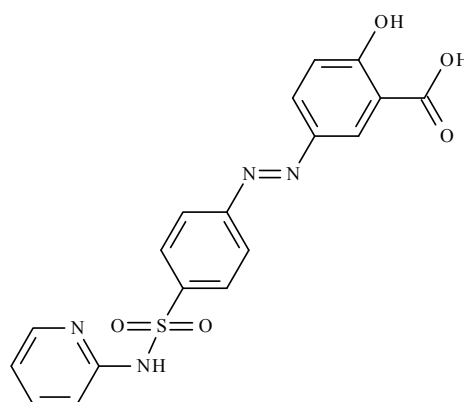
IUPAC systematic name: 2-Hydroxy-5-[2-[4-[pyridine-2-ylsulfonyl]phenyl]diazenyl]benzoic acid ([Lide, 2005](#); [European Pharmacopoeia, 2008](#))

United States nonproprietary name (USAN): Sulfasalazine

Synonyms: Benzoic acid, 2-hydroxy-5-[[4-[(2-pyridinylamino)sulfonyl]phenyl]azo]-; 5-[[p-(2-Pyridylsulamoyl)phenyl]azo] salicylic acid ([US Pharmacopeia, 2007, 2013](#))

See [WHO \(2007\)](#) for names in other languages.

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{18}H_{14}N_4O_5S$

Relative molecular mass: 398.39

From [O'Neil \(2001\)](#).

1.1.3 Chemical and physical properties of the pure substance

Description: Brownish-yellow, odourless crystals ([O'Neil, 2001](#); [European Pharmacopoeia, 2008](#)).

Melting-point: Decomposes at 240–245 °C ([O'Neil, 2001](#))

Density: 1.48 ± 0.1 g/cm³ at 20 °C ([SciFinder, 2013](#))

Spectroscopy data: Ultraviolet, mass, and nuclear magnetic resonance spectra have been reported ([McDonnell & Klaus, 1976](#); [European Pharmacopoeia, 2008](#))

Solubility: Practically insoluble in water, ether, benzene, chloroform; very slightly soluble in ethanol; soluble in alkali hydroxides ([McDonnell & Klaus, 1976](#); [O’Neil, 2001](#))

Octanol/water partition coefficient: Log $P = 3.88$ ([Rosenbaum, 2011](#))

Stability data: The compound did not degrade when dissolved in dimethylformamide and was subjected to thermal stress at 80 °C for 196 hours ([McDonnell & Klaus, 1976](#); [Jacoby, 2000](#))

- 2-hydroxy-3-[2-[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]benzoic acid
- 5-[2-[4',5-bis(pyridin-2-ylsulfamoyl)biphenyl-2-yl]diazenyl]-2-hydroxy benzoic acid
- salicylic acid
- 2-hydroxy-5-[2-(4-sulfophenyl)diazenyl]benzoic acid
- 4-amino-*N*-(pyridin-2-yl)benzenesulfonamide (sulfapyridine).

1.1.4 Technical products and impurities

(a) Trade names

Azulfidine EN-tabs; Azulfidine; Azaline; Sulfazine; Sulfazine EC; Apo-Sulfasalazine; PMS-Sulfasalazine; Salazopyrin En-Tabs; Salazopyrin; Azulfidina; Azulfin; Bomecon; Colo-Pleon; Disalazin; Falazine; Gastropyrin; Lazafin; Pyralin EN; Rosulfant; Salazine; Salazodin; Salazopirina; Salazopyrin Entabs; Salazopyrin-EN; Salazopyrina; Salazopyrine; Salivon; Salopyr; Salopyrine; Saridine-E; Sulcolon; Sulfasalazin; Sulfitis; Ulcol; Zopyrin ([Porter & Kaplan, 2013](#)).

(b) Impurities

Impurities as given in [European Pharmacopoeia \(2008\)](#):

- 4,4'-[(4-hydroxy-1,3-phenylene)bis(diazenediyl)]bis[*N*-(pyridin-2-yl)benzene sulfonamide
- 2-hydroxy-3,5-bis[2-[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]benzoic acid
- 2-hydroxy-5-[2-[4-(2-iminopyridin-1(2*H*)-yl)phenyl]diazenyl]benzoic acid
- 4-[2-(2-hydroxyphenyl)diazenyl]-*N*-(pyridin-2-yl)benzenesulfonamide
- 2-hydroxy-4'-(pyridin-2-ylsulfamoyl)-5-[2-[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]biphenyl-3-carboxylic acid

1.1.5 Analysis

Selected compendial and non-compendial methods of analysis are presented in [Table 1.1](#). Sulfasalazine in human plasma can be determined by high-performance liquid chromatography using ultraviolet detection ([Fukino et al., 2007](#)). It can also be analysed through liquid chromatography-tandem mass spectrometry in human plasma using electron spray ionization techniques in multiple reaction monitoring mode, with a limit of quantification of 10 ng/mL ([Gu et al., 2011](#)).

In urban water, sulfasalazine can be quantified by liquid chromatography-mass spectrometry using electron spray ionization. The limit of quantification is 65 ng/L ([Tuckwell et al., 2011](#)).

1.2 Production

1.2.1 Production process

Sulfasalazine does not occur in nature. Sulfasalazine is produced by reacting sulfanilamide with salicylic acid through a series of steps, with water as a solvent ([Novacek et al., 1991](#)).

1.2.2 Use

(a) Indications

Sulfasalazine is an aminosalicylate whose chief bioactive metabolite is 5-aminosalicylic acid (5-ASA). Sulfasalazine, mesalazine (5-ASA),

Table 1.1 Analytical methods for sulfasalazine

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
<i>Compendial methods</i>				
Assay	–	UV-visible spectroscopy Wavelength: 359 nm		European Pharmacopoeia (2008) , US Pharmacopoeia (2013)
Related substances	–	LC-UV Column: C ₁₈ Mobile phase A: sodium dihydrogen phosphate and sodium acetate in water Mobile phase B: mobile phase A : methanol (10 : 40) Flow rate: 1 mL/min Wavelength: 320 nm		European Pharmacopoeia (2008)
<i>Non-compendial methods</i>				
Biological samples:				
Human serum, breast milk	–	LC	2.5 or 1.3 µmol/L (LOD)	Esbjörner et al. (1987)
Human plasma	Centrifugation	LC-UV Column: C ₁₈ Mobile phase: 35% acetonitrile in 25 mM phosphate buffer pH 3.0 Flow rate: 0.12 mL/min Wavelength: 365 nm		Fukino et al. (2007)
Human plasma	Protein precipitation using methanol treatment, solid phase extraction (1 mL of methanol containing 5% ammonia)	ELISA Buffer solutions: phosphate buffer, carbonate/bicarbonate buffer, acetate buffer	0.51 ng/mL (sensitivity) 0.02 ng/mL (LOD)	Pastor-Navarro et al. (2007)
Human plasma	Proteins precipitation followed by centrifugation; supernatant was mixed with 100 µL of water in polypropylene tubes and transferred to the auto-sampler	LC-ESI-MS/MS Column: phenyl Mobile phases: 0.2% formic acid, 2 mM ammonium acetate in water, 2 mM ammonium acetate in methanol MRM mode 399 m/z, 381 m/z	10 ng/mL (LOQ)	Gu et al. (2011)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Mouse plasma	–	LC-UV Column: C ₁₈ Mobile phase: methanol and 25 mM phosphate buffer (64 : 36) pH 2.5 Flow rate: 1 mL/min	0.32 nmol/mL (LOD)	Zheng <i>et al.</i> (1993)
Rat plasma	–	LC-UV Column: C ₁₈ Mobile phase: 25 mM phosphate buffer : methanol (40 : 60) pH 3.0 Flow rate: 0.3 mL/min Wavelength: 360 nm	40 ng/mL (LOQ)	Lee <i>et al.</i> (2012)
Food samples:				
Pork meat	Pressurized liquid extraction with hot water, clean-up using oasis HLB cartridge (poly(divinylbenzene- <i>co</i> - <i>N</i> -pyrrolidone)	CE-ESI-MS ² Sheath liquid: methanol, water and formic acid (49.5 : 49.5 : 1) Electrolyte: 50 mM ammonium acetate pH 4.16 MRM mode [M+H] ⁺ 398 317 <i>m/z</i> , 156 <i>m/z</i> , 108 <i>m/z</i>	6.25 µg/kg (LOD) 21.3 µg/kg (LOQ)	Font <i>et al.</i> (2007)
Honey	Added 10% trichloroacetic acid, heated at 65 °C, followed by liquid–liquid extraction (acetonitrile, dichloromethane), organic phase was evaporated, reconstituted using methanol : water (20 : 80)	LC-APPI-MS/MS Column: C ₁₈ Mobile phase: 0.5% formic acid (v/v) and 1 mM nonylfluoropentanoic acid (solvent A) and a mixture of methanol/acetonitrile (50/50, v/v), containing 0.5% formic acid (solvent B) Flow rate: 300 µL/min (SRM) positive ionization	0.4–4.5 µg/kg (LOD) 1.2–15.0 µg/kg (LOQ)	Mohamed <i>et al.</i> (2007)
Venalink blister packs (monitored dosage system)	Dissolve drug in dimethylformamide, dilute with methanol. Internal standard was 1 mL of 0.1% (w/v) 4- <i>N,N</i> -dimethylaminobenzaldehyde	LC-UV Column: C ₁₈ Mobile phase: methanol, water, and acetic acid (70 : 29 : 1) Flow rate: 1.5 mL/min Wavelength: 365 nm	0.1 ng/mL (LOD) 1 ng/mL (LOQ)	Elmasry <i>et al.</i> (2011)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Environmental samples:				
Water	Aqueous sample was filtered, followed by SPE, and derivatized using acetonitrile and methanol	LC-ESI-MS ² Column: C ₁₈ Mobile phase A: 20 mM aqueous ammonium acetate, 0.1% formic acid Mobile phase B: 20 mM ammonium acetate in acetonitrile : methanol (2 : 1)	9–55.3 ng/mL (LOD)	Fatta <i>et al.</i> (2007)
Water	Vacuum extraction, then evaporation under gentle nitrogen stream. Reconstitution with methanol	LC-ESI-MS ^a Mobile phase: water and acetonitrile with 0.1% formic acid Flow rate: 0.2 mL/min Single parent ion (positive mode) [M+H] ⁺ 399	Effluent water, 150 ng/L River water, 65 ng/L (LOQ)	Tuckwell <i>et al.</i> (2011)

APPI, atmospheric pressure photospray ionization; CE-ESI-MS², capillary electrophoresis-electrospray ionization-quadrupole ion trap-tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MRM, multiple reaction monitoring; MS, mass spectrometry; MSⁿ, multistage mass spectrometry; SPE, solid-phase extraction; SPME, solid-phase microextraction; SRM, selected reaction monitoring; UV, ultraviolet

Table 1.2 Most commonly reported clinical indications for sulfasalazine in the USA, 2011–2012

Diagnosis	ICD code	Drug uses (in thousands)	Percentage of total
Rheumatoid arthritis	714.001	367	46.2
Psoriatic arthropathy	696.001	90	11.3
Colitis, ulcerative, NOS	556.004	83	10.5
Colitis, presumed infectious	009.101	69	8.7
Spondylitis, ankylopietic	720.001	28	3.5
Crohn disease	555.901	27	3.4
Surgery after intestinal disease	67.050	25	3.1
Arthritis, spine, NOS	721.901	14	1.7
Rheumatoid arthritis plus inflammatory polyarthritis, unspecified	714.901	11	1.4
All other diagnoses	–	81	10.2
Total with reported diagnoses	–	795	100

NOS, not otherwise specified

From [IMS Health \(2012a\)](#)

but also olsalazine and balsalazide are all 5-ASA drugs. As an anti-inflammatory and immunomodulatory agent, sulfasalazine is used in the treatment of autoimmune and inflammatory conditions, namely inflammatory bowel disease (IBD), most prominently ulcerative colitis and Crohn disease, as well as psoriatic and rheumatoid arthritis, including juvenile rheumatoid arthritis ([IMS Health, 2012a](#); [eMC, 2013](#); [Table 1.2](#)). The use of sulfasalazine for the treatment of urticaria has also been reported ([McGirt et al., 2006](#)). Sulfasalazine is recommended as a third-line medication in all the above conditions only when first- or second-line therapies have been ineffective.

(b) Dosage

Sulfasalazine is available as an oral dose at 250 or 500 mg, and as an oral suspension of 5 mL. There is a wide range of dosing regimens, varying from 500 mg once per day to 1000 mg four times per day, with 1000 mg twice per day being most common (35% of uses). The mean daily dosage for patients taking sulfasalazine is 2150 mg per day ([IMS Health, 2012a](#); [eMC, 2013](#)).

(c) Trends in use

Total worldwide sales of sulfasalazine were US\$ 222 million in 2012 ([IMS Health, 2012b](#)). The largest sales occurred in Japan (US\$ 63 million), followed by the USA (US\$ 32 million), the United Kingdom (US\$ 16 million), Germany (US\$ 12 million), Australia (US\$ 9 million), and Canada (US\$ 8 million). In the United Kingdom, 49 tonnes of sulfasalazine were prescribed in 2007 ([Tuckwell et al., 2011](#)).

Use of sulfasalazine has been relatively stable in the USA since 2005, at about 360 000 drug uses per year. In the USA, approximately 100 000 patients received sulfasalazine in 2012 ([IMS Health, 2012a](#)) and 1.1 million prescriptions for sulfasalazine were dispensed each year between 2008 and 2012 ([IMS Health, 2012c](#)).

1.3 Occurrence and exposure

Sulfasalazine has been found as a persistent residue in influent and effluent of a sewage treatment plant, at concentrations of 0.1 to 0.4 µg/L ([Tuckwell et al., 2011](#)).

Human exposure is largely limited to use as a medication. Workers in plants manufacturing sulfasalazine may be exposed.

1.4 Regulations and guidelines

Sulfasalazine has been widely approved by drug regulatory agencies around the world. In the USA, it was approved by the Food and Drug Administration in 1950 ([FDA, 2013](#)).

Sulfasalazine is listed as “known to cause cancer” by the State of California’s Office of Environmental Health Hazard Assessment, requiring public notice of potential environmental exposure ([OEHHA, 2013](#)).

2. Cancer in Humans

2.1 Background

Sulfasalazine, a member of the family of 5-ASA drugs (see Section 1.2.2), has been used since the 1950s to treat IBD (primarily ulcerative colitis) and, to a lesser extent, Crohn disease ([Hanauer, 2004](#)). IBD is associated with an increased risk of dysplasia and cancer of the colorectum. Risk factors for IBD-associated cancer of the colorectum include duration, severity and extent of colitis, the presence of coexistent primary sclerosing cholangitis, and a family history of cancer of the colorectum ([Dyson & Rutter, 2012](#)). Chronic inflammation has been proposed as a mechanism for colorectal cancer associated with IBD (or ulcerative colitis), and thus it has been suggested that 5-ASA drugs are chemopreventive agents, because of their anti-inflammatory, antioxidant, and pro-apoptotic properties ([Rubin et al., 2006](#); [Lakatos & Lakatos, 2008](#)).

The available epidemiological studies included a surveillance study, two cohort studies, three nested case–control studies and three case–control studies of cancer of the colorectum among patients with IBD or ulcerative colitis. Some studies on IBD included patients with Crohn disease in addition to patients with ulcerative colitis, but none of the studies stratified by IBD subtype. A case–control study of cancer of

the colorectum and exposure to dihydrofolate reductase inhibitors (sulfasalazine, triamterene, and methotrexate) was identified ([Coogan & Rosenberg, 2007](#)), but was not considered to be informative because it did not provide a risk estimate specifically for sulfasalazine; for further information, see the *Monograph* on triamterene in the present volume).

2.2 Longitudinal, cohort, and nested case–control studies

See [Table 2.1](#) and [Table 2.2](#). [Moody et al. \(1996\)](#) evaluated long-term treatment with sulfasalazine and risk of cancer of the colorectum in a retrospective cohort of 175 patients with ulcerative colitis diagnosed between 1972 and 1989 in Leicestershire, England. Clinical information, including compliance with sulfasalazine treatment and history of cancer, was obtained from case records. A patient was considered to be “non-compliant” if there was clear evidence that the patient had ceased taking the medication or was instructed by the physician to stop the medication without replacement by another 5-ASA drug. The crude proportion of cases of cancer of the colorectum in the sulfasalazine non-compliant group (31%) was significantly higher than in the compliant group (3%), and a significant effect of compliance was observed in survival analyses using log-ranked and Wilcoxon methods. [This study was limited by lack of a true unexposed group (the use of sulfasalazine in the non-compliant group was not known), small numbers, and limited information on use of sulfasalazine (e.g. time period, dose, duration), or other medications, and information on risk factors for cancer of the colorectum. It was not clear whether other 5-ASA drugs were used as a replacement for sulfasalazine in members of the compliant group who developed cancer, and whether the physician recommendations for stopping treatment with sulfasalazine would affect cancer outcome.]

Table 2.1 Surveillance and cohort studies of cancer and sulfasalazine

Reference Location, period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Moody et al. (1996) Leicester, England 1981–92	175	Case records – use and compliance	Colorectum	Crude proportion of cases (compliant vs non-compliant; χ^2) [OR for compliance] Sulfasalazine non-compliant group Sulfasalazine compliant group Survival analysis (cancer-free) for compliance (log ranked and Wilcoxon methods)	5 5	$P < 0.001$ [0.07 (0.02–0.30)] 5/16 (31%) 5/152 (3%) $P < 0.001$	Retrospective; cohort comprised patients with total ulcerative colitis or with limited colitis who were deceased; identified via colitis database (case ascertainment, 98%). Cancer cases or dysplasia identified via case records or registry; biopsy diagnosis confirmed on 10% sample. Survival analysis adjusted for age and sex
Lindberg et al. (2001) Sweden, Follow-up, 20 yr	143	Hospital records or questionnaires	Colorectum (cancer or dysplasia)	Sulfasalazine No sulfasalazine Sulfasalazine vs no sulfasalazine (<i>t</i> -test) [OR for sulfasalazine] Cumulative risk of CRC/dysplasia (sulfasalazine vs no treatment)	42 8	42/124 (34%) 8/18 (44%) $P = 0.38$ [0.6 (0.2–1.7)] $P = 0.40$	Surveillance study; patients with ulcerative colitis participating in 20-yr colonoscopic surveillance programme; 124 patients in the treatment group Cumulative risk analysis adjusted for primary sclerosing cholangitis, sex, duration of ulcerative colitis, colectomy
			Colorectum (cancer only)	Sulfasalazine vs no sulfasalazine (<i>t</i> -test) Sulfasalazine No sulfasalazine [OR for sulfasalazine]	5 2	NR 5/124 (4%) 2/18 (11%) [0.3 (0.06–1.87)]	Power, < 50%

Table 2.1 (continued)

Reference Location, period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
van Staa et al. (2005) United Kingdom, 1987–2001	33 905 with 5-ASA drug prescriptions 18 969	GPRD	Colorectum (153, 154, 159)	Reference cohort (number not reported); no history of IBD or prescription for 5-ASA drug	116	1	Patients with 5-ASA drug prescriptions and/or IBD identified from GPRD; patients with history of CRC excluded. Analysis adjusted for age and sex
				5-ASA drug/IBD cohort (5-ASA drug [excluding sulfasalazine] or sulfasalazine and IBD); <i>n</i> = 18 969	124	1.99 (1.54–2.56)	
				Sulfasalazine rheumatoid arthritis cohort (remaining sulfasalazine without IBD); number, NR	69	1.26 (0.94–1.70)	
	Nested case–control analysis (100 cases and 600 controls)			Sulfasalazine use 12 months before the index date:			Cases selected from 5-ASA drug/IBD cohort, controls randomly selected and matched for age, sex, and calendar year of index case. Analysis adjusted for BMI, IBD duration, history of colorectal polyps, NSAID, paracetamol, aspirin, immunosuppressive, glucocorticoids, prior hospitalization for gastrointestinal condition, physician visits, colonoscopy
				Regular use	22	0.67 (0.36–1.25)	
				6–12 prescriptions	3	0.95 (0.22–4.11)	
				13–30 prescriptions	5	0.41 (0.14–1.20)	
				> 30 prescriptions	14	0.77 (0.37–1.60)	
				Daily dose, < 2 g	6	0.84 (0.29–2.42)	
				Daily dose, ≥ 2 g	15	0.69 (0.35–1.37)	

5-ASA, 5-aminosalicylic acid; BMI, body mass index; CRC, colorectal cancer; GPRD, General Practice Research Database; IBD, inflammatory bowel disease; NR, not reported; NSAID, nonsteroidal anti-inflammatory drugs; OR, odds ratio; vs, versus

Table 2.2 Case-control studies of cancer of the colorectum and sulfasalazine

Reference Location, period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Pinczowski et al. (1994) Sweden, 1965–83	102 196	Nested case-control; ulcerative colitis cohort	Medical records	Colorectum	Sulfasalazine use, one or more treatment courses (> 3 months)	48	0.38 (0.20–0.69)	Age, number of exacerbations/yr Controls matched by sex, extent of ulcerative colitis at diagnosis, and yr of diagnosis
Jess et al. (2007) Copenhagen Denmark, 1962–97; Minnesota, USA, 1940–2004	43 102	Nested case-control: IBD cohort; ulcerative colitis or Crohn disease	Medical records	Colorectum (adenocarcinoma, adenoma, or dysplasia [combined])	Sulfasalazine use, cumulative dose: 2.9/1000 g (median) for cases vs 2.2 (median) for controls Sulfasalazine, regular use (> 2 g/day)	NR 36	1.1 (1.0–1.3) 1.1 (0.3–3.7)	Age and calendar yr of diagnosis Controls from the same regional cohort matched on sex, IBD (subtype, duration, calendar yr, and age of diagnosis). USA cohort followed until 2004, and Danish followed until 1997. Of the 43 cases, 23 were CRC, 13 adenoma, and 7 dysplasia
Eaden et al. (2000) England and Wales, [date, NR]	102 102	IBD patients	Medical records	Colorectum	Sulfasalazine use: < 2 g/day ≥ 2 g/day	7 32	0.93 (0.22–3.91) 0.85 (0.32–2.26)	Possible selection bias, source of population patients from physician interested in study, controls from IBD Leicestershire database. Controls matched for sex, age (within 10 yr), extent and duration of disease, but not hospital or yr of diagnosis. “Adjusted for most influential variables”, other 5-ASA drugs, contact with hospital doctor, colonoscopies diagnosis, relative with CRC

Table 2.2 (continued)

Reference Location, period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Rutter et al. (2004) England, 1988–2002	68 136	Hospital, colonoscopy surveillance	Medical records, interviews, and postal questionnaires	Colorectum (cancer, adenoma, and dysplasia) Colorectum (cancer)	Sulfasalazine use: ≤ 10 yr > 10 yr ≤ 10 yr > 10 yr	17 37 5 8	0.97 (0.41–2.26) 1.58 (0.71–3.51) 4.89 (0.47–51.00) 6.59 (0.64–67.92)	Controls matched for sex, extent and duration of ulcerative colitis, age of onset of ulcerative colitis, yr of index colonoscopy; also had to have intact colon and on surveillance within 5 yr of case diagnosis
Terdiman et al. (2007) USA, 2001–3	18 440 368 800 364 1172	Population, health-care database IBD patients	Administrative claims in database of large health-care insurance companies	Colorectum (ICD-9-CM)	Sulfasalazine use 1 yr before diagnosis Sulfasalazine use 1 yr before diagnosis, IBD patients No. of prescriptions: 0 1–2 3–4 ≥ 5 <i>P</i> for trend	64 44 320 12 11 21	2.33 (1.80–3.01) 1.19 (0.83–1.72) 1.0 (ref.) 1.65 (0.80–3.39) 1.01 (0.46–2.21) 1.10 (0.63–1.92) 0.27	Controls free of cancer and bowel surgery, matched to cases by age, sex, and calendar year (20 : 1); CRC but not IBD diagnosis internally validated. No adjustment in analyses of any use 1 yr before diagnosis. Dose–response analysis adjusted for age, sex, colonoscopy, physician visits, ulcerative colitis or Crohn disease, hospitalization, NSAID, glucocorticosteroids, and immunomodulators

5-ASA, 5-aminosalicylic acid; CRC, colorectal cancer; IBD, inflammatory bowel disease; ICD-9CM, International Classification of Diseases Ninth Revision, Clinical Modification; NR, not reported; NSAID, nonsteroidal anti-inflammatory drugs; ref., reference; vs, versus; yr, year

The association between sulfasalazine intake and colorectal cancer or dysplasia was evaluated in a study of 143 patients with ulcerative colitis who underwent regular colonoscopies and multiple biopsies in a 20-year surveillance programme in Sweden ([Lindberg *et al.*, 2001](#)). Of the 143 patients, 124 were in the group that had received treatment with sulfasalazine (treated for at least 6 months between onset of ulcerative colitis and start of surveillance by colonoscopy). Dysplasia or cancer of the colon developed in 51 patients. No statistically significant differences in the adjusted cumulative risk analysis for developing cancer or dysplasia of the colorectum, or the percentage of cancers in the two treatment groups (44% in the non-treatment group compared with 34% in the treatment group) were reported. [The study was limited by small numbers and limited exposure information.]

A cohort of users of 5-ASA drugs was identified from the General Practice Research Database in the United Kingdom ([van Staa *et al.*, 2005](#)). The cohort was divided into three subcohorts: (i) the 5-ASA drug/IBD cohort included 18 969 patients who either had a prescription for an 5-ASA drug (not including sulfasalazine) or who had taken sulfasalazine and had a diagnosis of IBD; (ii) the remaining patients who were taking sulfasalazine but did not have IBD; and (iii) a reference cohort consisting of patients without IBD or a prescription for a 5-ASA drug, matched by calendar year to participants receiving a 5-ASA drug. [The rationale for this approach was that sulfasalazine is used to treat IBD and other diseases in the United Kingdom, while the other 5-ASA drugs are only used to treat IBD.] Relative risks (RRs) for incidence of cancer of the colorectum were 1.99 (95% CI, 1.54–2.56) for the 5-ASA drug/IBD cohort, and 1.26 (95% CI, 0.94–1.70) for the sulfasalazine/non-IBD cohort.

A nested case-control analysis was conducted among the cohort of patients receiving 5-ASA drugs, which included 100 cases and 600 controls (matched to cases on age, sex, and

calendar year) who had had prescriptions in the 6 months preceding the case index date. The type of 5-ASA drug was classified according to the last prescription issued before the index date. Adjusted odds ratios (ORs) were < 1 for regular use or 6–12 prescriptions, 0.41 (0.14–1.20) for 13–30 prescriptions, and 0.77 (0.37–1.60) for > 30 prescriptions in the previous 12 months, and for daily doses of < 2 g and ≥ 2 g; however, they were not statistically significant and no clear exposure-response patterns were observed (see [Table 2.1](#)). Duration of IBD was a strong risk factor for cancer of the colorectum in the study and was controlled for in the analyses. [This study had several advantages, including the prospective design, population-base selection of subjects, analyses by different exposure categories for specific 5-ASA drugs, and good clinical information on each patient. However, information on drug use was limited to the last prescription, information on lifetime drug use was not available, and there was limited information on follow-up procedures (e.g. no information was provided on tracking individuals who moved out of the region of the United Kingdom database). There was also a potential for misclassification of disease; classification appeared to be based on the General Practice Research Database with diagnosis confirmed via physician questionnaire for a small subset of patients. The study had limited statistical power. Moreover, it was unclear whether all the variables in the statistical models were potential confounders, which may have further reduced the statistical power.]

Two case-control studies were nested in population-based cohorts of patients with IBD (see [Table 2.2](#)). A Swedish study identified 102 cases of cancer of the colorectum via linkage to the national Swedish cancer registry, among a cohort of patients with ulcerative colitis ([Pinczowski *et al.*, 1994](#)). Living controls ($n = 196$), with intact or partially intact colon, were matched to cases on sex, extent of disease, and time of diagnosis of disease. Information on pharmacological therapy

(including sulfasalazine), clinical features of disease, smoking, and family history of IBD, cancer of the colorectum, and other diseases, was collected from the patient's medical records. A decreased risk of cancer of the colorectum was found among individuals who had followed one or more treatment course of sulfasalazine (at least 3 months) (adjusted RR, 0.38; 95% CI, 0.20–0.69). [The strengths of the study were the prospective design, the use of a population-based cohort, and the use of controls matched for disease severity. The major limitations were the lack of detailed exposure information and small size.]

The second nested case–control study evaluated the risk of colorectal neoplasia (adenocarcinoma, adenoma, and dysplasia combined) in two cohorts of patients with IBD in Denmark and in Minnesota, USA ([Jess et al., 2007](#)). Both cohorts included patients with ulcerative colitis or Crohn disease. Cases were identified via linkage with cancer registries, and controls matched for sex, vital status, and age at diagnosis, and clinical factors related to IBD were identified from each regional cohort. Exposure and clinical information was obtained from medical records. The adjusted relative risk for colorectal neoplasia was close to unity for regular use (> 2 g/day) or cumulative dose of sulfasalazine. Primary sclerosing cholangitis was a strong risk factor for colorectal neoplasia. [The advantages and limitations of this study were similar to those of the Swedish study. An additional limitation of this study was that there was not a separate analysis for cancer of the colorectum alone; of the 43 cases of colorectal neoplasia, 23 were cancer.]

2.3 Case–control studies

See [Table 2.2](#)

Three studies selected cases and controls from patients with ulcerative colitis. [Eaden et al. \(2000\)](#) evaluated the risk of colorectal cancer among patients with ulcerative colitis and controls in England and Wales. Cases ($n = 102$)

were identified from records of consenting gastroenterologists throughout England and Wales, and 102 controls matched by sex, age (categories of 10 years), extent and duration of IBD were identified from the Leicestershire database of IBD patients. Data were extracted from medical records. Sulfasalazine therapy at both < 2 g/day and ≥ 2 g/day was inversely associated with increased risk of colorectal cancer in unadjusted analyses, while adjusted odds ratios were 0.93 (95% CI, 0.22–3.91) for the group at the lower dose and 0.85 (95% CI, 0.32–2.26) for the group at higher doses. [The limitations of the study were the potential for selection bias, inadequate matching of the controls (using categories of 10 years of age, and not matching on hospital or year of diagnosis of IBD), limited exposure information, limited documentation of covariates controlled in the analyses. Odds ratios were adjusted for the use of other 5-ASA drugs, but this may not be appropriate since these drugs could work via the same mechanisms as sulfasalazine. The study population may have overlapped with the cohort reported by [Moody et al. \(1996\)](#); both studies identified patients from the same database of patients, but the years of study recruitment were not reported in the study by [Eaden et al. \(2000\)](#).]

One study evaluated risk factors for colorectal neoplasia (cancer, adenoma, and dysplasia) among patients with chronic ulcerative colitis who were part of a colonoscopy surveillance programme ([Rutter et al., 2004](#)). Sixty-eight cases with neoplasia were identified and 136 controls from the surveillance population were matched on sex and clinical characteristics of IBD. Long-term use (> 10 years) of sulfasalazine was associated with a non-statistically significant elevated odds ratio for colorectal neoplasia (cancer, adenoma, or dysplasia) of 1.58 (95% CI, 0.71–3.51; 37 exposed cases); use of sulfasalazine for > 3 months to 10 years was associated with an odds ratio of 0.97 (95% CI, 0.41–2.26; 17 exposed cases). Analysis of the cancer cases only found that both exposure

categories were associated with elevated, but very imprecise odds ratios based on small numbers (see [Table 2.1](#)). [A significant association between severity of inflammation and colorectal neoplasia was noted, and this hindered interpretation of the association of colorectal cancer and treatment with sulfasalazine. Other concerns included the small numbers of exposed cancer cases, lack of adjustment for risk factors, and limitations in the generalizability of the findings due to the selection of subjects from a surveillance programme. In addition, drug use may have been related to duration of ulcerative colitis, and thus matching by duration of ulcerative colitis may bias the odds ratio towards the null.]

[Terdiman *et al.* \(2007\)](#) evaluated use of sulfasalazine in a population based case–control study consisting of 18 440 cases of cancer of the colorectum and 368 800 controls (matched by age, sex, and calendar year) identified from two administrative databases covering all regions in the USA, including 364 cases and 1172 controls with a diagnosis of IBD. Information on claims for sulfasalazine prescriptions and other clinical variables was obtained from the claim database. A statistically significant increased risk of cancer of the colorectum was found among all patients using sulfasalazine 1 year before diagnosis (crude OR, 2.33; 95% CI, 1.80–3.01), while among patients with IBD only the adjusted odds ratio for sulfasalazine treatment was 1.19 (95% CI, 0.83–1.72); no exposure–response relationship was observed with number of prescriptions, regardless of adjustment for multiple covariates (*P* for trend, 0.27). [The strengths of this study were the large size and population-based design, and information on many (but not all) potential confounders, and some exposure–response analyses. The major limitation was the short exposure duration (1 year before diagnosis); in addition, information was not available on several key potential confounders, such as family history of colon cancer or IBD and other factors not related to medications.]

2.4 Meta-analysis

A meta-analysis of four cohort studies assessed the association between long-term treatment with sulfasalazine and risk of colorectal cancer ([Diculescu *et al.*, 2003](#)). [The Working Group could not interpret this study due to the lack of information on analytical methods and the inclusion of studies that were not specific for treatment with sulfasalazine. Furthermore, summary measures of association were not calculated.]

3. Cancer in Experimental Animals

3.1 Mouse

See [Table 3.1](#)

Oral administration

In one study, groups of 50 male and 50 female B6C3F₁ mice (age, 6 weeks) were given sulfasalazine (USP grade) at a dose of 0, 675, 1350, or 2700 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 104 weeks. There was a 6–18% decrease in mean body weight in male and female mice at the highest dose compared with controls. The incidence of hepatocellular adenoma in males and females, and the incidence of hepatocellular adenoma or carcinoma (combined) in males and females, were significantly greater than those in controls, and the incidences increased with a positive trend. The incidence of hepatocellular carcinoma was significantly increased in female mice ([Iatropoulos *et al.*, 1997](#); [NTP, 1997a](#)).

In a first experiment in a group of related studies, groups of 50–60 male B6C3F₁ mice (age, 6 weeks) were given sulfasalazine (USP grade) at a dose of 0 or 2700 mg/kg bw by gavage in corn oil once per day, 5 days per week, for 103 weeks (~2 years); in a second experiment, an unexposed

Table 3.1 Studies of carcinogenicity in mice given sulfasalazine by gavage

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence, (%) and/or multiplicity of tumours	Significance	Comments
B6C3F ₁ (M, F) 104 wk NTP (1997a) , Iatropoulos et al. (1997)	Sulfasalazine (in corn oil) at a dose of 0, 675, 1350, or 2700 mg/kg bw per day, 5 days per wk, for 104 wk 50 M and 50 F/group	<i>Males</i> Hepatocellular adenoma: 13/50 ¹ , 32/50*, 28/50**, 42/50* Hepatocellular carcinoma: 13/50, 15/50, 23/50, 8/50 Hepatocellular adenoma or carcinoma (combined): 24/50 ¹ , 38/50***, 38/50****, 44/50* <i>Females</i> Hepatocellular adenoma: 12/50 ¹ , 28/50*, 25/50**, 28/49* Hepatocellular carcinoma: 2/50, 10/50****, 10/50****, 9/49**** Hepatocellular adenoma or carcinoma (combined): 14/50 ² , 32/50*, 28/50*, 29/49*	* <i>P</i> < 0.001 (Poly-3 test) ** <i>P</i> = 0.002 (Poly-3 test) *** <i>P</i> = 0.004 (Poly-3 test) **** <i>P</i> = 0.005 (Poly-3 test) ***** <i>P</i> ≤ 0.05 (Poly-3 test) ¹ <i>P</i> < 0.001 (Poly-3 trend test) ² <i>P</i> = 0.005 (Poly-3 trend test)	Purity, USP grade
B6C3F ₁ (M) Up to 156 wk NTP (1997b) , Abdo & Kari (1996)	<i>Exp. 1:</i> sulfasalazine (in corn oil) at a dose of 0 or 2700 mg/kg bw per day, 5 days per wk, for 103 wk (~2 yr), fed ad libitum <i>Exp. 2:</i> unexposed group fed such that mean body weight matched that of the treated group fed ad libitum <i>Exp. 3</i> (dietary restriction): two groups of 110 mice (one control and one dosed) were given identical quantities of feed such that the control group would attain body weights of approximately 80% that of the ad libitum-fed controls; 60 mice per group were evaluated at 103 wk (~2 yr) and the remaining 50 mice per group at 156 wk (3 yr), or when survival reached 20% 50–60 M/group	Hepatocellular adenoma: <i>Exp. 1:</i> 13/50, 42/50* <i>Exp. 2:</i> 8/50, 42/50* <i>Exp. 3:</i> 13/52, 9/50 (~2 yr) <i>Exp. 3:</i> 10/48, 14/50 (up to 3 yr) Hepatocellular carcinoma: <i>Exp. 1:</i> 13/50, 8/50 <i>Exp. 2:</i> 6/50, 8/50 <i>Exp. 3:</i> 7/52, 1/50 (~2 yr) <i>Exp. 3:</i> 16/48, 6/50 (up to 3 yr) Hepatocellular adenoma or carcinoma (combined): <i>Exp. 1:</i> 24/50, 44/50* <i>Exp. 2:</i> 14/50, 44/50* <i>Exp. 3:</i> 18/52, 9/50 (~2 yr) <i>Exp. 3:</i> 21/48, 18/50 (up to 3 yr)	* <i>P</i> < 0.001 (increase, logistic regression test)	Purity, USP grade

bw; body weight; Exp., experiment; F, female; M, male; wk, week; USP, United States Pharmacopoeia; yr, year

group was fed such that the mean body weight of the group matched that of the treated group fed ad libitum. In a third experiment (dietary restriction), two groups of 110 animals, one control group and one group given sulfasalazine at a dose of 2700 mg/kg bw in corn oil were offered identical quantities of feed such that the control group would attain body weights of approximately 80% those of the control group fed ad libitum. Sixty mice from each group were evaluated at 103 weeks and the remaining 50 mice from each group were evaluated at 156 weeks (3 years), or at the time when survival reached 20%.

The mean body weight at 1 year and survival at 103 weeks (~2 years) for the mice treated with sulfasalazine were decreased by 15% and 19%, respectively, relative to controls. The body weight and survival of the weight-matched vehicle-control group were similar to those of the treated group fed ad libitum. Under the dietary restriction protocol, the control and treated groups weighed 42 g and 34 g at 1 year and had respective survival rates of 84% and 88% after 103 weeks.

Exposure to sulfasalazine under ad-libitum feeding conditions for 103 weeks (~2 years) caused significantly increased incidences of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) in exposed mice compared with the controls fed ad libitum and the weight-matched controls. In contrast to the findings of the first two experiments, the incidence of hepatocellular tumours in dietary-restricted mice was significantly decreased in the exposed group after 103 weeks, and was similar to that in non-treated mice after 3 years ([Abdo & Kari, 1996](#); [NTP, 1997b](#)).

3.2 Rat

See [Table 3.2](#)

Oral administration

In one study, groups of 50 male and 50 female F344/N rats (age, 6 weeks) were given sulfasalazine (USP grade) at a dose of 0, 84, 168, or 337.5 mg/kg bw by gavage in corn oil once per day, 5 days per week, for 105 weeks (core study; continuous exposure). An additional group of male rats (stop-exposure group) was treated with sulfasalazine in corn oil at 337.5 mg/kg bw for 26 weeks, and then with corn oil only for the remainder of the study (79 weeks). Survival of male rats at the highest dose in the core study was significantly lower than that of controls, with most deaths occurring during the last 8 weeks of the study. Survival of all other treated groups was similar to that of controls.

The incidence of transitional cell papilloma of the urinary bladder in the core study was increased with a positive trend in the groups of treated male rats; the incidence in the group at the highest dose was significantly increased. The transitional cell neoplasms of the urinary tract observed in the core study were not observed in the stop-exposure group. In exposed females, there were also low incidences of [rare] transitional cell papilloma of the kidney and of the urinary bladder. All rats with transitional cell papillomas of the urinary tract also had grossly visible concretions (calculi) in the kidney and/or urinary bladder ([Iatropoulos et al., 1997](#); [NTP, 1997a](#)).

In a first experiment in a group of related studies, groups of 50–60 male F344/N rats (age, 6 weeks) were given sulfasalazine (USP grade) at a dose of 0 or 337.5 mg/kg bw in corn oil by gavage once per day, 5 days per week, for up to 104 weeks; in a second experiment, an unexposed group was fed such that the mean body weight of the group matched that of the treated group fed ad libitum. In a third experiment (dietary restriction), two groups of 110 rats, one control group and one group given sulfasalazine at a dose of 337.5 mg/kg bw in corn oil were offered identical

Table 3.2 Studies of carcinogenicity in rats given sulfasalazine by gavage

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: Incidence, (%) and/or multiplicity of tumours	Significance	Comments
F344/N (M, F) 105 wk NTP (1997a) , Iatropoulos et al. (1997)	Sulfasalazine (in corn oil) at a dose of 0, 84, 168, or 337.5 mg/kg bw, once per day, 5 days per wk, for 105 wk (core study); an additional group of male rats (stop-exposure group) was treated with sulfasalazine (in corn oil) at 337.5 mg/kg bw for 26 wk and then with corn oil only for the remainder of the study (79 wk) 50 M and 50 F/group	<i>Males</i> Transitional cell papilloma of the urinary bladder: 0/50 ^a , 0/49, 2/50 (4%), 6/50 (12%)*; stop exposure, 0/47 <i>Females</i> Transitional cell papilloma of the urinary bladder ^a : 0/49, 0/50, 2/50 (4%), 0/50 Transitional cell papilloma of the kidney ^b : 0/50, 0/50, 0/50, 2/50 (4%)	¹ <i>P</i> < 0.001 (Poly-3 trend test) [*] <i>P</i> = 0.011 (Poly-3 test)	Purity, USP grade
F344/N (M) Up to 130 wk NTP (1997b) , Abdo & Kari (1996)	Exp. 1: sulfasalazine in corn oil at a dose of 0, or 337.5 mg/kg bw, once per day, 5 days per wk for up to 104 wk, fed ad libitum Exp. 2: unexposed group fed such that mean body weight matched that of the treated group fed ad libitum. Exp. 3 – dietary restriction: two groups of 110 rats (one control and one dosed) were given identical quantities of feed such that the control group would attain body weights of approximately 80% those of the ad libitum-fed controls; 60 rats/group were evaluated at 104 wk (~2 yr) and the remaining 50 rats/group at 130 wk, or when survival reached 20% 50–60 males/group	Transitional cell papilloma of the urinary bladder: <i>Exp. 1:</i> 0/50, 6/50* <i>Exp. 2:</i> 0/50, 6/50* <i>Exp. 3:</i> 0/51, 0/50 (~2 yr) <i>Exp. 3:</i> 0/49, 1/49 (up to 130 wk)	[*] <i>P</i> = 0.011 (logistic regression)	Purity, USP grade

^a Historical incidence for 2-year studies by the NTP in rats fed corn oil by gavage (vehicle control groups): 3/903 (0.3% ± 0.8%); range, 0–2%

^b Historical incidence for 2-year studies by the NTP in rats fed corn oil by gavage (vehicle control groups): 0/920

bw, body weight; Exp., experiment; F, female; M, male; NTP, National Toxicology Program; wk, week; USP, United States Pharmacopoeia; yr, year

quantities of feed such that the control group would attain body weights of approximately 80% that of the controls fed ad libitum. Sixty rats from each group were evaluated at 104 weeks and the remaining 50 rats from each group were evaluated at 130 weeks, or at the time when survival reached 20%.

After 1 year, mean body weights for the control and treated rats in the first experiment were similar. Since there was negligible body weight loss throughout the study, no adjustments were made to the weight-matched control group, thereby yielding a redundant control group. Survival at 2 years in the first experiment was 70% and 46% for the control and treated rats, respectively.

The incidence of transitional cell papilloma of the urinary bladder was significantly greater in exposed rats than in the controls fed ad libitum in the first experiment, or weight-matched controls in the second experiment. All rats with transitional cell papilloma of the urinary bladder also had grossly visible concretions in the kidney and/or urinary bladder. In the third experiment, no significant increase in the incidence of transitional cell papilloma of the urinary bladder was observed ([Abdo & Kari, 1996](#); [NTP, 1997b](#)).

3.3 Studies of co-carcinogenicity

A group of 12 male Wistar rats was given 1,2-dimethylhydrazine at a dose of 40 mg/kg bw as a single subcutaneous injection each week, concurrently with sulfasalazine at a dose of 60 mg/kg bw per day by gavage, for 20 weeks. One control group of 11 male Wistar rats was given 1,2-dimethylhydrazine only. Development of “colon tumours” (mainly adenocarcinomas) was assessed histologically at week 21. All rats developed tumours of the intestine. In the control group receiving 1,2-dimethylhydrazine only, there were 70 tumours of the intestine with a tumour multiplicity of 6.4 ± 0.69 , while in the group given 1,2-dimethylhydrazine plus

sulfasalazine there were 141 tumours of the intestine ($P \leq 0.05$, ANOVA test) with a tumour multiplicity of 11.8 ± 2.16 ($P < 0.05$, *t*-test) ([Davis et al., 1992](#)).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

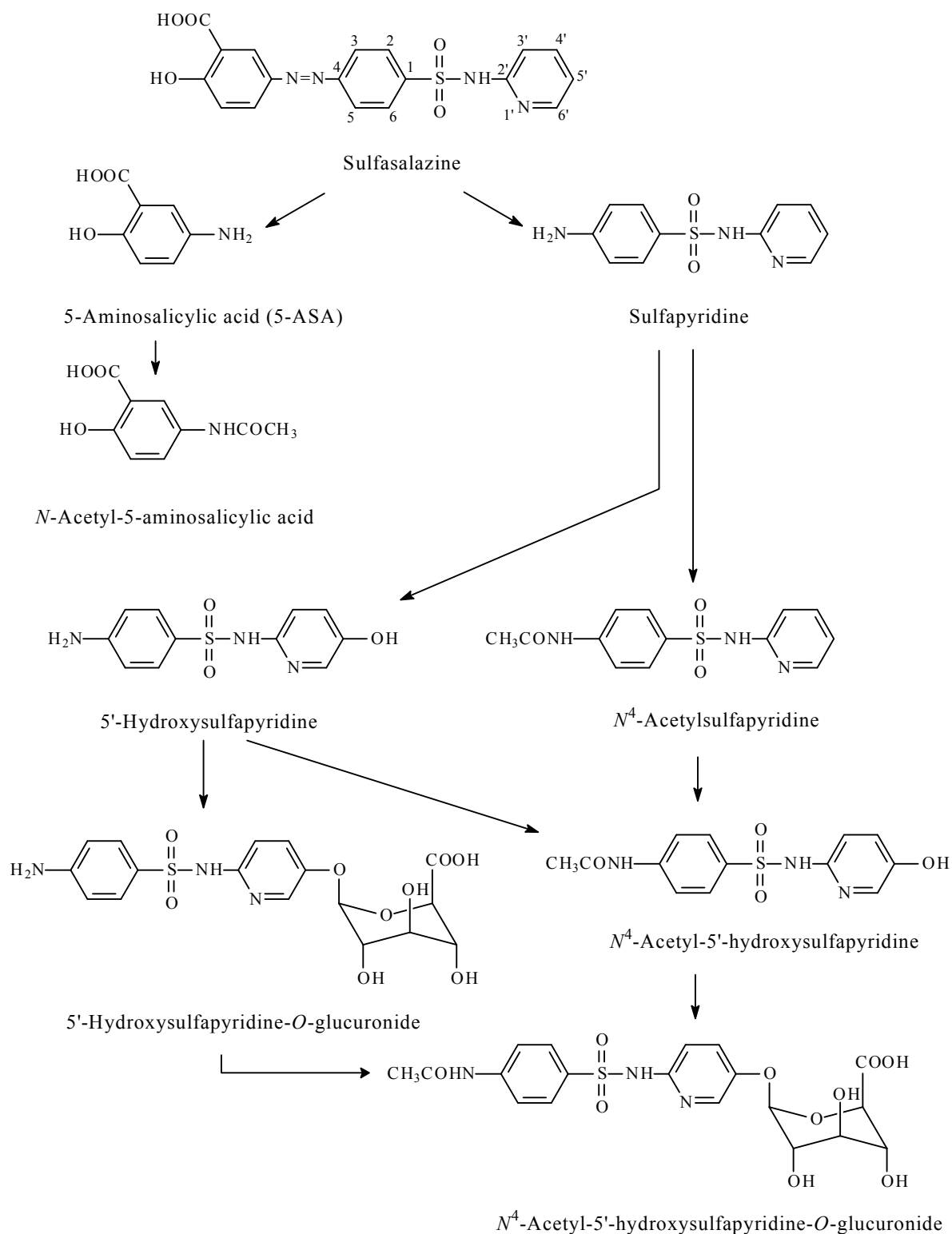
4.1.1 Humans

(a) Absorption, distribution, metabolism, and excretion

The metabolic scheme for sulfasalazine in humans is shown in [Fig. 4.1](#) ([Das & Dubin, 1976](#); [NTP, 1997a](#)).

Sulfasalazine is not absorbed to any significant extent from the stomach ([Das & Dubin, 1976](#)). Slow absorption of small amounts (~10–30%) via the small intestine has been reported before enterohepatic recycling, and with the majority of unchanged drug reaching the colon ([Das & Dubin, 1976](#); [Azad Khan et al., 1982](#)).

The sulfasalazine molecule comprises 5-ASA and sulfapyridine moieties, linked by an azo bond, which is cleaved by bacterial azoreductases in the colon, releasing 5-ASA and sulfapyridine ([Azad Khan et al., 1982](#)). This cleavage is the rate-limiting step for clearance of sulfasalazine ([Das & Dubin, 1976](#)). Most of the 5-ASA is excreted; approximately 50% directly in the faeces, and at least 25% via the kidneys (after absorption and acetylation in the liver) ([Das & Dubin, 1976](#); [Azad Khan et al., 1982](#)). In contrast, sulfapyridine is almost completely absorbed. In the liver, sulfapyridine undergoes hydroxylation and/or *N*-acetylation to 5'-hydroxysulfapyridine, *N*⁴-acetylsulfapyridine, and *N*⁴-acetyl-5'-hydroxysulfapyridine subsequently forming glucuronic acid conjugates, before excretion mainly in the urine ([Das & Dubin, 1976](#); [Azad Khan et al., 1982](#)).

Fig. 4.1 Metabolic pathways of sulfasalazine in humans

From [Das & Dubin \(1976\)](#), [NTP \(1997a\)](#). With kind permission from Springer Science and Business Media.

In studies of serum from 10 healthy male volunteers given single oral doses of 4 g of sulfasalazine, parent drug was detectable at 1.5 hours after dosing, and at maximum concentrations at 3–5 hours in nine subjects, and after 7 hours in one subject ([Schröder & Campbell, 1972](#)). Metabolites (sulfapyridine, and acetylated and glucuronidated derivatives) were detected in the serum at 3–5 hours after dosing ([Schröder & Campbell, 1972](#)). The pharmacokinetics of rectally administered sulfasalazine have been studied in three healthy male Japanese volunteers ([Tokui et al., 2002](#)). Sulfasalazine (6.5 mmol), given as a single suppository, reached maximum plasma concentration ($2.5 \pm 0.4 \mu\text{M}$) in 5 hours (T_{max}), and an area under the curve (AUC) of $27.4 \pm 4.8 \mu\text{M.h}$. Parent drug was almost completely hydrolysed in the colon, and the urinary recovery was only approximately 0.2%. Maximum plasma concentration (C_{max}) of the metabolite *N*-acetyl-5-aminosalicylic acid was $0.5 \pm 0.2 \mu\text{M}$, reached in 12 hours, while that of sulfapyridine was $1.2 \pm 0.4 \mu\text{M}$, reached in 5 hours. 5-ASA was not detected in the serum. Administration of an enema containing 6.5 mmol of 5-ASA, resulted in C_{max} $5.8 \pm 2.0 \mu\text{M}$ in 1 hour and an AUC of $29.4 \pm 11.1 \mu\text{M.h}$. In the urine, approximately 0.3% was recovered unchanged. The C_{max} for the acetylated metabolite, *N*-acetyl-5-aminosalicylic acid, was $13.3 \pm 3.6 \mu\text{M}$ in 7 hours. More than 10% of 5-ASA was excreted in the urine as acetyl-5-aminosalicylic acid, suggesting that absorption of 5-ASA is favoured when administered rectally ([Tokui et al., 2002](#)).

A study to compare the absorption and metabolism of oral preparations of sulfasalazine, mesalazine (5-ASA) and olsalazine (a dimer of 5-ASA) used regularly by patients ($n = 12, 13$, and 8, respectively) for treatment of ulcerative colitis, showed considerably greater absorption of 5-ASA and less acetylation, in patients receiving mesalazine than in those receiving olsalazine or sulfasalazine ([Stretch et al., 1996](#)).

(a) *Variation in absorption, distribution, metabolism, and excretion*

(i) *IBD and rheumatoid arthritis*

The characteristics of absorption, metabolism, and excretion of the parent drug in four patients with IBD (ulcerative colitis or Crohn disease) were similar to those in four healthy subjects, each given a single oral dose of sulfasalazine (3 or 4 g). However, absorption and urinary excretion of the metabolite, sulfapyridine, was decreased in patients with IBD. The metabolism of sulfasalazine was markedly reduced in patients taking antibiotics and after removal of the large bowel ([Azad Khan et al., 1982](#)).

Pharmacokinetic studies of sulfasalazine and its principal metabolites in 13 patients with rheumatoid arthritis and 8 patients with IBD given sulfasalazine as a single oral dose of 2 g ([Astbury et al., 1990](#)), showed that patients with rheumatoid arthritis had a significantly higher (and more sustained) plasma concentration of sulfapyridine than did patients with IBD (medians of 14.0 $\mu\text{g/mL}$ and 7.4 $\mu\text{g/mL}$, respectively). Two factors may have contributed to high peak plasma concentrations of sulfapyridine in patients with rheumatoid arthritis: firstly, the metabolism of sulfapyridine may be impaired, and secondly, a larger quantity of sulfasalazine may reach the lower bowel leading to higher concentrations of the subsequent cleavage compounds. The pharmacokinetics of sulfasalazine were variable among patients; maximum plasma concentrations of sulfapyridine ranged from 8 to 22 $\mu\text{g/mL}$ in patients with rheumatoid arthritis, and from 5 to 18 $\mu\text{g/mL}$ in patients with IBD. The elimination half-life of sulfasalazine ranged from 3 to 8 hours in patients with rheumatoid arthritis, and from 4 to 9 hours in patients with IBD ([Astbury et al., 1990](#)).

(ii) Pregnancy

Sulfasalazine and its primary metabolites are able to cross the placenta ([Azad Khan & Truelove, 1979](#); [Järnerot *et al.*, 1981](#)). In five patients with ulcerative colitis treated with sulfasalazine (0.5 g, four times per day) throughout and after pregnancy, sulfasalazine was detected in the umbilical cord blood (mean concentration, 50% of that in maternal serum) and at very low concentrations in the amniotic fluid ([Azad Khan & Truelove, 1979](#)). Analyses of metabolites showed that total concentrations of sulfapyridine were equal in maternal and cord sera, but concentrations of free sulfapyridine were significantly lower ($P < 0.02$) in cord sera. Total concentrations of acetylated sulfapyridine were significantly higher ($P < 0.025$) in cord sera than maternal sera. Total concentrations of 5-ASA were very low in all fluids analysed ([Azad Khan & Truelove, 1979](#)). In the study by [Järnerot *et al.* \(1981\)](#) of 11 pregnant patients with IBD treated with sulfasalazine (1 g daily), concentrations of sulfasalazine were almost identical in cord and maternal serum. Seven of the women were analysed at a later date (4–24 months) after pregnancy; plasma concentrations of sulfasalazine remained the same, but concentrations of sulfapyridine had increased, probably reflecting different extents of protein binding of these compounds, and the different distribution volume in pregnancy ([Järnerot *et al.*, 1981](#)).

Small quantities of sulfasalazine and sulfapyridine have also been detected in breast milk ([Azad Khan & Truelove, 1979](#); [Järnerot & Into-Malmberg, 1979](#)). Mean concentrations of sulfasalazine and total sulfapyridine in milk, compared with concentrations in maternal serum, were approximately 30% and 50%, respectively, as reported by [Azad Khan & Truelove \(1979\)](#), and negligible and 40%, respectively as reported by [Järnerot & Into-Malmberg \(1979\)](#). The various metabolites of sulfapyridine were present in approximately the same proportions

as in maternal serum ([Azad Khan & Truelove, 1979](#)). It was estimated that an infant would receive sulfapyridine at dose of 3–4 mg/kg bw, after a maternal dose of 2 g of sulfasalazine per day ([Järnerot & Into-Malmberg, 1979](#)). Sulfapyridine and its acetylated and glucuronidated metabolites have been shown to be excreted by babies, 1–2.5 months after maternal dosing ([Järnerot & Into-Malmberg, 1979](#)).

(c) Genetic polymorphisms

(i) N-Acetyltransferases

The sulfasalazine molecule may be considered as a slow-release carrier for sulfapyridine, but there is large inter-individual variation in the rate of metabolism of sulfapyridine, which can affect steady-state serum concentrations ([Das & Dubin, 1976](#)). The rate of *N*-acetylation of 5-ASA to *N*-acetyl-5-aminosalicylic acid is under genetic control ([Das & Dubin, 1976](#)). The cytosolic *N*-acetyltransferase (NAT) family comprises NAT1 and NAT2, which catalyse the transfer of acetyl groups from activated acetyl-coenzyme A to the nitrogen of primary amines, hydrazines, or hydrazides ([Kuhn *et al.*, 2010](#)). Single nucleotide polymorphisms have been detected in NAT2. The wildtype has been designated NAT2*4, but NAT2*5, NAT2*6, and NAT2*7 alleles encode *N*-acetyltransferase enzymes with amino-acid changes that cause reduced activity (slow acetylating function). Patients with a “slow” acetylator phenotype generally show significantly higher, and more sustained plasma concentrations of sulfapyridine and its non-acetylated metabolites. The elimination half-life of sulfasalazine in patients with a slow-acetylator phenotype may be approximately 50–100% longer than in those with a fast-acetylator phenotype ([Taggart *et al.*, 1992](#)).

A 1–3 year study of 185 patients with ulcerative colitis undergoing daily treatment with sulfasalazine (2 g per day) demonstrated that serum concentrations of sulfapyridine (both free

and total) were higher in patients with a slow-acetylator phenotype ([Azad Khan et al., 1983](#)). Concentrations of sulfasalazine and of 5-ASA were not significantly different in fast and slow acetylators. These findings were confirmed by later studies ([Taggart et al., 1992](#)).

The frequency of polymorphism in NAT2 varies in different racial or ethnic populations ([Ma et al., 2009](#)). Studies have shown that about 60% of patients with IBD, studied in Edinburgh, Scotland, are slow acetylators ([Das et al., 1973](#)), and a similar proportion was found in healthy volunteers in a study in Liverpool, England ([Schröder & Evans, 1972](#)). In Germany, a study of NAT2 genotype and acetylation, using sulfasalazine as probe drug, showed that 24 out of 44 healthy volunteers (54.5%) were slow acetylators, in accordance with the “slight prevalence of slow acetylators in central European (Caucasian) populations” which has been reported in several studies ([Kuhn et al., 2010](#)).

The NAT2 genotype has also been investigated in Asian populations. Studies in 21 Japanese subjects (8 healthy subjects and 13 patients with IBD) given a single oral dose of sulfasalazine at 40 mg/kg bw demonstrated generally good correlation between three NAT2 genotypes (rapid, intermediate, slow acetylators) and the plasma or urinary concentrations of sulfapyridine and *N*⁴-acetyl sulfapyridine ([Tanigawara et al., 2002](#)). Similar analyses in seven healthy Japanese subjects after 8 days of continued (“multiple dosing”) oral doses of 1 g of sulfasalazine once per day also demonstrated correlation with genotype ([Kita et al., 2001](#)). In 18 healthy Chinese men given 1 g of sulfasalazine as a single oral dose, the NAT2 gene was shown to be an important determinant of metabolite profiles; the frequency of slow acetylators in the Chinese population was lower than in Caucasians, but higher than in the Japanese population ([Ma et al., 2009](#)).

The effects of age and acetylator status on the pharmacokinetics of sulfasalazine were

compared in patients with rheumatoid arthritis (8 young and 12 elderly, with equal numbers of slow and fast acetylators in both age groups), each receiving sulfasalazine at 2 g per day for 21 days ([Taggart et al., 1992](#)). In the elderly, the elimination half-life of sulfasalazine was increased [possibly partly due to slow cleavage of the azo bond ([Tett, 1993](#))], and steady-state serum concentrations of *N*-acetyl-5-aminosalicylic acid were higher. The pharmacokinetics of sulfapyridine were unchanged with age, but were influenced by acetylation status, in particular, with increased steady-state serum concentrations in slow acetylators. Although age is a determinant of the steady-state concentration of salicylate moieties, the acetylator phenotype seems to play the greater role in determining serum concentrations of sulfapyridine ([Taggart et al., 1992](#)).

(ii) *Role of transporter proteins*

An efflux ATP-binding cassette (ABC) transporter, the breast cancer resistance BCRP protein (encoded by the *ABCG2* gene), has a role in the pharmacokinetics of various drugs, including sulfasalazine. The BCRP protein is expressed at the luminal membrane of cells with key functions in drug transport, namely, placental trophoblast cells, hepatocyte bile canaliculi, kidney cells, and enterocytes.

The poor bioavailability of sulfasalazine has long been attributed to its low solubility and poor permeability ([Das & Dubin, 1976](#)). However, treatment of human T-cells with sulfasalazine was shown to cause cellular drug resistance that was mediated by induction of BCRP (*ABCG2*), suggesting that sulfasalazine may be a substrate for human BCRP ([van der Heijden et al., 2004](#); [Urquhart et al., 2008](#)).

A subsequent study in vitro, using data on expression in human tissue, indicated an association between reduced cell surface expression of the 421C > A variant and reduced BCRP-mediated transport of sulfasalazine in patients

carrying an A allele at position 421 of the *BCRP* [*ABCG2*] gene ([Urquhart et al., 2008](#)).

Variation in the *ABCG2* gene may impair the transport of drugs that are substrates for the ABC transporter, leading to increased intestinal absorption, and/or decreased biliary excretion, and result in high plasma concentrations, as demonstrated in 37 healthy Japanese people selected according to *ABCG2* and *NAT2* genotype and given a single oral dose of 2 g of sulfasalazine ([Yamasaki et al., 2008](#)). They showed that in *ABCG2*-A/A subjects, mean plasma AUC_{0-48h} and C_{max} values for sulfasalazine were significantly higher, and the AUC for sulfapyridine was lower (except in those who also had the slow-acetylator *NAT2* genotype) than in individuals without the *ABCG2* variant. The increased AUC for sulfasalazine in *ABCG2*-A/A subjects may result from increased oral availability and/or decreased hepatic clearance, since BCRP is expressed on enterocytes and hepatocytes. The ratios of $AUC_{acetylsulfapyridine}/AUC_{sulfapyridine}$ were significantly higher in subjects with the rapid-acetylator *NAT2* phenotype than in those with intermediate or slow genotypes, demonstrating that inter-individual variability in the pharmacokinetics of sulfasalazine can be attributed to genetic polymorphism in drug transport and metabolism ([Yamasaki et al., 2008](#)).

4.1.2 Experimental systems

(a) Absorption, distribution, metabolism, and excretion

Experimental studies in Sprague-Dawley rats given diets containing sulfasalazine have shown that most of the sulfasalazine is reductively cleaved by intestinal bacteria to two compounds, 5-ASA (which is poorly absorbed) and sulfapyridine (which is well absorbed) ([Peppercorn & Goldman, 1972](#)). After metabolism by mammalian enzymes, 5-ASA and sulfapyridine are excreted mainly in the faeces, and in the urine, respectively ([Peppercorn & Goldman, 1972](#)).

In male and female B6C3F₁ mice given sulfasalazine as an intravenous dose at 5 mg/kg bw, plasma concentrations of the parent drug rapidly declined with a mean elimination half-life of 0.5 hour in males and 1.2 hour in females ([Zheng et al., 1993](#)). The sex-specific differences in clearance rate were reflected in $AUC_{sulfasalazine}$ values (9.21 $\mu\text{M}\cdot\text{h}^{-1}$ and 21.39 $\mu\text{M}\cdot\text{h}^{-1}$, in males and females, respectively).

In male and female B6C3F₁ mice given sulfasalazine by gavage at various doses (67.5, 675, 1350, or 2700 mg/kg bw), the bioavailability of the parent drug was approximately 17% (range, 16–18%) at the lowest dose (67.5 mg/kg bw), and was lower (range, 3–9%) at the higher doses. Both sulfapyridine and *N*⁴-acetylsulfapyridine were identified in the plasma. Sulfapyridine was eliminated more slowly than the parent compound, and thus accumulated in all mice given multiple doses of sulfasalazine. The differential between plasma concentrations of sulfapyridine and sulfasalazine varied, however, between males and females: the AUCs for sulfapyridine, at all four doses of sulfasalazine, were higher than those of parent drug by 21–32 times in males and by 5–25 times in females, while maximum plasma concentrations were higher than those of the parent drug by 6–8 times in male mice, and up to 4 times in females. Plasma concentrations of *N*⁴-acetylsulfapyridine were very low compared with those of sulfapyridine. [This indicated slow acetylation of sulfapyridine by B6C3F₁ mice, comparable to that found in humans with the slow-acetylator phenotype.] The pharmacokinetic pattern in B6C3F₁ mice given multiple oral doses of sulfasalazine (i.e. daily doses for three consecutive days) was similar to that in mice given a single oral dose, but accumulation of sulfapyridine was evident, producing greater $AUC_{sulfapyridine}$ values in the multiple-dose study than in the single-dose study ([Zheng et al., 1993](#)).

In a study by the [NTP \(1997a\)](#), male F344/N rats were given sulfasalazine as an intravenous dose at 5 mg/kg bw, and pharmacokinetic

parameters were compared with those in the study in male B6C3F₁ mice by [Zheng et al. \(1993\)](#). The rats retained sulfasalazine longer than mice; the AUC for sulfasalazine in rats was double that in mice, while values for systemic clearance and apparent volume of distribution in mice were double those in rats. The rate of elimination of sulfasalazine was similar in rats and mice; plasma elimination rate constants were 1.47 per hour and 1.28 per hour, respectively, and elimination half-lives, 0.53 hour and 0.54 hour, respectively. In F344/N rats given a low oral dose of 67.5 mg/kg bw, sulfasalazine and its metabolites were undetectable; however, after a higher dose (675 mg/kg bw), plasma concentrations of parent compound were detectable within 12 hours ([NTP, 1997a](#)).

(b) Role of transporter proteins

BCRP is a member of the ATP-dependent efflux transporters, which includes P-glycoprotein or multi-drug resistance protein 1 (*MDR1/ABCB1*) and multidrug resistance-associated protein 2 (*MRP2/ABCC2*). These transporter proteins have significant roles in the processes of drug absorption, distribution, and clearance, and are expressed at the apical membrane of cells in the liver, kidney, brain, placenta, colon, and intestine. From the latter location, on the villus tip of the apical brush-border membrane of intestinal enterocytes, they actively cause efflux of drugs from gut epithelial cells back into the intestinal lumen.

In experiments in *Bcrp*^{-/-} [*Abcg2*^{-/-}] knockout mice given sulfasalazine, the AUC for sulfasalazine was greater than in wildtype mice by 13-fold after an intravenous dose (5 mg/kg bw) and 111-fold after an oral dose (20 mg/kg bw) ([Zaher et al., 2006](#)). This treatment in the *mdr1a* [*Abcb1a*] knockout mouse did not significantly change the AUC for sulfasalazine. Furthermore, studies in wildtype mice treated with an inhibitor of Bcrp (gefitinib) before an oral dose of sulfasalazine resulted in a 13-fold increase in the AUC of plasma sulfasalazine compared with nontreated

controls. This work thus demonstrated that Bcrp has a key role in controlling [i.e. maintaining a low ([Dahan & Amidon, 2010](#))] oral bioavailability of sulfasalazine ([Zaher et al., 2006](#)).

In Caco-2 cells, sulfasalazine normally exhibits a basolateral-to-apical permeability that is 19 times higher than apical-to-basolateral permeability, indicative of net mucosal secretion ([Dahan & Amidon, 2009](#)). In this study of three ATP-dependent efflux transporters (in Caco-2 cells and rat jejunum), specific inhibitors of BCRP and of MRP2 were shown to disrupt the normal direction of sulfasalazine permeability. The presence of both MRP2 and BCRP inhibitors produced an efflux ratio of 1, indicating no efflux of sulfasalazine. Inhibitors of P-glycoprotein had no effect on the movement of sulfasalazine. The results thus suggested that efflux transport of sulfasalazine is mediated by BCRP and MRP2 ([Dahan & Amidon, 2009](#)). A more recent study of sulfasalazine absorption has shown that curcumin is a potent inhibitor of human BCRP. Curcumin not only increased the plasma AUC_{0-8h} eightfold in wildtype mice (but not in *Bcrp*^{-/-} mice), but also increased the plasma AUC_{0-24h} by twofold at microdoses of sulfasalazine and by 3.2-fold at therapeutic doses in humans ([Kusuhara et al., 2012](#)).

Further studies of the absorption characteristics of sulfasalazine in the isolated mouse intestine, have indicated that both influx and efflux transporters are involved in the intestinal absorption of sulfasalazine ([Tomaru et al., 2013](#)). OATP2B1 is a multispecific organic anion influx transporter. Like BCRP, it is localized at the brush-border membrane of intestinal epithelial cells, and mediates uptake of many endogenous and xenobiotic substrates from the lumen. Like BCRP, sulfasalazine is a substrate for OATP2B1 ([Kusuhara et al., 2012](#); [Tomaru et al., 2013](#)). The study by [Kusuhara et al. \(2012\)](#) was inspired by the finding that pharmacokinetic data (plasma AUC for parent drug) from human subjects given sulfasalazine, at either a microdose (100 µg

suspension) or a therapeutic dose (2 g as tablets), demonstrated nonlinearity between doses in the AUC of plasma sulfasalazine ([Kusuhara et al., 2012](#)). Investigations of sulfasalazine transport were performed in three systems in vitro, namely: (i) ATP-dependent uptake of sulfasalazine by membrane vesicles expressing human BCRP; (ii) oral bioavailability of sulfasalazine in vivo, in wildtype and *Bcrp*^{-/-} mice; and (iii) uptake of sulfasalazine in HEK293 cells transfected with the influx transporter *OATP2B1* (*SLCO2B1*). The results indicated that sulfasalazine is a substrate for OATP2B1, and that saturation of the influx transporter OATP2B1 at the therapeutic dose is a possible mechanism underlying nonlinearity in the dose–exposure relationship for sulfasalazine ([Kusuhara et al., 2012](#)).

The nonsteroidal anti-inflammatory drug indomethacin (an inhibitor of the MRP family that includes MRP2) has been shown to change, in a concentration-dependent manner, the direction of membrane permeability to sulfasalazine in Caco-2 cells; high concentrations of indomethacin substantially reduced efflux of sulfasalazine. Efflux was not however abolished, due to the contribution of BCRP to the control of absorption. Additionally, an indomethacin-induced increase in sulfasalazine permeability through the gut wall was also shown in the rat jejunal perfusion model. The concomitant intake of indomethacin and sulfasalazine may lead to increased absorption of sulfasalazine in the small intestine, reducing its concentration in the colon, and potentially altering its therapeutic effect ([Dahan & Amidon, 2010](#)).

4.2 Genetic and related effects

Several studies, particularly those conducted in vivo, have demonstrated genotoxicity associated with sulfasalazine and some of its metabolites. The mutagenicity of sulfasalazine and its two major metabolites, sulfapyridine and 5-ASA, was reviewed by [Iatropoulos et al. \(1997\)](#).

4.2.1 Humans

See [Table 4.1](#)

[Mitelman et al. \(1982\)](#) reported that there was no clear evidence of chromosomal damage in lymphocytes of patients receiving sulfasalazine for 1 month or 4 months at 3 g per day, although an effect could not be ruled out.

Increased frequencies of micronucleus formation and sister chromatid exchange in patients with IBD receiving sulfasalazine have been reported, but confounding factors were apparent in the study ([Erskine et al., 1984](#)).

4.2.2 Experimental systems

See [Table 4.1](#)

(a) Mutagenicity

Sulfasalazine was not mutagenic in assays for gene mutation in bacteria, including a variety of strains of *Salmonella typhimurium*, *Escherichia coli*, and *Klebsiella pneumoniae*, in a variety of protocols, with or without metabolic activation ([Voogd et al., 1980](#); [Zeiger et al., 1988](#); [Iatropoulos et al., 1997](#)). In addition, treatment with sulfasalazine did not result in an increase in mutations conferring 6-thioguanine resistance in mouse lymphoma L5178Y cells, with or without metabolic activation ([Iatropoulos et al., 1997](#)).

(b) Chromosomal damage

[Mackay et al. \(1989\)](#) reported positive results in a test for induction of sister chromatid exchange in cultured human lymphocytes treated with sulfasalazine at a concentration of 20–160 µg/mL in the absence of metabolic activation. In contrast, [Bishop et al. \(1990\)](#) observed no increase in the frequency of sister chromatid exchange in Chinese hamster ovary cells treated with sulfasalazine at up to 1000 µg/mL, with or without metabolic activation. An increase in the formation of micronuclei in cultured human

Table 4.1 Genetic and related effects of sulfasalazine

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>In vitro</i>				
<i>Salmonella typhimurium</i> TA100, TA1535, TA97, TA98, reverse mutation	–	– ^c	6666 µg/plate	Zeiger <i>et al.</i> (1988)
<i>Salmonella typhimurium</i> TA100, TA1535, TA97, TA98, reverse mutation	–	–	6250 µg/plate	Iatropoulos <i>et al.</i> (1997) ^d
<i>Escherichia coli</i> ,WP2 <i>uvrA</i> -p, reverse mutation	–	–	6250 µg/plate	Iatropoulos <i>et al.</i> (1997) ^d
<i>Klebsiella pneumoniae</i> , fluctuation test, base substitution mutation	–	NT	0.5 g/L	Voogd <i>et al.</i> (1980)
Mouse lymphoma L5178Y cells, 6-thioguanine resistance	–	–	700 µg/mL (–S9) 500 µg/mL (+S9)	Iatropoulos <i>et al.</i> (1997) ^d
Sister chromatid exchange, Chinese hamster ovary cells	–	–	160 µg/mL (–S9) 1000 µg/mL (+S9)	Bishop <i>et al.</i> (1990)
Sister chromatid exchange, human lymphocytes	+	NT	20 µg/mL	Mackay <i>et al.</i> (1989)
Micronucleus formation, human lymphocytes	+	NT	40 µg/mL	Mackay <i>et al.</i> (1989)
Chromosomal aberration, Chinese hamster ovary cells	–	–	1000 µg/mL	Bishop <i>et al.</i> (1990)
Chromosomal aberration, human lymphocytes	–	–	100 µg/mL	Iatropoulos <i>et al.</i> (1997) ^d
<i>In vivo</i>				
Micronucleus formation, male B6C3F ₁ mouse, bone-marrow cells	+		1000 mg/kg bw, po × 2	Bishop <i>et al.</i> (1990)
Micronucleus formation, male and female B6C3F ₁ mouse, peripheral blood erythrocytes	+		675 mg/kg bw, po × 13 wk	Bishop <i>et al.</i> (1990)
Micronucleus formation (kinetochore-positive), male B6C3F ₁ mouse, bone-marrow cells	+		1389 mg/kg bw, po × 3	Witt <i>et al.</i> (1992a)
Micronucleus formation (total micronuclei), male B6C3F ₁ mouse, bone-marrow cells	+		1389 mg/kg bw, po × 3	Witt <i>et al.</i> (1992a)
Micronucleus formation (kinetochore-negative), male B6C3F ₁ mouse, bone-marrow cells	+		5634 mg/kg bw, po × 3	Witt <i>et al.</i> (1992a)
Micronucleus formation, male B6C3F ₁ mouse, bone-marrow cells	+		1000 mg/kg bw, po × 3	NTP (1997a)
Micronucleus formation, female B6C3F ₁ mouse, bone-marrow cells	+		1000 mg/kg bw, po × 3	NTP (1997a)
Micronucleus formation, male B6C3F ₁ mouse, bone-marrow cells	–		4000 mg/kg bw, po × 1	NTP (1997a)

Table 4.1 (continued)

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, male F344 rat, bone-marrow cells	?		3000 mg/kg bw, po × 3 ^e	NTP (1997a)
Chromosomal aberration, male B6C3F ₁ mouse, bone marrow cells	–		1000 mg/kg bw, po × 1	Bishop <i>et al.</i> (1990)
Chromosomal aberration, male B6C3F ₁ mouse, bone-marrow cells	–		4000 mg/kg bw, po × 3	NTP (1997a)
Chromosomal aberration, male and female Sprague-Dawley rat, bone-marrow cells	–		500 mg/kg bw, po × 1 ^f	Iatropoulos <i>et al.</i> (1997) ^d
Chromosomal aberration, human lymphocytes	–		3 g/day for 4 months	Mitelman <i>et al.</i> (1980)

^a +, positive; –, negative; ?, inconclusive

^b In-vitro test, µg/mL; in-vivo test, mg/kg bw per day

^c S9 from liver of Aroclor 1254-treated Sprague-Dawley rats and Syrian hamsters

^d Studies conducted by Pharmacia, not published; results summarized in [Iatropoulos *et al.* \(1997\)](#)

^e Two trials were conducted; the first trial gave positive results at the highest dose of 2700 mg/kg bw (po × 3), and the second gave negative results (HID, 3000 mg/kg bw, po × 3). The overall result was equivocal on the basis of nonreproducibility of the positive response

^f Chromosomal aberration was measured at 6, 24, and 48 hours after treatment

HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; po, oral

lymphocytes after treatment with sulfasalazine (effective concentration range, 40–160 µg/mL) in the absence of metabolic activation was reported by [Mackay *et al.* \(1989\)](#). No significant increases in the frequency of chromosomal aberration were observed in cultured Chinese hamster ovary cells ([Bishop *et al.*, 1990](#)), or cultured human lymphocytes ([Iatropoulos *et al.*, 1997](#)), treated with sulfasalazine (concentration, up to 1000 or 100 µg/mL, respectively). Thus the results of tests for chromosomal damage in vitro after treatment with sulfasalazine were generally negative, although sporadic positive results were reported.

In vivo, consistent with results reported in assays in vitro, no increases in the frequency of chromosomal aberration were observed in male mice or male and female rats treated with sulfasalazine by gavage at doses of up to 4000 mg/kg bw per day ([Bishop *et al.*, 1990](#); [Iatropoulos *et al.*, 1997](#); [NTP, 1997a](#)).

The results of assays for micronucleus formation in male and female mice treated with sulfasalazine were uniformly positive when multiple treatments (at least two) were employed ([Bishop *et al.*, 1990](#); [Witt *et al.*, 1992a](#), [NTP, 1997a](#)). Further investigation of the nature of the induced micronuclei revealed that the majority were kinetochore-positive, suggesting that the micronuclei contained whole chromosomes rather than fragments, and were primarily due to aneuploidy events rather than chromosome breakage ([Witt *et al.*, 1992a](#)). This observation was consistent with the negative results in assays for chromosomal aberration with sulfasalazine in vitro and in vivo ([Mitelman *et al.*, 1980](#); [Bishop *et al.*, 1990](#); [Iatropoulos *et al.*, 1997](#); [NTP, 1997a](#)). The negative results of one test in male mice given a single dose of sulfasalazine at 4000 mg/kg bw underscored the need for multiple treatments to induce an observable increase in micronucleus formation ([NTP, 1997a](#)).

In addition to the necessity for multiple treatments, sulfasalazine may also demonstrate selective activity in mice; the results of a study on

micronucleus formation in bone marrow of male rats given three doses of sulfasalazine (highest dose, 3000 mg/kg bw) were judged to be equivocal ([NTP, 1997a](#)); in this assay, an initial trial gave a positive response at the highest dose of 2700 mg/kg bw, but a second trial, with a highest dose of 3000 mg/kg bw, gave negative results. This apparent preferential activity in mice was consistent with the observation that mice have a greater systemic exposure than rats to sulfapyridine, the active moiety, after administration of similar doses ([Zheng *et al.*, 1993](#)).

In one study, no evidence for genotoxicity was obtained for sulfasalazine when tested for the induction of micronuclei in mouse bone marrow, with or without pretreatment with folate. Likewise, no evidence for formation of DNA adducts was detected by ³²P-postlabelling in rat and mouse liver and urinary bladder ([Iatropoulos *et al.*, 1997](#)). [The nuclease P1 enrichment procedure was used in the ³²P-postlabelling method. Assuming that N-hydroxylation of sulfapyridine occurred in vivo, adducts derived from this metabolite would probably be lost.]

4.2.3 Genotoxicity of sulfasalazine metabolites

See [Table 4.2](#)

Sulfasalazine has two major metabolites, sulfapyridine (a carrier molecule that allows transport of sulfasalazine to the intestine, where it is activated) and 5-ASA, the therapeutically active moiety.

(a) 5-ASA

5-ASA has not shown activity in any assay for genotoxicity in vitro or in vivo. It does not induce mutations in any of a variety of *Salmonella typhimurium* strains, with or without metabolic activation or in *Klebsiella pneumoniae* in the absence of metabolic activation ([Voogd *et al.*, 1980](#)). No induction of sister chromatid exchange, micronucleus formation, or chromosomal aberration

Table 4.2 Genetic and related effects of metabolites of sulfasalazine

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>5-Aminosalicylic acid</i>				
<i>Salmonella typhimurium</i> TA100, TA1537, TA98, reverse mutation	–	–	10 g/L (TA1537, TA98) 5 g/L (TA100)	Voogd et al. (1980)
<i>Klebsiella pneumoniae</i> , fluctuation test, base-substitution mutation	–	NT	0.5 g/L	Voogd et al. (1980)
Sister chromatid exchange, Chinese hamster ovary cells, in vitro	–	–	280 µg/mL	Witt et al. (1992b)
Sister chromatid exchange, human lymphocytes, in vitro	–	NT	16 µg/mL	Mackay et al. (1989)
Micronucleus formation, human lymphocytes, in vitro	–	NT	16 µg/mL	Mackay et al. (1989)
Chromosomal aberration, Chinese hamster ovary cells, in vitro	–	–	280 µg/mL	Witt et al. (1992b)
Micronucleus formation, male B6C3F ₁ mouse, bone-marrow cells, in vivo	–		250 mg/kg bw, ip × 3	Witt et al. (1992b)
<i>Sulfapyridine</i>				
Sister chromatid exchange, Chinese hamster ovary cells, in vitro	+	–	1667 µg/mL	Witt et al. (1992b)
Chromosomal aberration, Chinese hamster ovary cells, in vitro	–	–	5000 µg/mL	Witt et al. (1992b)
Sister chromatid exchange, human lymphocytes, in vitro	+	NT	200 µg/mL	Mackay et al. (1989)
Micronucleus formation, human lymphocytes, in vitro	–	NT	400 µg/mL	Mackay et al. (1989)
Micronucleus formation, male B6C3F ₁ mouse, bone-marrow cells, in vivo	+		1000 mg/kg bw, ip × 3	Witt et al. (1992b)
Micronucleus formation (kinetochore-positive), male B6C3F ₁ mouse, bone-marrow cells, in vivo	+		714 mg/kg bw, po × 3	Witt et al. (1992a)
Micronucleus formation (total micronuclei), male B6C3F ₁ mouse, bone-marrow cells, in vivo	+		714 mg/kg bw, po × 3	Witt et al. (1992a)
Micronucleus formation (kinetochore negative), male B6C3F ₁ mouse, bone-marrow cells, in vivo	+		1429 mg/kg bw, po × 3	Witt et al. (1992a)
<i>N⁴-Acetylsulfapyridine</i>				
Sister chromatid exchange, human lymphocytes, in vitro	+	NT	80 µg/mL	Mackay et al. (1989)
Micronucleus formation, human lymphocytes, in vitro	+	NT	20 µg/mL	Mackay et al. (1989)
<i>N⁴-Acetyl-5'-hydroxysulfapyridine</i>				
Sister chromatid exchange, human lymphocytes, in vitro	+	NT	80 µg/mL	Mackay et al. (1989)
Micronucleus formation, human lymphocytes, in vitro	–	NT	80 µg/mL	Mackay et al. (1989)

Table 4.2 (continued)

Test system	Results ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>5'-Hydroxysulfapyridine</i>				
Sister chromatid exchange, human lymphocytes, in vitro	–	NT	80 µg/mL	Mackay <i>et al.</i> (1989)
Micronucleus formation, human lymphocytes, in vitro	–	NT	80 µg/mL	Mackay <i>et al.</i> (1989)
<i>N-Acetyl-5-aminosalicylic acid</i>				
Sister chromatid exchange, human lymphocytes, in vitro	–	NT	8 µg/mL	Mackay <i>et al.</i> (1989)
Micronucleus formation, human lymphocytes, in vitro	–	NT	8 µg/mL	Mackay <i>et al.</i> (1989)

^a +, positive; –, negative

^b In-vitro test, µg/mL; in-vivo test, mg/kg bw per day

HID, highest ineffective dose; ip, intraperitoneal; LED, lowest effective dose; po, oral

has been reported in human lymphocytes or Chinese hamster ovary cells in vitro ([Mackay et al., 1989](#); [Witt et al., 1992b](#)). In vivo, no increase in the frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of male mice treated with 5-ASA (dose range, 125–250 mg/kg bw per day for 3 days) by intraperitoneal injection ([Witt et al., 1992b](#)).

(b) Sulfapyridine

Sulfapyridine has been reported to induce sister chromatid exchange in Chinese hamster ovary cells and cultured human lymphocytes in the absence of metabolic activation ([Mackay et al., 1989](#); [Witt et al., 1992b](#)); no increase in the frequency of sister chromatid exchange was noted in the presence of metabolic activation in Chinese hamster ovary cells ([Witt et al., 1992b](#)). Sulfapyridine did not induce chromosomal aberration in Chinese hamster ovary cells, with or without metabolic activation ([Witt et al., 1992b](#)). [Mackay et al. \(1989\)](#) reported that sulfapyridine did not induce micronucleus formation in cultured human lymphocytes in the absence of metabolic activation, at concentrations that reached 400 µg/mL. Sulfapyridine induced a strong, dose-related increase in the frequency of micronucleated polychromatic erythrocytes when administered either as multiple intraperitoneal injections ([Witt et al., 1992b](#)) or by gavage ([Witt et al., 1992a](#)). As with sulfasalazine, the majority of micronucleated erythrocytes induced by sulfapyridine in mice were shown to contain kinetochores ([Witt et al., 1992a](#)), implying that sulfapyridine-induced micronucleus formation resulted from failure of mitotic chromosomal segregation, rather than chromosome breakage.

(c) Metabolites of 5-ASA and sulfapyridine

[Mackay et al. \(1989\)](#) also tested four acetylated and/or hydroxylated metabolites of sulfapyridine and 5-ASA for their ability to induce sister chromatid exchange and micronucleus formation in cultured human

lymphocytes. *N*⁴-acetylsulfapyridine was capable of inducing both sister chromatid exchange and micronucleus formation, while *N*⁴-acetyl-5'-hydroxysulfapyridine only induced sister chromatid exchange. 5'-Hydroxysulfapyridine and *N*⁴-acetyl-5-aminosalicylic acid did not induce either sister chromatid exchange or micronucleus formation at the concentrations tested.

4.3 Other mechanistic data relevant to carcinogenicity

4.3.1 Adverse effects

In humans, sulfasalazine is associated with a wide range of adverse side-effects that include agranulocytosis ([Kaufman et al., 1996](#)), hepatotoxicity ([de Abajo et al., 2004](#); [Jobanputra et al., 2008](#)), nephrotoxicity ([Gisbert et al., 2007](#)), neurotoxicity ([Liedorp et al., 2008](#)), and pulmonary toxicity ([Parry et al., 2002](#)). Sulfasalazine is also associated with reversible infertility in men and in male experimental animals ([O'Moráin et al., 1984](#)). Reactions to sulfasalazine may result from an idiosyncratic delayed-type hypersensitivity reaction that may affect internal organs in variable ways ([Jobanputra et al., 2008](#)).

Case reports of serious hepatotoxicity associated with sulfasalazine are frequent and occur predominantly within the first month of starting therapy; the pattern of liver injury can be hepatocellular or cholestatic, and may lead to liver failure. Serious hepatotoxicity, which could be a part of the DRESS (drug rash, eosinophilia and systemic symptoms) syndrome is described in approximately 0.1% of users, but the estimated incidence is higher (0.4%) in patients with inflammatory arthritis ([de Abajo et al., 2004](#); [Jobanputra et al., 2008](#)).

Renal toxicity associated with sulfasalazine treatment may be irreversible. Although the sulfapyridine moiety is thought to be responsible for most of the adverse effects of sulfasalazine, several case reports in patients with IBD

indicate that renal toxicity in humans may occur from treatment with both sulfasalazine and 5-ASA ([Gisbert *et al.*, 2007](#)). Clinically, 5-ASA-associated nephrotoxicity is typically expressed as interstitial nephritis, glomerulonephritis, nephritic syndrome, and acute renal failure ([Barbour & Williams, 1990](#); [Birketvedt *et al.*, 2000](#); [Augusto *et al.*, 2009](#)). The incidence of clinically restrictive renal impairment has been estimated at < 1 per 500 patients ([World *et al.*, 1996](#)). The mechanism is unclear, although both a delayed cell-mediated response, and a dose-dependent effect have been considered ([Corrigan & Stevens, 2000](#)). 5-ASA-related nephrotoxicity appeared to be dose-related in female rats given a single intravenous injection of the sodium salt of 5-ASA at doses of up to 5.7 mmol/kg bw ([Calder *et al.*, 1972](#)); however, dose-dependency may require the administration of doses much higher than those given to humans. Of note, 5-ASA combines the structural features of a salicylate and a phenacetin, both of which have well documented nephrotoxic potential ([Corrigan & Stevens, 2000](#)).

It has been hypothesized that oxidative stress may be a factor in sulfasalazine-induced renal and hepatic injury. Treatment-related alterations in the levels of biomarkers of oxidative stress were detected in kidney and liver tissues of male Sprague-Dawley rats given sulfasalazine as daily oral doses at 0, 300, or 600 mg/kg bw for 14 days. At the highest dose, there were significant decreases in the activities of renal and hepatic superoxide dismutase, and significant increases in catalase activity, thiobarbituric acid-reactive substances, and in the oxidized/reduced glutathione ratio ([Linares *et al.*, 2009](#)).

Sulfasalazine can cause haemolytic anaemia ([Das *et al.*, 1973](#); [Mechanick, 1985](#)) and methaemoglobinaemia ([Miller *et al.*, 1971](#); [Kater, 1974](#); [Azad Khan *et al.*, 1983](#)). In a group of 50 patients receiving sulfasalazine at 2.5 g per day as maintenance therapy for ulcerative colitis, approximately 40% had elevated levels of methaemoglobin

([Pounder *et al.*, 1975](#)). Although sulfonamide-induced haemolysis can be severe in patients with glucose-6-phosphate dehydrogenase deficiency, this study showed that sulfasalazine-induced erythrocyte damage also occurred in patients with normal levels of this enzyme ([Pounder *et al.*, 1975](#)).

The role of metabolites in sulfasalazine-mediated toxicity was investigated in vitro, using human erythrocytes and mononuclear leukocytes as target cells in the presence of human liver microsomes; methaemoglobin formation and cytotoxicity were selected as toxicity end-points. In addition to sulfasalazine, the study included the metabolites 5-ASA, sulfapyridine, and 5'-hydroxysulfapyridine ([Pirmohamed *et al.*, 1991](#)). Bioactivation by human liver microsomes that are dependent on reduced nicotinamide adenine dinucleotide phosphate (NADPH) to a species that caused methaemoglobinaemia and cytotoxicity was only observed with sulfapyridine. Chromatographic analysis demonstrated that sulfapyridine was converted to a short-lived intermediate ($t_{1/2}$, 8.1 minutes at pH 7.4) with elution characteristics identical to those of synthetic sulfapyridine hydroxylamine. This hydroxylamine (10–500 μ M) caused a concentration-dependent increase in both methaemoglobinaemia (2.9–24.4%) and cytotoxicity in the absence of a microsomal system; neither sulfasalazine nor any of the other test metabolites had such effects. When the microsomal incubations were conducted in the presence of micromolar concentrations of reducing agents (e.g. ascorbic acid, glutathione, or *N*-acetylcysteine), sulfapyridine-induced cytotoxicity was decreased in mononuclear leukocytes, but there was no effect upon the levels of methaemoglobinaemia. This suggested that sulfapyridine hydroxylamine could readily penetrate erythrocytes, where it may undergo redox cycling to nitroso-sulfapyridine, the species ultimately responsible for the production of methaemoglobin. These observations further suggested that

N-hydroxylation of sulfapyridine may account for some of the adverse effects associated with sulfasalazine ([Pirmohamed et al., 1991](#)). [Of note, *N*-hydroxylation is thought to play a key role in the bioactivation of aromatic amine carcinogens, such as 4-aminobiphenyl ([IARC, 2012](#)).]

4.3.2 Effects upon folate pathways

Sulfasalazine has been shown to inhibit the activity of dihydrofolate reductase, methylenetetrahydrofolate reductase, and serine transhydroxymethylase, and also the cellular uptake of folate ([Selhub et al., 1978](#); [Jansen et al., 2004](#); [Urquhart et al., 2010](#)).

4.3.3 Urolithiasis

An increased incidence of transitional cell papilloma of the urinary bladder in male rats treated with sulfasalazine has been correlated ($P < 0.01$) with increased incidences of concretions (calculi) in the urinary bladder ([NTP, 1997a](#); see also Section 3). In a subsequent study, there was decreased incidence of urinary hyperplasia in male rats subjected to caloric restriction and treated with sulfasalazine, and little evidence of urinary bladder concretion, compared with rats fed ad libitum ([NTP, 1997b](#); see also Section 3). [These data suggested that chronic inflammation associated with urolithiasis may be a factor in sulfasalazine-induced carcinogenesis of the bladder in male rats.]

4.4 Susceptibility

The adverse effects of sulfasalazine have been linked to sulfapyridine ([Das et al., 1973](#)). This metabolite, which is well absorbed from the colon, is inactivated by *NAT2*-mediated *N*-acetylation. *NAT2* polymorphisms have been associated with different susceptibilities to the adverse effects of sulfasalazine. People with the slow-acetylator genotype have higher serum concentrations of free sulfapyridine and lower concentrations of

acetylated sulfapyridine than fast acetylators (see also Section 4.1.1), and appear more likely to experience toxic symptoms when treated with equivalent doses of sulfasalazine ([Das & Dubin, 1976](#); [Azad Khan et al., 1983](#); [Ricart et al., 2002](#); [Tanaka et al., 2002](#); [Kumagai et al., 2004](#); [Chen et al., 2007](#); [Soejima et al., 2008](#)).

4.5 Mechanistic considerations

Sulfasalazine was reported to act as a co-carcinogen at a dose of 60 mg/kg bw per day in the 1,2-dimethylhydrazine model of colon carcinogenesis in rats. In the same study, 5-ASA, the active pharmacophore unit of sulfasalazine, acted as a co-carcinogen at a dose of 30 mg/kg bw per day, but not at 60 mg/kg bw per day, which suggested that 5-ASA exerts a protective effect on the colon mucosa, provided a sufficient amount of the compound reaches the colon ([Davis et al., 1992](#)). On the basis of early proposals that localized tissue folate deficiency may account for carcinogenesis ([Lashner et al., 1989](#)), it was hypothesized that sulfasalazine may be co-carcinogenic due to its anti-folate characteristics. Sulfasalazine inhibited dihydrofolate reductase, methylenetetrahydrofolate reductase, and serine transhydroxymethylase, and also the cellular uptake of folate ([Selhub et al., 1978](#); [Jansen et al., 2004](#); [Urquhart et al., 2010](#)). Reduced levels of *S*-adenosylmethionine or 5,10-methylenetetrahydrofolate, required for thymidine synthesis, might account for the effect; however, colonic cells may not be completely dependent on blood stream nutrients ([Meenan, 1993](#)). In the rat, colonic bacterial folate is incorporated in the hepatic folate pool ([Rong et al., 1991](#)), and this could counteract sulfasalazine-induced folate depletion ([Meenan, 1993](#)). In patients with ulcerative colitis, folate concentrations measured in colonic epithelial cells obtained from endoscopic colon biopsies were not decreased in sulfasalazine-treated patients compared with controls; this contrasted with serum concentrations of

folate, which were reduced in patients receiving sulfasalazine ([Meenan *et al.*, 1996](#)). These data suggested that the potentially protective effects of folate supplementation against colorectal carcinogenesis in patients with ulcerative colitis were not due to correction of localized folate deficiency.

Two-year studies in male and female F344/N rats given sulfasalazine by gavage indicated some evidence for carcinogenic activity on the basis of increased incidences of transitional cell papilloma of the urinary bladder, and clear evidence for carcinogenic activity in male and female B6C3F₁ mice on the basis of increased incidences of hepatocellular adenoma and hepatocellular carcinoma ([NTP, 1997a](#); see also Section 3). The data on mutagenicity of sulfasalazine and its metabolite, sulfapyridine, suggested that the parent drug and the metabolite are predominantly aneugens ([Bishop *et al.*, 1990](#); [Witt *et al.*, 1992a, b](#)). Increased frequencies of micronucleus formation and sister chromatid exchange in patients with IBD receiving sulfasalazine have been reported, but confounding factors were apparent in the study ([Erskine *et al.*, 1984](#)).

Folate deficiency was considered as a possible explanation for the induction of micronucleus formation by sulfasalazine in vivo. However, patients reported to have an elevated frequency of sister chromatid exchange and micronucleus formation had serum folate concentrations that were at the low end of the normal range, and the observation of reticulocytosis in a 90-day study in mice suggested an erythropoietic effect not characteristic of folate deficiency ([Bishop *et al.*, 1990](#)).

The increased incidence of transitional cell papilloma of the urinary bladder in male rats treated orally with sulfasalazine was correlated with increased incidence of concretions (calculi) in the urinary bladder ([NTP, 1997a](#)). Chronic inflammation associated with urolithiasis may be a factor in sulfasalazine-induced carcinogenesis of the bladder in male rats.

5. Summary of Data Reported

5.1 Exposure data

Sulfasalazine is a synthetic aminosalicylate used as an oral anti-inflammatory drug. The most common use of sulfasalazine is for the treatment of autoimmune arthritis. Prescriptions have been stable over the past decade, with global sales of US\$ 222 million in 2012. Environmental contamination with sulfasalazine in ground-water has been noted, but exposure is likely to be predominantly through use as a medication.

5.2 Human carcinogenicity data

The available studies of exposure to sulfasalazine included a surveillance study, two cohort studies, three nested case-control studies, and three case-control studies on cancer of the colorectum among patients with inflammatory bowel disease or ulcerative colitis. These studies were hampered in their ability to evaluate the association between exposure to sulfasalazine and risk of cancer of the colorectum by the small numbers of exposed cases, imprecise risk estimates, and little information on exposure or the dose or duration of sulfasalazine use. There were also concerns about selection bias in some studies based on clinical populations.

The best designed studies were a nested case-control study from a large cohort from the General Practice Research Database in the United Kingdom, and a large population-based case-control study among patients with inflammatory bowel disease in the USA that evaluated exposure-response relationships. Conflicting findings were reported across studies, with four studies reporting relative risks of less than unity, two studies reporting estimates close to unity, and two studies reporting a relative risk of greater than unity. Most of these relative risks were not statistically significant. In the studies that evaluated dose-response relationships, no clear patterns were observed.

5.3 Animal carcinogenicity data

In one study in male and female mice given sulfasalazine by gavage, there was a significant increase in the incidence of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined) in both sexes; there was also an increase in the incidence of hepatocellular carcinoma in females.

In a study of dietary restriction in male mice given diets containing sulfasalazine ad libitum, there were significant increases in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) in exposed mice compared with the controls fed ad libitum and the weight-matched controls. In contrast, the incidence of hepatocellular tumours in dietary-restricted mice was significantly decreased after 2 years in the group exposed to sulfasalazine, and was similar to that in non-treated mice after 3 years.

In one study in male and female rats given sulfasalazine by gavage, there was a significant increase in the incidence of transitional cell papilloma of the urinary bladder in males; there was also a non-significant increase in the incidences of rare transitional cell papilloma of the kidney and of rare transitional cell papilloma of the urinary bladder in female rats.

In a study of dietary restriction in male rats, the incidence of transitional cell papilloma of the urinary bladder was significantly greater in rats receiving sulfasalazine than in controls fed ad libitum. No significant increase in the incidence of transitional cell papilloma of the urinary bladder was observed in rats subjected to dietary restriction and given sulfasalazine.

In a co-carcinogenicity study in male rats, sulfasalazine increased the total number and multiplicity of 1,2-dimethylhydrazine-induced intestinal tumours.

5.4 Mechanistic and other relevant data

The sulfasalazine molecule contains a 5-aminosalicylic acid moiety linked by an azo bond to a sulfapyridine moiety. Cleavage of the azo bond by bacterial azoreductases in the colon releases two pharmacologically active compounds: 5-aminosalicylic acid and sulfapyridine. Sulfapyridine is absorbed, and *N*-acetylated by the highly polymorphic *N*-acetyl transferase 2 (NAT2), resulting in considerable inter-individual variation in the pharmacokinetics of sulfasalazine.

Sulfasalazine is not mutagenic in standard bacterial assays for gene mutation, with or without exogenous metabolic activation. Tests for chromosomal damage in vitro after treatment with sulfasalazine were generally negative, although sporadic positive results have been reported. Likewise, no increases in the frequency of chromosomal aberration were observed in male mice or rats treated with sulfasalazine. Positive results were consistently obtained in assays for micronucleus formation in male and female mice in vivo when multiple treatments with sulfasalazine were given; the results suggested that these results were primarily due to aneuploidy events rather than chromosome breakage.

Sulfasalazine inhibits the activity of dihydrofolate reductase, methylenetetrahydrofolate reductase, and serine transhydroxymethylase, and also the cellular uptake of folate. However, folate deficiency does not appear to account for the effects of sulfasalazine in humans and mice.

The sulfasalazine metabolite sulfapyridine has been shown to undergo *N*-hydroxylation when incubated with human liver microsomes in the presence of NADPH. *N*-Hydroxylation is known to account for the bioactivation of carcinogenic aromatic amines to DNA-binding species, and such a pathway would be consistent with the target organs associated with sulfasalazine-induced carcinogenicity in rats. However, no

evidence of DNA-adduct formation was detected in rat and mouse liver or urinary bladder.

Male rats treated orally with sulfasalazine had an increased incidence of transitional cell papilloma of the urinary bladder, and this was correlated with increased incidences of calculi in the urinary bladder. Chronic inflammation associated with urolithiasis may be a factor in sulfasalazine-induced carcinogenesis of the bladder in male rats.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

There is *sufficient evidence* for the carcinogenicity of sulfasalazine in experimental animals.

6.3 Overall evaluation

Sulfasalazine is *possibly carcinogenic* to humans (Group 2B).

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