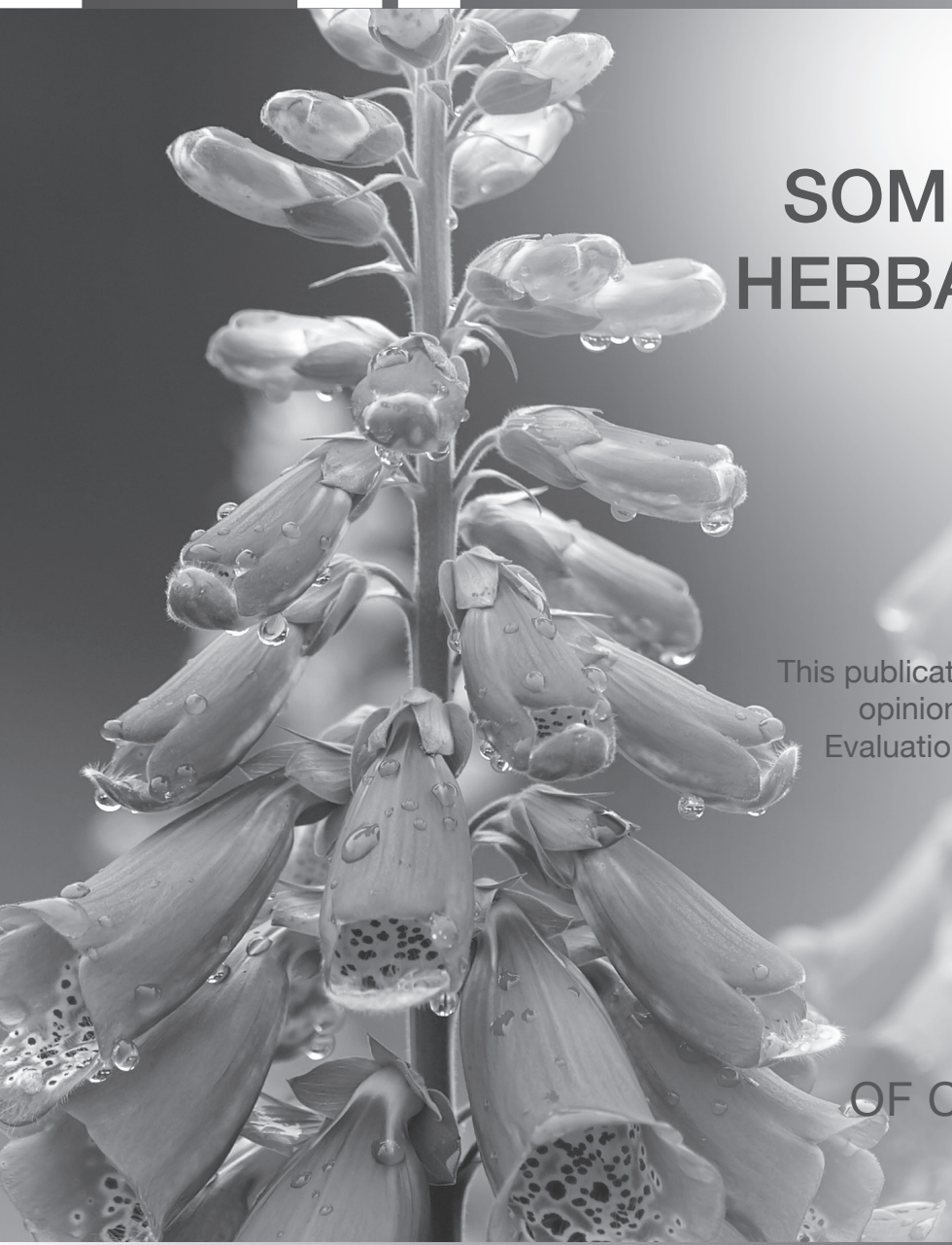
IARC MONOGRAPHS



SOME DRUGS AND HERBAL PRODUCTS

VOLUME 108

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 4–11 June 2013

Lyon, France - 2016

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

PIOGLITAZONE AND ROSIGLITAZONE

1. Exposure Data

Thiazolidinediones are a class of synthetic compounds that exert direct effects on the mechanisms of insulin resistance, and result in improved insulin action and reduced hyperinsulinaemia. In the present *Monograph*, the Working Group evaluated pioglitazone and rosiglitazone, two thiazolidinediones that initially showed great promise as receptor-mediated oral therapy for type 2 diabetes mellitus. Rosiglitazone and pioglitazone were introduced to the market at about the same time (1999 in the USA, and 2001–2002 in Taiwan, China; Tseng, 2012d). Some patients may therefore have been exposed to both drugs, which were sometimes prescribed sequentially. The Working Group did not consider other thiazolidinediones, such as troglitazone, which was marketed for only a short period (1997–2000), before being withdrawn from the world market subsequent to reports of fatal hepatotoxicity (Julie *et al.*, 2008).

1.1 Chemical and physical data on pioglitazone

1.1.1 Nomenclature

(a) Pioglitazone

Chem. Abstr. Serv. Reg. No.: 111025-46-8 (SciFinder, 2013).

Chem. Abstr. Serv. Name: 2,4-Thiazolidinedione, 5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]- (SciFinder, 2013).

IUPAC systematic Name: 5-[[4-[2-(5-Ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Drugbank, 2013; Pubchem, 2013).

WHO International Nonproprietary Name (INN): Pioglitazonum (WHO, 2007).

(b) Pioglitazone hydrochloride

Chem. Abstr. Serv. Reg. No.: 112529-15-4 (SciFinder, 2013).

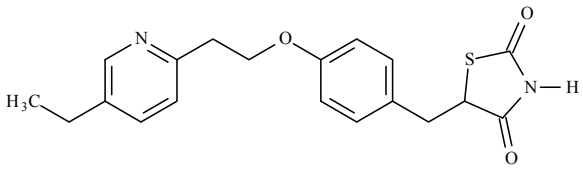
Chem. Abstr. Serv. Name: 2,4-Thiazolidinedione,5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-, hydrochloride (1:1) (SciFinder, 2013).

Proprietary Names: Actos; Glustin; Zactose (Drugbank, 2013; Pubchem, 2013).

United States Nonproprietary Name (USAN): Pioglitazone hydrochloride

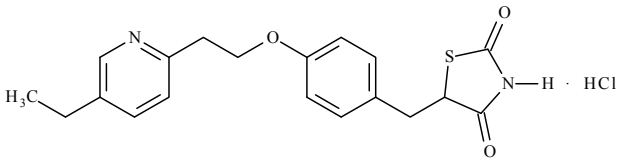
1.1.2 Structural and molecular formulae and relative molecular mass

(a) Pioglitazone



$C_{19}H_{20}N_2O_3S$ (O'Neil, 2006)
Relative molecular mass: 356.44

(b) Pioglitazone hydrochloride



$C_{19}H_{20}N_2O_3S$. HCl
Relative molecular mass: 392.90

1.1.3 Chemical and physical properties of the pure substance

(a) Pioglitazone

Description: Colourless needles from dimethylformamide and water (O'Neil, 2006)

Density: 1.260 g/cm³ at 20 °C (Langchem, 2013)

Melting point: 183–184 °C (O'Neil, 2006; Milne, 2000)

Spectroscopy data: Ultraviolet (UV) (Venkatesh *et al.*, 2006), proton nuclear magnetic resonance (^{1}H NMR) (Madivada *et al.*, 2009), ^{13}C NMR (Madivada *et al.*, 2009), infrared (IR) (Madivada *et al.*, 2009), and mass spectrometry (MS) (Wang & Miksa, 2007; Thevis *et al.*, 2005) have been reported

Solubility: 14.05 µg/mL (water); 25.07 µg/mL (0.15 M NaCl); 10.61 µg/mL (0.1 M phosphate buffer) (Seedher & Kanojia, 2008); 46.85 mg/L at 25 °C (EMA CHMP, 2012)

The solubility is highly dependent on pH, and is greater at lower pH. Solubility according to pH: 52.60 µg/mL (pH 1.83); 38.63 µg/mL (pH 2.57); 4.55 µg/mL (pH 3.92); 6.35 µg/mL (pH 7.39); 19.19 µg/mL (pH 8.82); 49.96 µg/mL (pH9.52) (Seedher & Kanojia, 2009); 100 µg/mL in 1:1 dimethyl sulfoxide:phosphate-buffered saline (pH 7.2); 2.5 mg/mL in dimethylformamide and dimethyl sulfoxide (Cayman SDS, 2013)

Stability data: Exposure to heat (105 °C) results in a change of appearance; exposure to heat and UV light results in a slight drop (1.5–2%) in assay; exposure to 0.1 N sodium hydroxide results in degradation; exposure to heat (105 °C) and peroxide results in a slight increase in total impurities (EMA CHMP, 2012)

Octanol/water partition coefficient: Log P = 2.72–3.73 (Giaginis *et al.*, 2007)

Vapour pressure: 2.88×10^{-14} mm Hg at 25 °C, estimated (EMA CHMP, 2012)

(b) Pioglitazone hydrochloride

Description: White crystalline powder, odourless (Physicians Desk Reference, 2012); colourless prisms from ethanol (O'Neil, 2006)

Density: 1.26 g/cm³ (ChemicalBook, 2013)

Melting point: 193–194 °C (O'Neil, 2006; Milne, 2000)

Solubility: Practically insoluble in water, insoluble in ether, slightly soluble in ethanol, very slightly soluble in acetone and acetonitrile (O'Neil, 2006); very soluble in dimethylformamide (Physicians Desk Reference, 2012)

Vapour pressure: 3.0×10^{-13} mm Hg at 25 °C (ChemicalBook, 2013)

1.1.4 Technical products and impurities

Pioglitazone hydrochloride is used to formulate the finished dosage forms described below.

(a) Trade names

Actos; Glustin; Glizone; Pioz; Zactose (Rx List, 2013).

(b) Impurities

Three impurities were detected up to concentration of 0.1% by reversed-phase high-performance liquid chromatography (HPLC) and were characterized by ^{1}H NMR, ^{13}C NMR, MS, and IR spectral data (Kumar *et al.*, 2004):

- 5-(4-Hydroxybenzyl)-1,3-thiazolidine-2,4-dione
- 5-(4-Fluorobenzyl)-1,3-thiazolidine-2,4-dione
- 2-[2-(4-Bromophenoxy) ethyl-5-ethyl] pyridine.

Four impurities in pioglitazone were prepared and characterized by NMR spectroscopy (Richter *et al.*, 2007):

- 5-{4-[2-(5-Ethyl-6-{4-[2-(5-ethylpyridin-2-yl)-ethoxy]phenyl}pyrid-2-yl)ethoxy] benzyl}-1,3-thiazolidine-2,4-dione
- 5-{4-[2-(5-Ethyl-4-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}pyrid-2-yl)ethoxy] benzyl}-1,3-thiazolidine-2,4-dione
- 5-{6,4′-bis-[2-(5-Ethyl-pyridin-2-yl)ethoxy] biphenyl-3-ylmethyl}-1,3-thiazolidine-2,4-dione
- 5-{4-[2-(5-Ethyl-pyridin-2-yl)ethoxy] benzyl}-3-[2-(5-ethyl-pyridin-2-yl) ethyl]-1,3-thiazolidine-2,4-dione.

1.2 Analysis of pioglitazone

Physical properties used for the identification of the substance, e.g. IR and melting point, are presented in Section 1.1.3. Selected non-compendial methods are presented in Table 1.1.

There are numerous methods including HPLC with UV or MS detection for the analysis of pioglitazone in different matrices, such as formulations, plasma, and serum. The limit of quantitation (LOQ) in serum using the method by Palem *et al.* (2011) is 1 ng/mL; Lin *et al.* (2003) reported a LOQ of 0.5 ng/mL in human plasma. Other reported techniques for analysing formulations include capillary electrophoresis (Radhakrishna *et al.*, 2002a) and potentiometric sensors (Mostafa & Al-Majed, 2008).

1.3 Production and use of pioglitazone

1.3.1 Production

Pioglitazone exists in two polymorphic crystal forms. Polymorph 1 is used in the manufacture of the finished drug product. The manufacture of pioglitazone consists of six steps, the last in combination with hydrochloride to yield pioglitazone hydrochloride (EMEA, 2012).

1.3.2 Use

(a) Indications

Pioglitazone acts as an "insulin sensitizer", providing a means to improve glycaemic control by reducing insulin resistance and thus decreasing hyperglycaemia in patients with type 2 diabetes mellitus (Sweetman, 2011; FDA, 2013a). Pioglitazone acts only in the presence of endogenous insulin. It is indicated particularly for overweight patients as an adjunct to diet and exercise to improve glycaemic control. It is contraindicated in patients with type 1 diabetes mellitus, and for the treatment of diabetic ketoacidosis. It

Table 1.1 Analytical methods for pioglitazone and rosiglitazone

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Non-compendial methods				
Human plasma	Pre-activation of SPE column with acetonitrile and KH_2PO_4, addition of IS and KH_2PO_4 to pioglitazone solution in plasma, extraction using SPE column, elution using acetonitrile and water, filtration, and analysis of filtrate	LC-UV Column: C_{18} Mobile phase: methanol, acetonitrile and mixed phosphate buffer (pH 2.6, 10 mM) (40 : 12 : 48, v/v/v) Flow rate: 1.2 mL/min Wavelength: 269 nm	50 ng/mL (LLOQ)	Sripalakit *et al.* (2006)
Human plasma	Addition of pioglitazone standard solutions, IS, diethylether, mixing, centrifugation, addition of NaOH to organic layer, mixing, centrifugation, and injection of aqueous layer in HPLC	LC-UV Column: C_{18} Mobile phase: acetonitrile and 140 mM KH_2PO_4 (pH 4.45) (40 : 60, v/v) Flow rate : 1.4 mL/min Wavelength: 269 nm	25 ng/mL (LLOQ)	Souri *et al.* (2008)
Human plasma	Addition of IS, 0.1 M ammonium acetate, pH adjustment, extraction with methyl *tert*-butyl ether and butyl chloride, centrifugation, evaporation, residues dissolved in mobile phase, centrifugation	LC-ESI-MS Column: C_{18} Mobile phase: acetonitrile : water (60 : 40) with 10 mM ammonium acetate and 0.02% TFA Flow rate: 0.2 mL/min SRM transition: 357 m/z →134 m/z (positive mode)	0.5 ng/mL (LLOQ)	Lin *et al.* (2003)
Human serum	Samples diluted 1 : 1 (v/v) with acetonitrile containing IS	LC-ESI-MS Column: C_{18} Mobile phase: 50% of 10 mM ammonium acetate in acetonitrile: water (10 : 90) and 50% of water : acetonitrile (10 : 90) Flow rate: 1/35 mL/min SRM transition: 357 m/z → 134 m/z (positive mode)	9 ng/mL (LLOQ)	Xue *et al.* (2003)
Pig serum	Addition of IS, NaOH solution (1 M), dichloromethane, centrifugation, separation of organic layer, evaporation, reconstitution with methanol and analysis	LC-UV Column: C_{18} Mobile phase: acetonitrile : 50 mM ammonium acetate buffer (pH 5) (67 : 33, v/v) Flow rate: 1 mL/min Wavelength: 240 nm	1 ng/mL (LLOQ)	Palem *et al.* (2011)
Dog serum	Serum samples loaded on the column, elution with acetonitrile, eluate mixed with purified water, and analysis	LC-UV Column: C_{18} Mobile phase: Acetonitrile : water (41 : 59, v/v) containing 1.2 mL/L acetic acid (pH 6.0 ± 0.05) Flow rate: 1.0 mL/min Wavelength: 229 nm	25 ng/mL (LLOQ)	Zhong & Lakings (1989)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Rat serum	Addition of IS solution (rosiglitazone), precipitation by addition of ethylacetate, centrifugation, and analysis	LC-UV Column: C_{18} Mobile phase: Methanol : ammonium acetate (30 mM, pH 5) (60 : 40, v/v) Flow rate: 1.0 mL/min Wavelength: 269 nm	15 ng/mL (LOD) 50 ng/mL (LOQ)	Ravikanth *et al.* (2011)
Human serum and urine	Serum sample: activation of SPE column, addition of phosphate buffer, elution with methanol and 0.02 M sodium acetate, addition of acetic acid, evaporation, dissolve residue in 0.1 M KH_2PO_4, extraction in diethylether, evaporation and addition of IS Urine sample: addition of 0.1 M KH_2PO_4, extraction with mixture of diethylether and dichloromethane (4 : 1, v/v), evaporation, dissolution in IS solution, and analysis	LC-UV Column: C_{18} Mobile phase: 0.05 M phosphate buffer (pH 6.0) : methanol (9 : 1, v/v) and 0.05 M phosphate buffer (pH 6.0): methanol : acetonitrile (4 : 2 : 4, v/v) Flow rate: 1.0 mL/min Wavelength: 269 nm	Serum: 0.01–0.05 µg/mL Urine: 0.1–0.5 µg/mL	Yamashita *et al.* (1996)
Bulk and pharmaceutical formulation	Bulk sample: sample in mixture of aqueous 0.1% *ortho*-phosphoric acid and acetonitrile at 1 : 1 (v/v) Formulation: 20 weighed tablets ground to a fine powder, extraction with diluting solution, centrifugation	HPLC Column: C_{18} Mobile phase: 10 mM KH_2PO_4 : acetonitrile (pH 6.0) Flow rate: 1 mL/min Wavelength: 225 nm		Radhakrishna *et al.* (2002a)
Tablet formulation	Tablets ground to fine powder, dissolve in methanol, sonication, filtration, dilution, and analysis	HPLC Column: C_{18} Mobile phase: methanol : phosphate buffer (pH 4.3) (75 : 25, v/v) Flow rate: 1.0 mL/min Wavelength: 258 nm		Jain *et al.* (2008)
Tablet	Finely powdered tablets, addition of methanol, sonication, centrifugation, supernatant diluted with 60% methanol, injection on column	HPLC Column: C_{18} Mobile phase: ammonium formate buffer (0.05 M, pH 4.1) : acetonitrile (45 : 55, v/v) Flow rate 1.0 mL/min Wavelength: 266 nm	42 ng/mL (LOD)	Jedlicka *et al.* (2004)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Formulation	Powder transferred to volumetric flask, volume adjustment with acetonitrile and methanol (1 : 1), sonication, filtration, addition of IS and injection onto HPLC column	HPLC Column: C_{18} Mobile phase: formic acid, (0.05 M, pH 3.0), water : acetonitrile (5 : 95, v/v) and water : methanol (10 : 90, v/v) Flow rate : 1.0 mL/min Wavelength: 260 nm		Venkatesh *et al.* (2006)
Environmental sample	Addition of acetonitrile to stabilize sample, store at 4 °C, filtration, addition of rosiglitazone as IS, and analysis	HPLC-TOF-MS Column: C_{18} Mobile phase: acetonitrile (containing formic acid 0.1%, v/v) and an aqueous 10 mM ammonium formate solution (containing formic acid 0.1%, v/v) Flow rate: 0.7 mL/min	LOQ Waste water: 1.1 ng/L River water: 1.2 ng/L Tap water: 3.1 ng/L	Martín *et al.* (2012)
Formulation	Addition of mobile phase to flask containing tablet powder, sonication, filtration, and analysis	RP-UPLC Column: C_{18} Mobile phase: acetonitrile:buffer (pH 3.2) (20 : 80, v/v) Flow rate: 0.2 mL/min Wavelength: 220 nm	0.01 µg/mL (LOD) 0.05 µg/mL (LOQ)	Xavier & Basavaiah (2012)
Formulation	Crushing of tablet, transfer of powder to beaker, dissolve in acidic water solution, sonication, pH adjustment to 3.0 using phosphate buffer	Potentiometric sensors The sensing membranes incorporate ion association complexes of pioglitazone cation and sodium tetraphenylborate or phosphomolybdic acid or phosphotungstic acid as electroactive material.		Mostafa & Al-Majed (2008)
Marketed formulation	Tablet finely powdered, extraction with methanol, further dilutions to achieve a concentration of 100 µg/mL of pioglitazone using methanol	Chiral normal-phase HPLC Column: chiral Mobile phase: hexane and *n*-propyl alcohol (80 : 20, v/v) Flow rate: 1.0 mL/min Wavelength: 233 nm	100 ng/mL (LOD) 400 ng/mL (LOQ)	Gowramma *et al.* (2012a, b)
Bulk and pharmaceutical formulation	Twenty weighed tablets ground to a fine powder, extraction with diluting solution, centrifugation	Capillary electrophoresis Separation mode: micellar electrokinetic chromatographic fused-silica capillary Background electrolyte: 80 parts of 20 mM sodium borate (pH 9.3) containing 50 mM SDS and 20 parts of acetonitrile. Wavelength: 210 nm Voltage: 25 kV	Pioglitazone unsaturated impurity: 0.29 µg/mL (LOD) 0.74 µg/mL (LOQ)	Radhakrishna *et al.* (2002a)

HPLC, high-performance liquid chromatography; HPLC-TOF-MS, high-performance liquid chromatography time of flight mass spectrometry; IS, internal standard; KH_2PO_4, monopotassium phosphate; LC-UV, liquid chromatography ultraviolet spectroscopy; LC-ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LOD, limit of detection; LLOQ, lower limit of quantification; LOQ, limit of quantification; NaOH, sodium hydroxide; RP-UPLC, reverse phase-ultra high pressure liquid chromatography; SDS, sodium dodecyl sulfate; SPE, solid-phase extraction; SRM, selected reaction monitoring; TFA, trifluoroacetic acid; UV, ultraviolet

Table 1.2 Most commonly reported clinical indications for pioglitazone and rosiglitazone in the USA, 2011–2012

Diagnosis[a]	ICD-9 code[b]	Drug uses (in thousands)		Percentage of total	
		Pioglitazone	Rosiglitazone	Pioglitazone	Rosiglitazone
Diabetes mellitus NOS	250.001	3500	90	54.0	28.4
Diabetes type II, non-insulin dependent	250.003	2585	126	39.9	39.8
Diabetic nephropathy	250.302	66	7	1.0	2.1
Diabetic neuropathy	250.503	60	–	0.9	–
Diabetic kidney disease	250.301	57	13	0.9	4.1
Diabetes type I, insulin dependent	250.002	45	–	0.7	–
Metabolic/insulin resistant syndrome	277.701	35	60	0.5	18.9
Elevated glucose	790.201	27	–	0.4	–
Polycystic ovary syndrome	256.401	–	7	–	2.1
All other diagnoses	–	110	14	1.7	–
Total with reported diagnoses	–	6484	316	100.0	100.0

[a] No diagnosis was stated for 0.3% of drug uses.
[b] The ICD-9 codes given are a more detailed, proprietary version developed by IMS Health.
NOS, not otherwise specified
From IMS Health (2012b)

is also contraindicated in patients with advanced congestive heart failure (FDA, 2013a).

Apart from its approved indication for treatment of type 2 diabetes mellitus, pioglitazone is also used for other off-label indications (Table 1.2).

(b) Dosage

Pioglitazone is available as tablets of 15, 30 and 45 mg dosage titrated on adequacy of therapeutic response (Sweetman, 2011). Pioglitazone is also available in combination products, including pioglitazone and metformin (Actoplus Met), pioglitazone and glimeperide (Duetact), and pioglitazone and alogliptin (Oseni) (FDA, 2013a; ChemSpider, 2013).

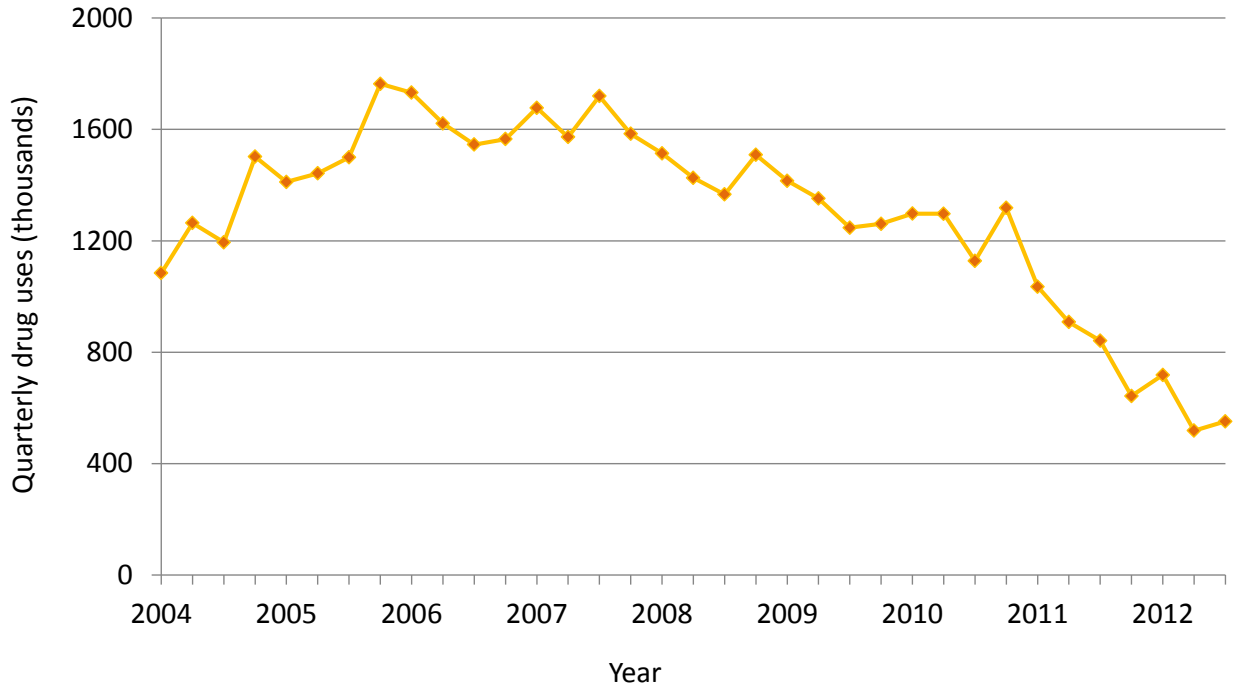
(c) Trends in use

Pioglitazone was one of the most widely used drugs for the treatment of type 2 diabetes among adults in 2000–2005. However, prescription sales of thiazolidinediones in general, and of pioglitazone in particular, have declined following several studies that suggested links to congestive heart failure (Singh *et al.*, 2007a), and fractures (Loke *et al.*, 2009), and subsequent warnings about the risk of cancer of the bladder (FDA, 2013a). Prescription trends from the Netherlands also declined after regulatory warnings concerning fractures and development of cancer of the bladder (Ruiter *et al.*, 2012).

Total worldwide sales of pioglitazone were US$ 3.34 billion in 2012, with 71% occurring in the USA (US$ 2.37 billion). Other nations with significant sales of pioglitazone included Japan (US$ 322 million), India (US$ 106 million), United Kingdom (US$ 71 million) and Italy (US$ 66 million) (IMS Health, 2012a).

Pioglitazone was reported in 2.4 million drug uses in the USA in 2012, a decline from 6.7 million reported uses in 2006, according to IMS Health National Disease and Therapeutic Index data. Based on these same data, approximately 600 000 patients in the USA were taking pioglitazone in 2012 (IMS Health, 2012b). See also Fig. 1.1.

Fig. 1.1 Trends in use of pioglitazone by office-based physicians in the USA



Prepared by the Working Group from data obtained from IMS Health, National Disease and Therapeutic Index (IMS Health, 2012b).

1.4 Occurrence and exposure to pioglitazone

1.4.1 Natural occurrence

Pioglitazone is not reported to occur naturally. The production and medicinal use of pioglitazone may contaminate the environment through various waste streams (Pubchem, 2013). If released into the air, pioglitazone is removed by wet or dry deposition, since it exists solely in the particulate phase in the atmosphere. Pioglitazone does not volatilize from dry soil surfaces based upon its vapour pressure. If released into the water, pioglitazone is expected to adsorb to suspended solids and sediment.

1.4.2 Occupational exposure

Occupational exposure to pioglitazone may occur through inhalation and dermal contact at workplaces where pioglitazone is produced or used (Pubchem, 2013). No information was available to the Working Group on the potential number of workers exposed.

1.5 Regulations and guidelines for pioglitazone

Pioglitazone was first approved for use in the USA on 15 July 1999 (FDA, 2013b). The Food and Drug Administration (FDA) approved a risk evaluation and mitigation strategy (REMS) for pioglitazone to ensure that the benefits of this drug outweighed the risks; however, this REMS was later rescinded.

The French Agency for the Safety of Health Products (AFFSAPS) suspended the use of medications containing pioglitazone in 2011, on the basis of a French study linking pioglitazone to cancer of the bladder (AFFSAPS, 2013). Following this study, the Federal Institute for Drugs and Medical Devices (BrFAM) in Germany also

recommended the suspension of sales of pioglitazone (BrFAM, 2011).

1.6 Chemical and physical data on rosiglitazone

1.6.1 Nomenclature

(a) Rosiglitazone

Chem. Abstr. Serv. Reg. No.: 122320-73-4 (SciFinder, 2013)

Chem. Abstr. Serv. name: 2,4-Thiazolidinedione, 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl] (SciFinder, 2013)

IUPAC Systematic Name: 5-[[4-[2-[Methyl(pyridin-2-yl)amino]ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Pubchem, 2013)

WHO INN: Rosiglitazone (WHO, 2007)

(b) Rosiglitazone maleate

Chem. Abstr. Serv. Reg. No.: 155141-29-0 (SciFinder, 2013)

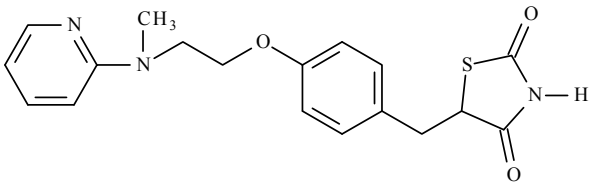
Chem. Abstr. Serv. Name: 2,4-Thiazolidinedione, 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-, (2Z)-2-butenedioate (1:1) (SciFinder, 2013)

IUPAC Systematic Name: (*Z*)-But-2-enedioic acid;5-[[4-[2-[methyl(pyridin-2-yl)amino]ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Pubchem, 2013)

Proprietary Names: Gaudil (Pubchem, 2013); Rezult (SciFinder, 2013); Avandia (GSK, 2012)

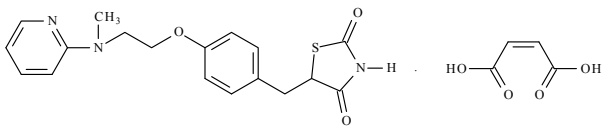
1.6.2 Structural and molecular formulae and relative molecular mass

(a) Rosiglitazone



$C_{18}H_{19}N_3O_3S$
Relative molecular mass: 357.43

(b) Rosiglitazone maleate



$C_{18}H_{19}N_3O_3S.\ C_4H_4O_4$
Relative molecular mass: 473.50
From O'Neil (2006), SciFinder (2013)

1.6.3 Chemical and physical properties of the pure substance

(a) Rosiglitazone

Description: Solid, colourless crystals from methanol (O'Neil, 2006)

Melting point: Rosiglitazone: 151–155 °C (O'Neil, 2006)

Density: 1.315 ± 0.06 g/cm^3 at 20 °C and pressure 760 Torr (SciFinder, 2013)

Spectroscopy data: UV (Venkatesh *et al.*, 2006), ^{1}H NMR, ^{13}C NMR, IR (potassium bromide), and MS have been reported. (Pang *et al.*, 2009; Wang & Miksa, 2007)

Solubility: 30.67 µg/mL in water at 25 °C; 3.79 µg/mL in 0.1 M phosphate buffer at 25 °C (Seedher & Kanojia, 2008; Seedher & Kanojia, 2009); 35.09 µg/mL in 0.15 M NaCl at 25 °C (Seedher & Kanojia, 2008); soluble in the mg/mL range in ethanol, dimethyl sulfoxide, and dimethylformamide (Cayman SDS, 2013); 10.45 mg/L in water at 25 °C (NLM, 2013)

Octanol/water partition coefficient: Log P = 2.78–3.02 (Giaginis *et al.*, 2007)

Vapour pressure: 1.14 × 10^{-13} mm Hg at 25 °C (SciFinder, 2013)

(b) Rosiglitazone maleate

Description: White to off-white solid (O'Neil, 2006; GSK, 2012)

Melting point: 122–123 °C (O'Neil, 2006)

Solubility: Readily soluble in ethanol and in buffered aqueous solution at pH 2.3; solubility decreases with increasing pH in the physiological range (O'Neil, 2006; GSK, 2012)

Stability data: Stable for 2 years when stored at 4 °C. Stock solutions are stable for up to 3 months when stored at –20 °C (Enzo PDS, 2012)

1.6.4 Technical products and impurities

Rosiglitazone maleate is used to formulate the finished dosage forms described below.

(a) Trade names

Avandia (GSK, 2012); Roglit 4; Romerol; Rosit-2; Sensulin; Tazone-4 (BDdrugs, 2013)

(b) Impurities

- Desmethyl impurity or 5-(4-(2-(pyridin-2-yl-amino)ethoxy)benzyl)thiazolidine-2,4-dione (Krishna *et al.*, 2008)
- Dimer impurity or 5-((2,4-dioxothiazolidin-5-yl)(4-(2-(methyl(pyridin-2-yl)amino)ethoxy)phenyl)methyl)-5-(4-(2-(methyl(pyridin-2-yl)amino)ethoxy)benzyl)thiazolidine-2,4-dione (Krishna *et al.*, 2008)
- Succinate impurity or 2-(5-(4-(2-(methyl-(pyridin-2-yl)amino)ethoxy)benzyl)-2,4-dioxothiazolidin-3-yl)succinic acid (Krishna *et al.*, 2008)
- 4-(2-(Methyl(pyridin-2-yl)amino)ethoxy)benzaldehyde (Radhakrishna *et al.*, 2002b)

1.7 Analysis of rosiglitazone

Physical properties used for the identification of the substance, e.g. IR and melting point, are presented in Section 1.6.3.

Selected non-compendial methods are presented in Table 1.1. Rosiglitazone can be analysed in different matrices such as plasma, serum, urine and formulations, by HPLC and with detection by UV or MS. Detection and quantification limits for determination of rosiglitazone in human serum by HPLC method with UV detection are 0.033 µg/mL and 0.102 µg/mL, respectively (Sultana *et al.*, 2011). Rosiglitazone can be analysed in human plasma with lower limit of quantification of 1.00 ng/mL using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) (O'Maille *et al.*, 2008). Other analytical methods for detection in human urine include square-wave adsorptive stripping voltammetry method (Al-Ghamdi & Hefnawy, 2012); analysis of formulation can be also achieved with capillary electrophoresis with UV detection (Yardımcı *et al.*, 2007) or by UV spectroscopy (Sireesha *et al.*, 2011).

1.8 Production and use of rosiglitazone

1.8.1 Production

Rosiglitazone is produced by dissolving 2-*N*-methyl-2-pyridylaminoethanol in dimethylformamide and adding sodium hydroxide under atmospheric nitrogen (Cantello *et al.*, 1994; Pubchem Substance, 2013). 4-Fluorobenzaldehyde is added. The resulting mixture is then dissolved in toluene and piperidine is added. The compound obtained is dissolved in dioxane and hydrogenated at room temperature and atmospheric pressure. Rosiglitazone is separated by filtration, vacuum concentration and recrystallization.

1.8.2 Use

(a) Indications

Rosiglitazone maleate is an antidiabetic agent indicated for the improvement of glycaemic control in adults with type 2 diabetes mellitus, as an adjunct to diet and exercise. Rosiglitazone maleate decreases hyperglycaemia by reducing insulin resistance in the presence of endogenous insulin (Avandia, 2010; Sweetman, 2011).

In the USA, rosiglitazone maleate is only indicated for patients already using rosiglitazone, or for those not taking rosiglitazone and who have been unable to achieve adequate glycaemic control using other diabetes medications, or who have decided not to take pioglitazone (Avandia, 2010). In the USA, the most commonly reported indication for rosiglitazone is type 2 diabetes mellitus (see Table 1.2).

Rosiglitazone is also used for off-label indications such as polycystic ovarian syndrome and insulin resistance syndrome.

Side-effects include fluid retention, congestive heart failure, and liver disease (Pubchem Substance, 2013).

(b) Dosage

Rosiglitazone is available as tablets of 2 mg, 4 mg, and 8 mg. Rosiglitazone is also available in combination with glimeperide or with metformin. Rosiglitazone is started at a dose of 4 mg and can be titrated up to a dose of 8 mg, if inadequate response is obtained in combination with metformin or sulfonylureas (Sweetman, 2011).

(c) Trends in use

Total worldwide sales of rosiglitazone in 2012 were US$ 43 million, a decline from much higher levels in the previous decade (IMS Health, 2012a). In 2012, there were limited, if any, sales in most countries of the European Union as a consequence of the decision of the European Medicines Agency to suspend marketing of this medication in September 2010 (EMA, 2010). Countries with appreciable continuing sales included China (US$ 12 million), Canada (US$ 11 million), Mexico (US$ 7 million), Australia (US$ 3 million), USA (US$ 3 million), and Argentina (US$ 3 million). The use of rosiglitazone rapidly declined in the USA after increased risk of cardiovascular disease associated with use of rosiglitazone was reported in May 2007 (Nissen & Wolski, 2007; Singh *et al.*, 2007b).

Rosiglitazone was reported as 105 000 drug uses in the USA in 2012, down from 6.7 million uses in 2005 (IMS Health, 2012b; see Fig. 1.2). Approximately 10 000 patients in the USA were taking rosiglitazone in 2012, down from about 40 000 in 2011 (IMS Health, 2012b).

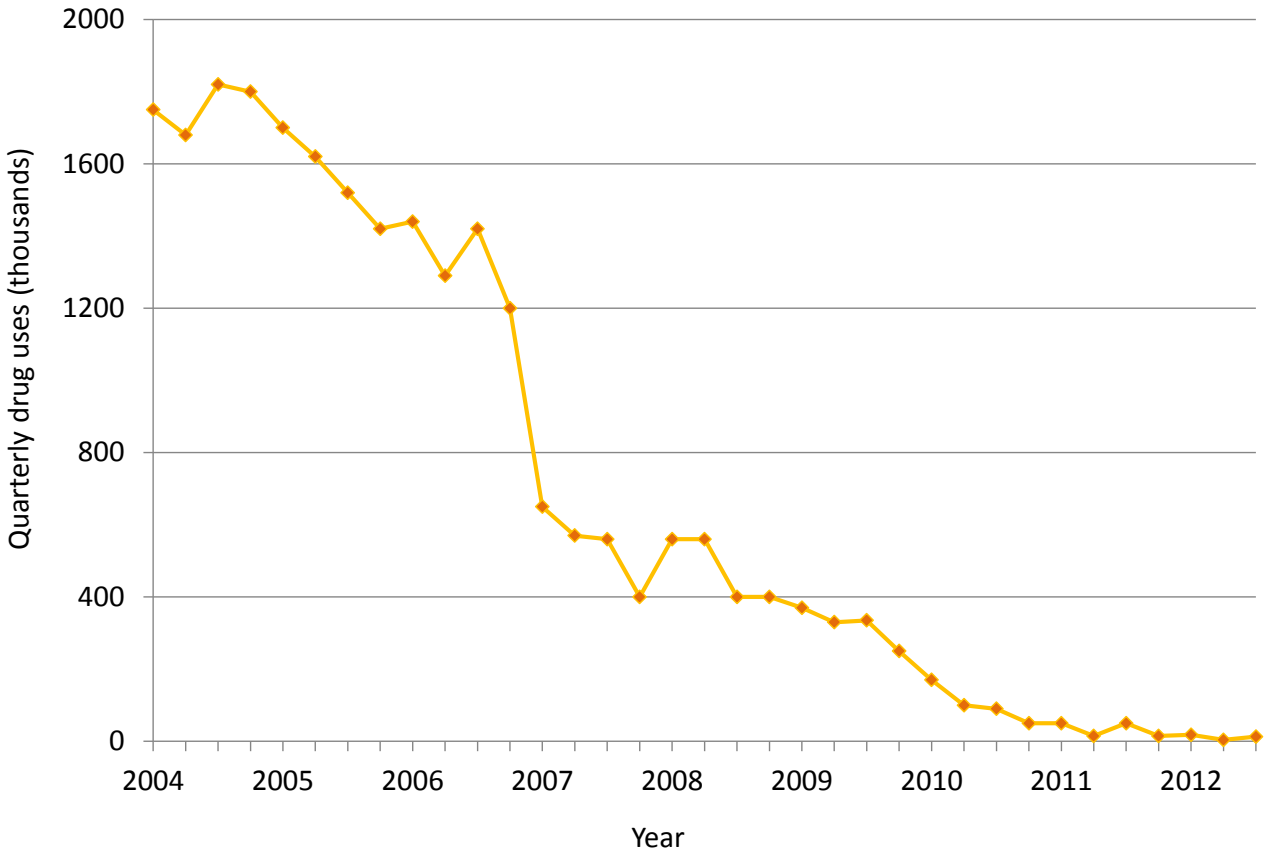
Prescribing trends from the Netherlands show a decline in prescriptions for rosiglitazone after regulatory warnings (Ruiter *et al.*, 2012). Since the marketing authorization for rosiglitazone was suspended, rosiglitazone is no longer available for use in Europe (EMA, 2010). Prescriptions for rosiglitazone have also declined in several other countries across Asia, such as Taiwan, China (Lu & Li, 2013).

1.9 Occurrence and exposure to rosiglitazone

1.9.1 Natural occurrence and environmental fate

Rosiglitazone is not reported to occur naturally. The production and use of rosiglitazone may result in its release to the environment through various waste streams. If released to air, it will exist solely in the particulate phase and will be removed from the atmosphere by wet or dry deposition (Pubchem Substance, 2013).

Fig. 1.2 Trends in use of rosiglitazone by office-based physicians in the USA



Prepared by the Working Group from data obtained from IMS Health, National Disease and Therapeutic Index (IMS Health, 2012b).

1.9.2 Occupational exposure

Occupational exposure to rosiglitazone may occur through inhalation of dust and via dermal contact at workplaces where rosiglitazone is produced or used (Pubchem Substance, 2013). No information was available to the Working Group on the potential number of workers exposed.

1.10 Regulations and guidelines for rosiglitazone

In the USA, rosiglitazone is only available under a REMS, and approved by FDA on the basis of safety and effectiveness (DHHS/FDA, 2007; Woodcock *et al.*, 2010). The marketing authorization for rosiglitazone was withdrawn in Europe (EMA, 2010), where rosiglitazone is no longer in use after being linked in several studies to an increase in the risk of myocardial infarction (Nissen & Wolski, 2007; Singh *et al.*, 2007b).

In June 2013, an FDA advisory committee meeting was held to discuss the re-adjudication of data on cardiovascular events associated with rosiglitazone from a large randomized controlled trial (FDA, 2013c). The advisory committee recommended that restrictions on rosiglitazone be lessened, since re-adjudication did not reveal a statistically significant increase in the risk of cardiovascular events.

2. Cancer in Humans

Thiazolidinediones (rosiglitazone, pioglitazone and troglitazone) have been used as orally administered glucose-lowering drugs in patients with type 2 diabetes mellitus. Rosiglitazone and pioglitazone were introduced to the market at about the same time (1999 in the USA, and 2001 and 2002 for rosiglitazone and pioglitazone, respectively, in Taiwan, China; Tseng, 2012d). Some patients may therefore have been exposed to both drugs, which were sometimes prescribed sequentially. Troglitazone was marketed for only a short period (1997–2000), before being withdrawn from the world market subsequent to reports of fatal hepatotoxicity (Julie *et al.*, 2008).

There was a concern regarding the potential for ascertainment bias in the observational studies, since differences in the intensity and frequency of ascertainment between the pioglitazone and control groups were unknown. Since pioglitazone is associated with an increased risk of oedema and congestive heart failure, patients taking pioglitazone were potentially more likely to undergo more frequent urine analysis, which could lead to detection of microscopic haematuria, more frequent cystoscopies, and eventually a diagnosis of cancer of the bladder.

Pioglitazone and rosiglitazone may have different effects on the risk of cancer and the Working Group therefore evaluated these compounds separately, whenever data were available. Studies that reported results for non-specific thiazolidinediones were considered uninformative by the Working Group and are not cited in this *Monograph*.

Several of the studies on these agents were based on analyses of large databases from France, the United Kingdom, the USA, and Taiwan, China, which are briefly described below. Associations of multiple cancers with specific thiazolidinediones were reported (see Table 2.1)

The French health insurance databases SNIIRAM (*Système national d'information inter-régimes de l'Assurance maladie*) and PMSI (*Programme de médicalisation des systèmes d'information*) cover all employees and represent approximately 75% of the French population. These databases contain all reimbursement data for the patients' health expenditure, including medication and outpatient medical and nursing care prescribed or performed by health-care professionals. International Classification of Diseases 10th Revision (ICD-10) codes are applied in the databases and hospital discharge information can be linked. To evaluate the association between the use of pioglitazone or rosiglitazone and risk of various cancers, a cohort of 1 491 060 diabetic patients (aged 40–79 years on 31 December 2006) from this national health insurance scheme was created. Patients included had filled at least one prescription for an antidiabetic drug (i.e. metformin, sulfonylurea, pioglitazone, rosiglitazone, other oral antidiabetic drugs and/or insulin) in 2006. Patients were excluded if they had cancer of the bladder diagnosed before study entry or within the first 6 months after study entry. Diagnosis of cancer of the bladder or other cancers was followed up until 31 December 2009 (Neumann *et al.*, 2012). [The Working Group noted that the period of follow-up was only 3 years. It was unclear which drugs patients may have used in the past, before enrollment into the cohort.]

In the United Kingdom, The Health Improvement Network (THIN) database (since 2003), managed by the Medicines and Healthcare Products Regulatory Agency, MHRA) is similar in structure and content to the General Practice Research Database (GPRD, 1994–2002), which provides electronic medical records of approximately 10 million patients living in the United Kingdom [the Working Group estimated a 50% overlap in the two databases]. Data available include demographic information, medical diagnoses (using Read codes, a standard classification

Table 2.1 Cohort studies of cancer and exposure to pioglitazone or rosiglitazone

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Bladder cancer							
Lewis *et al.* (2011)[a] KPNC, USA, 1997–2008	193 099	Prescription records	Bladder (linkage with KPNC cancer registry)	*Pioglitazone*			Included diabetic patients aged ≥ 40 yr between 1997 and 2002. Numbers of cases reported as median incidence rate per 100 000 person-years.
				Never use	791	1.00 (ref.)	
				Ever use	90	1.2 (0.9–1.5)	
				Time since starting (mo):			
				< 18	NR	1.2 (0.8–1.7)	
				18–36	NR	1.4 (0.9–2.1)	
				> 36	NR	1.3 (0.9–1.8)	
				P for trend		0.07	
				Duration of therapy (mo):			
				< 12	NR	0.8 (0.6–1.3)	
				12–24	NR	1.4 (0.9–2.1)	
				> 24	NR	1.4 (1.03–2.0)	
				P for trend		0.03	
				Cumulative dose (mg):			
				1–10 500	NR	1.0 (0.7–1.5)	
				10 501–28 000	NR	1.2 (0.8–1.8)	
				> 28 000	NR	1.4 (0.96–2.1)	
				P for trend		0.08	
Neumann *et al.* (2012) France, 2006–9	1 491 060 (pioglitazone exposed: *n* = 155 535)	Prescription records	Bladder (discharge diagnosis with ICD-10 C67, combined with specific aggressive treatment)	*Pioglitazone (+) vs (–):*			Age, sex (when applicable), and exposure to glucose-lowering drugs. Patients with diabetes in two large national linked databases: health insurance system (SNIIRAM) and hospitalization (PMSI), 2006–2009. Age range: 40–79 yr; lack of consideration of potential confounders like smoking and comorbidities.
				Both sexes	2016	1.22 (1.05–1.43)	
				Men	1790	1.28 (1.09–1.51)	
				Women	226	0.78 (0.44–1.37)	
				Rosiglitazone (+) vs (–):			
				Both sexes	2016	1.08 (0.92–1.26)	
				Men	1790	1.10 (0.93–1.30)	
				Women	226	0.89 (0.53–1.49)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Neumann et al. (2012) France, 2006–9 (cont.)				*Pioglitazone (–), both sexes*		1.00 (ref.)	Age, sex (when applicable), level of pioglitazone use (i.e. cumulative dose and duration of exposure, respectively) and exposure to other glucose-lowering drugs
				Cumulative dose (mg):			
				< 10 500	NR	1.12 (0.89–1.40)	
				10 500–27 999	NR	1.20 (0.93–1.53)	
				≥ 28 000	NR	1.75 (1.22–2.50)	
				Duration of exposure (d):			
				< 360	NR	1.05 (0.82–1.36)	
				360–719	NR	1.34 (1.02–1.75)	
				≥ 720	NR	1.36 (1.04–1.79)	
				Pioglitazone (–), men		1.00 (ref.)	
				Cumulative dose (mg):			
				< 10 500	NR	1.17 (0.92–1.48)	
				10 500–27 999	NR	1.24 (0.96–1.60)	
				≥ 28 000	NR	1.88 (1.30–2.71)	
				Duration of exposure (d):			
				< 360	NR	1.10 (0.84–1.43)	
				360–719	NR	1.39 (1.06–1.84)	
				≥ 720	NR	1.44 (1.09–1.91)	
				Pioglitazone (–), women		1.00 (ref.)	
				Cumulative dose (mg):			
				< 10 500	NR	0.77 (0.36–1.65)	
				10 500–27 999	NR	0.84 (0.35–2.06)	
				≥ 28 000	NR	0.57 (0.08–4.11)	
				Duration of exposure (d):			
				< 360	NR	0.76 (0.34–1.72)	
				360–719	NR	0.87 (0.32–2.35)	
				≥ 720	NR	0.71 (0.22–2.23)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Wei *et al.* (2013)[b] General Practice Research Database, United Kingdom, 2001–10	207 714 (pioglitazone exposed, 23 548; unexposed, 184 166)	Prescription records	Bladder (database records)	Pioglitazone (yes vs no)	869	1.16 (0.83–1.62)	Type 2 diabetes patients aged ≥ 40 yr. HR, 1.22 (95% CI, 0.80–1.84) in a propensity-matched analysis done in a group of patients without missing data on baseline characteristics
Tseng (2012a)[c] Taiwan, China, 2006–9	54 928	Medical reimbursement records in the Taiwan, China, National Health Insurance database	Bladder (ICD-9 188)	*Pioglitazone*			
				Never-users	155	1.00 (ref.)	
				Ever-users	10	1.31 (0.66–2.58)	
				Time since starting (mo):			
				< 18	4	1.38 (0.49–3.83)	
				18–36	5	1.54 (0.62–3.84)	
				> 36	1	0.65 (0.09–4.77)	
				P for trend		0.6352	
				Duration of therapy (mo):			
				< 12	8	1.54 (0.73–3.26)	
				≥ 12	2	0.82 (0.20–3.35)	
				P for trend		0.6919	
				Cumulative dose (mg):			
				1–10 500	8	1.45 (0.69–3.06)	
				> 10 500	2	0.94 (0.23–3.84)	
				P for trend		0.7125	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Tseng (2013a)[d] Taiwan, China, 2006–9	547 584 diabetic men	Medical reimbursement records in the Taiwan, China, National Health Insurance database	Bladder (ICD-9 188)	Pioglitazone (yes vs no) Rosiglitazone (yes vs no)	1869 1869	1.02 (0.75–1.39) 1.12 (0.92–1.37)	Benign prostatic hyperplasia is a significant risk factor for bladder cancer in diabetic men. The hazard ratios for pioglitazone and rosiglitazone are estimated in diabetic men with benign prostatic hyperplasia.
Fujimoto *et al.* (2013) Japan, 2000–11	21 335	Hospital records	Bladder	Pioglitazone (yes vs no)	170	1.75 (0.89–3.45)	NR, probably not adjusted. Single centre, lack of adjustment for confounders.
Colorectal cancer							
Neumann *et al.* (2012)[e] France, 2006–9	1 485 146	Prescription records	Colorectum (ICD-10 C18 to C21)	Pioglitazone (+) vs (–) Rosiglitazone (+) vs (–)	10 618 10 618	0.97 (0.90–1.05) 0.88 (0.82–0.95)	Age, sex (when applicable), and exposure to glucose-lowering drugs
Tseng (2012b)[e] Taiwan, China, 2003–5	995 843	Reimbursement databases	Colon (ICD-9 153)	Pioglitazone (yes vs no) Rosiglitazone (yes vs no)	3 versus 2386 29 vs 2360	0.78 (0.25–2.49) 1.22 (0.81–1.84)	
Ferrara *et al.* (2011) KPNC, USA, 1997–2005	252 467	Prescription records	Colon	Never-use of other TZD Ever-use of other TZD Never-use of pioglitazone Ever-use of pioglitazone	 1260 1260	1.00 (ref.) 1.1 (0.8–1.5) 1.00 (ref.) 0.9 (0.7–1.1)	
Lung cancer							
Ferrara *et al.* (2011)[f] KPNC, USA, 1997–2005	252 467	Prescription records	Lung/bronchus (linkage with KPNC cancer registry)	Never-use of other TZD Ever-use of other TZD Never-use of pioglitazone Ever-use of pioglitazone	 1637 1637	1.00 (ref) 0.9 (0.6–1.3) 1.00 (ref.) 1.0 (0.8–1.3)	Ten categories of cancer sites, diabetic patients aged ≥ 40 yr
Neumann *et al.* (2012) France, 2006–9	1 493 472	Prescription records	Lung (ICD-10 C33 and C34)	Pioglitazone (+) vs (–) Rosiglitazone (+) vs (–)	9298 9298	0.94 (0.87–1.02) 0.91 (0.84–0.99)	Age, sex, and exposure to glucose-lowering drugs

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Prostate cancer							
Ferrara *et al.* (2011)[f] KPNC, USA, 1997–2005	252 467	Prescription records	Prostate (linkage with KPNC cancer registry)	Never-use of other TZD Ever-use of other TZD Never-use of pioglitazone Ever-use of pioglitazone	 2105 2105	1.00 (ref.) 1.0 (0.7–1.3) 1.00 (ref.) 1.0 (0.8–1.2)	Ten categories of cancer sites. Diabetic patients aged ≥ 40 yr
Tseng (2011)[g] Taiwan, China, 2003–5	494 630	Medical reimbursement records in the Taiwan, China, National Health Insurance database	Prostate (ICD-9 185)	Pioglitazone (yes vs no) Rosiglitazone (yes vs no)	889 889	0.77 (0.10–5.75) 0.88 (0.43–1.80)	
Breast cancer							
Ferrara *et al.* (2011) KPNC, USA, 1997–2005	252 467	Prescription records	Female breast (linkage with KPNC cancer registry)	Never-use of other TZD Ever-use of other TZD Never-use of pioglitazone Ever-use of pioglitazone	 1561 1561	1.0 (ref.) 0.9 (0.7–1.2) 1.0 (ref.) 1.0 (0.8–1.3)	
Neumann *et al.* (2012) France, 2006–9	671 510	Prescription records	Female breast (ICD-10 C50)	Pioglitazone (+) vs (–) Rosiglitazone (+) vs (–)	6820 6820	0.91 (0.83–1.00) 0.80 (0.73–0.88)	Age, sex, and exposure to glucose-lowering drugs

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Other cancers							
Ferrara *et al.* (2011) KPNC, USA, 1997–2005	252 467	Prescription records	Linkage with KPNC cancer registry	Never-use of other TZD		1.00 (ref.)	
			NHL	Ever-use of other TZD	569	0.7 (0.4–1.2)	
			Corpus uterus	Ever-use of other TZD	552	1.2 (0.8–1.9)	
			Pancreas	Ever-use of other TZD	431	1.0 (0.6–1.8)	
			Kidney/renal pelvis	Ever-use of other TZD	430	1.3 (0.7–2.3)	
			Rectum	Ever-use of other TZD	390	0.7 (0.4–1.5)	
			Melanoma	Ever-use of other TZD	373	1.0 (0.5–1.8)	
				Never-use of pioglitazone		1.00 (ref.)	
			NHL	Ever-use of pioglitazone	569	1.3 (1.0–1.8)	
			Corpus uterus	Ever-use of pioglitazone	552	1.1 (0.8–1.5)	
			Pancreas	Ever-use of pioglitazone	431	1.2 (0.8–1.7)	
			Kidney/renal pelvis	Ever-use of pioglitazone	430	0.7 (0.4–1.1)	
			Rectum	Ever-use of pioglitazone	390	1.2 (0.8–1.8)	
			Melanoma	Ever-use of pioglitazone	373	1.3 (0.9–2.0)	
Neumann *et al.* (2012) France, 2006–9	1 495 787	Prescription records	Kidney (ICD-10 C64)	Pioglitazone (+) vs (–)	2861	0.91 (0.79–1.06)	Age, sex, and exposure to glucose-lowering drugs
				Rosiglitazone (+) vs (–)	2861	0.98 (0.86–1.13)	
	1 495 411		Head and neck (ICD-10 C00 to C14)	Pioglitazone (+) vs (–)	2868	0.85 (0.73–0.99)	Age, sex, and exposure to glucose-lowering drugs
				Rosiglitazone (+) vs (–)	2868	0.79 (0.67–0.92)	
Tseng (2012c)[h] Taiwan, China, 1996–2005	999 730	Reimbursement databases	Thyroid (ICD-9 193)	Pioglitazone (yes vs no)	943	0.52 (0.07–3.93)	
				Rosiglitazone (yes vs no)		0.67 (0.23–1.95)	

Table 2.1 (continued)

Reference Location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Tseng (2013c)[i] Taiwan, China, 2003–5	998 540	Reimbursement databases	Oral cavity, lip, and pharynx (ICD-9 140, 141, 143, 144, 145, 146, 148, and 149)	Pioglitazone (yes vs no)	766		
				Men		1.70 (0.22–13.20)	
				Women		no incident cases of oral cancer	
				Rosiglitazone (yes vs no)			
				Men		1.15 (0.44–3.04)	
				Women		0.90 (0.20–3.98)	

[a] Age, sex, race/ethnicity, current smoking, renal function, bladder condition, congestive heart failure, income, baseline A1C, newly diagnosed with diabetes at start of follow-up, duration of diabetes, other cancer before baseline, other diabetic medications (other TZDs, metformin, sulfonylureas, other oral hypoglycaemic drugs, insulin).

[b] Age, sex, duration of diabetes, smoking status and BMI before entry into the study, and insulin treatment and number and type of different oral hypoglycaemic drug classes used during the follow-up period.

[c] Age, sex, diabetes duration, nephropathy, urinary-tract disease, hypertension, COPD, cerebrovascular disease, IHD, peripheral arterial disease, eye disease, dyslipidaemia, heart failure, rosiglitazone, sulfonylurea, meglitinide, metformin, acarbose, insulin, statin, fibrate, angiotensin-converting enzyme inhibitor/angiotensin-receptor blocker, Ca-channel blocker, region of residence, occupation, and other cancer before baseline.

[d] Age, diabetes duration, nephropathy, urinary-tract diseases, hypertension, COPD, cerebrovascular disease, IHD, peripheral arterial disease, eye disease, dyslipidaemia, heart failure, obesity, alcohol-related diagnosis, non-alcohol-related chronic liver disease, rosiglitazone/pioglitazone, sulfonylurea, meglitinide, metformin, acarbose, insulin, statin, fibrate, ACEI/ARB, Ca-channel blockers, α-blockers, 5-α reductase inhibitors, clopidogrel, ticlopidine, dipyridamole, cyclophosphamide, diuretics, other cancer before baseline and potential detection examinations.

[e] Age, sex, diabetes, hypertension, COPD, asthma, stroke, nephropathy, IHD, peripheral arterial disease, eye disease, dyslipidaemia, obesity, statin, fibrate, ACEI/ARB, Ca-channel blocker, aspirin, dipyridamole, clopidogrel/ticlopidine, NSAIDs, sulfonylurea, metformin, insulin, acarbose, rosiglitazone, region of residence, occupation, and colon-cancer detection examinations.

[f] Age, ever use of other diabetes medications, year of cohort entry, sex, race/ethnicity, income, current smoking, baseline HbA1c, diabetes duration, new diabetes diagnosis, creatinine, and congestive heart failure.

[g] Age, diabetes duration, hypertension, COPD, stroke, nephropathy, IHD, peripheral arterial disease, eye disease, obesity, dyslipidaemia, statin, fibrate, ACEI/ARB, Ca-channel blocker, sulfonylurea, metformin, insulin, acarbose, rosiglitazone, region of residence, and occupation.

[h] Age, sex, diabetes, living region, occupation, detection examination, hypertension, COPD, stroke, nephropathy, IHD, peripheral arterial disease, eye disease, obesity, dyslipidaemia, benign thyroid disease, other cancer, sulfonylurea, metformin, insulin, acarbose, pioglitazone/rosiglitazone, statin, fibrate, ACEI/ARB, Ca-channel blocker, aspirin, ticlopidine, clopidogrel, NSAIDs.

[i] Age, diabetes, obesity, hypertension, COPD, alcohol-related diagnoses, stroke, nephropathy, IHD, peripheral arterial disease, eye disease, dyslipidaemia, statin, fibrate, ACEI/ARB, Ca-channel blockers, sulfonylurea, metformin, insulin, acarbose, pioglitazone/rosiglitazone, living region, occupation, potential detection.

ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; BMI, body mass index; Ca, calcium; COPD, chronic obstructive pulmonary disease; d, day; HbA1c, glycated haemoglobin; HR, hazard ratio; IHD, ischaemic heart disease; KPNC, Kaiser Permanente Northern California; mo, month; NHL, non-Hodgkin lymphoma; NR, not reported; NSAIDs, nonsteroidal anti-inflammatory drugs; PMSI, *Programme de médicalisation des systèmes d'information*; ref., reference; SNIIRAM, *Système national d'information inter-régimes de l'Assurance maladie*; TZD, thiazolidinediones; vs, versus; yr; year

system used in the United Kingdom primary-care settings), lifestyle, and measures taken during clinical practice. The database is regularly updated and practitioners contributing data receive training for consistency in data recording. Studies conducted by Wei *et al.* (2013) and Azoulay *et al.* (2012) used this database.

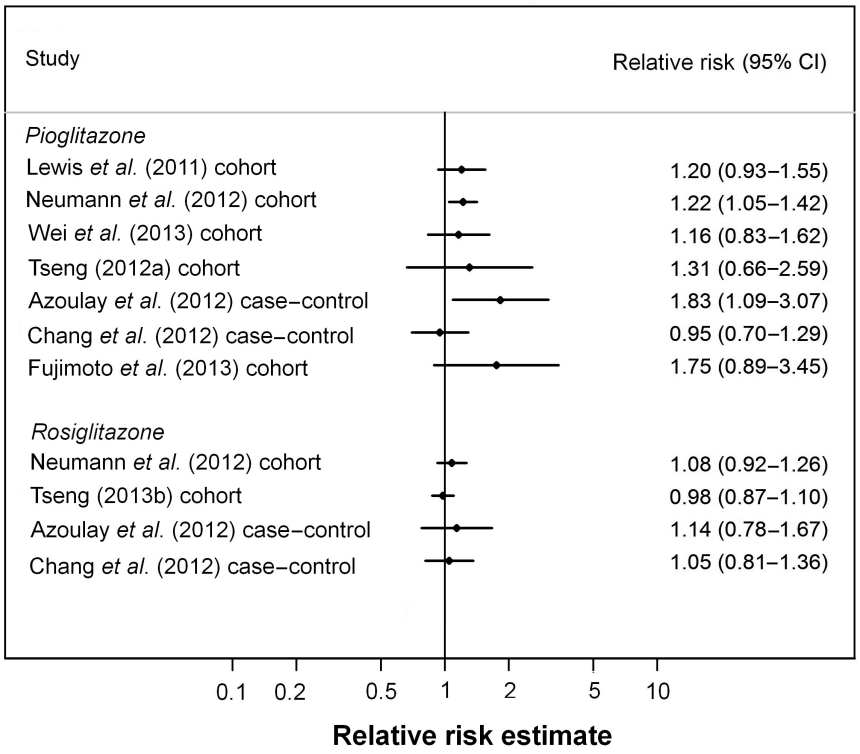
In the USA, the Kaiser Permanente Northern California (KPNC) pharmacy database covers 3.2 million members of this health plan (approximately 30% of the population of the area) and includes outpatient prescriptions dispensed at a KPNC pharmacy. Approximately 95% of members fill their prescriptions at KPNC pharmacies. The KPNC diabetes registry gathers longitudinal electronic medical records and clinically related data for patients with diabetes from the following sources: primary hospital-discharge diagnoses of diabetes, two or more outpatient-visit diagnoses of diabetes, any prescription of a diabetes-related medication, or any record of glycated haemoglobin (HbA1c) > 6.7%. The database includes information of cancer registries, pharmacy records, laboratory records, and inpatient and outpatient medical diagnoses. Patients who met any of the following criteria were eligible for forming a cohort for the analysis of the association between pioglitazone use and risk of cancer referred to in this Monograph: (i) as of 1 January 1997, diagnosed with diabetes, aged ≥ 40 years, and members of KPNC; (ii) diagnosed with diabetes, reached age 40 years between 1 January 1997 and 31 December 2002, and were KPNC members on their 40th birthday; or (iii) having diabetes and aged ≥ 40 years when joining KPNC between 1 January 1997 and 31 December 2002. A total of 193 099 patients (30 173 ever-users and 162 926 never-users of pioglitazone) were followed and a mid-point interim analysis was published in 2011 (Lewis *et al.*, 2011).

Several independent groups (Tseng, 2011, 2012a, 2012b, 2013a; Chang *et al.*, 2012) used the reimbursement databases of the National Health Insurance of Taiwan, China, for evaluating the association between use of thiazolidinediones and risk of various cancers. Since March 1995, a compulsory and universal system of health insurance (National Health Insurance) has been implemented in Taiwan, China. All contracted medical institutes must submit computerized and standard claim documents for reimbursement. More than 99% of the population of 23 million people were enrolled in this insurance system, and > 98% of the hospitals nationwide were under contract with the insurance. The average number of annual physician visits in Taiwan, China, is one of the highest around the world, at approximately 15 visits per year per capita in 2009. The National Health Research Institute is the only institute approved, as per local regulations, for handling these reimbursement databases for academic research. The databases contain detailed records on every visit for each patient, including outpatient visits, emergency-department visits, and hospital admission. The databases also include principal and secondary diagnostic codes, prescription orders, and claimed expenses. Certain computerized databases, including a database from the national cancer registry (with a high level of completeness), are also available for data linkage. Most studies used the International Classification of Diseases Ninth Revision, Clinical Modification (ICD-9-CM) codes for disease or cancer diagnosis, with or without data linkage with the cancer registry. [The Working Group noted that there may have been a detection bias associated with this database because of the high number of patient visits per capita.]

2.1 Cancer of the bladder

See Fig. 2.1

Fig. 2.1 Risk of cancer of the bladder associated with ever-use or never-use of pioglitazone or rosiglitazone, by study design[a]



[a] Weights are from random-effects analysis
Compiled by the Working Group

2.1.1 Clinical trial

The potential risk of cancer of the bladder associated with exposure to pioglitazone in humans was first raised by the large randomized PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive), which showed an imbalance of incident cases of cancer of the bladder, with greater number in patients randomized to pioglitazone than to placebo (14 cases versus 6 cases, or 0.5% versus 0.2%; P = 0.069) (Dormandy *et al.*, 2005). [Insufficient data were provided to calculate a risk estimate. The aim of the PROactive clinical trial was primarily to evaluate macrovascular events associated with use of pioglitazone. The Working Group also noted that 11 out of the 20 cases of cancer of the bladder were diagnosed within 1 year of randomization, which might have precluded a cause–effect relationship.] After excluding one case of previously diagnosed cancer of the bladder in the placebo group (found later to show benign histology), Hillaire-Buys *et al.* (2011) recalculated the crude relative risk of cancer of the bladder for pioglitazone versus placebo in PROactive as 2.83 (95% CI, 1.02–7.85; P value for Fisher exact test = 0.04). [In a randomized controlled trial, the confounders are balanced at baseline and any differences in ascertainment are likely to be non-differential. The Working Group noted these findings in light of the very low incidence of cancer of the bladder. After excluding the eleven cases of cancer of the bladder diagnosed within 1 year of randomization, one with benign histology in the placebo

group, and six with known risk factors for cancer of the bladder, only three cases remained – two in the group receiving pioglitazone and one in the placebo group.]

2.1.2 Cohort studies

See Table 2.1

Lewis *et al.* (2011) studied the incidence of cancer of the bladder among 193 099 members of the KPNC health plan who were enrolled in the plan's diabetes registry and were aged ≥ 40 years between 1997 and 2002. The registry routinely compiled electronic medical records data from various sources, including cancer registries, pharmacy records, laboratory records, and medical diagnoses. Incident cases of cancer of the bladder were identified from 2002 to 2008 via the KPNC cancer registry and ever-use of specific diabetes medications (defined as two or more prescriptions within 6 months) was determined from the pharmacy database. In the mid-point interim analysis of a 10-year longitudinal KPNC cohort study in the USA (Lewis *et al.*, 2011), the overall hazard ratio was 1.2 (95% CI, 0.9–1.5) for cancer of the bladder for ever-users of pioglitazone versus never-users; patients who used pioglitazone for > 24 months showed a risk with adjusted hazard ratio of 1.4 (95% CI, 1.03–2.0). [Although adjustment for smoking was a strength of this study, only current smoking was considered, which may not have fully controlled for confounding.]

Neumann *et al.* (2012) analysed the risk of cancer of the bladder associated with exposure to pioglitazone and rosiglitazone in a cohort of 1 491 060 diabetic patients aged 40–79 years who had been prescribed at least one dose of glucose-lowering drugs in 2006. Subjects were followed between 2006 and 2009 using the French national health insurance information system (SNIIRAM) linked with the French hospital discharge database (PMSI). Overall, 2016 cases of cancer of the bladder were identified (men, 1790 cases; and women, 226 cases). Patients were excluded if they had an occupationally related cancer of the bladder, or if they were diagnosed before entry or within the first 6 months after study entry. Patients were followed up for a mean of 39.9 months (27.4 months due to exposure), starting 6 months after study entry. Ten percent of the patients (155 535 out of 1 491 060) took a minimum of two prescriptions for pioglitazone over 6 consecutive months. Compared with non-use, the estimated hazard rate ratio for cancer of the bladder associated with use of pioglitazone was 1.22 (95% CI, 1.05–1.43) and for rosiglitazone was 1.08 (95% CI, 0.92–1.26), after adjustment for age, sex, and exposure to glucose-lowering drugs. Dose–response analyses were available only for pioglitazone and showed increasing hazard ratios with increasing duration and cumulative dose. The hazard ratios for cumulative doses of < 10 500, 10 500–27 999 and ≥ 28 000 mg compared with never-users of pioglitazone was 1.12 (95% CI, 0.89–1.40), 1.20 (95% CI, 0.93–1.53) and 1.75 (95% CI, 1.22–2.50), respectively; and were 1.05 (95% CI, 0.82–1.36), 1.34 (95% CI, 1.02–1.75) and 1.36 (95% CI, 1.04–1.79), respectively, for duration of exposure < 360, 360–719 and ≥ 720 days compared with never-users. [Smoking was not accounted for in the analyses and therefore may have confounded the reported results. Sex-specific analyses suggested an association observed only in men, but not in women. Data on smoking were not available for adjustment. Since pioglitazone is usually used as a second- or third-line antidiabetic drug, users of pioglitazone may have had longer duration of diabetes, poorer glycaemic control, and higher rates of chronic diabetic complications and comorbidities. All these characteristics may affect the risk of cancer of the bladder (Perez, 2013; Tseng, 2012d). The length of follow-up limited evaluation of the long-term impact of treatment.]

In a matched cohort study by Wei *et al.* (2013) that used a propensity score approach (derived from baseline characteristics of age, sex,

smoking, body mass index, and diabetes duration), the association between use of pioglitazone and risk of cancer of the bladder was assessed in patients with type 2 diabetes using the General Practice Research Database. Between 2001 and 2010, 207 714 patients aged ≥ 40 years were studied: 23 548 users of pioglitazone and 184 166 patients receiving other antidiabetic medications. Follow-up started at the date of first prescription for pioglitazone or other oral antidiabetic drugs during the study period and ended in December 2010. Patients with a cancer diagnosis before the entry date or less than 90 days of follow-up time were excluded. Incident cases of cancer of the bladder were obtained from general practitioner records during follow-up. Hazard ratios were computed, comparing the risk of developing cancer of the bladder in the group receiving pioglitazone and in the group receiving treatment with other oral antidiabetic drugs. A propensity score matched analysis was used in patients without missing data on baseline characteristics to minimize confounding by indication (n = 34 498). The following potential confounders were included: smoking status, age, sex, duration of diabetes from first diagnosis to the first treatment with oral antidiabetic drug during the study period, body mass index before entry into the study, and insulin treatment and number and type of different oral antidiabetic drug classes used during follow-up. During the study period, 66 new cases of cancer of the bladder (mean follow-up time, 3.5 years) occurred in the pioglitazone group, and 803 cases in the group receiving other oral antidiabetic drugs (mean follow-up time, 5.3 years) (adjusted HR, 1.16; 95% CI, 0.83–1.62). [The use of a propensity score to control for confounding by adjustment was a strength of this study. There was a potential overlap in the studied population because the authors used a similar database to that used by Azoulay *et al.* (2012).]

The National Health Insurance of Taiwan, China, was used to conduct several analyses of cancer of the bladder associated with the use of thiazolidinediones (Tseng, 2012a, 2013a, b).

Tseng (2012a) followed a random sample of 54 928 patients with type 2 diabetes in the reimbursement databases of the National Health Insurance for 4 years from 1 January 2006 to 31 December 2009. Among 165 incident cases of cancer of the bladder, 10 (0.39%) were ever-users and 155 (0.30%) were never-users of pioglitazone, and were not necessarily using other antidiabetic drugs. The hazard ratio for ever-users versus never-users of pioglitazone was 1.31 (95% CI, 0.66–2.58) after adjustment for age, sex, diabetes duration, various comorbidities, and medications. Dose–response relationships were also evaluated, but no trend was observed. [Smoking and body mass index were not available for analyses from the databases.]

In a second study drawn from the entire database of the National Health Insurance of Taiwan, China, Tseng (2013a) evaluated the risk of cancer of the bladder associated with use of pioglitazone and rosiglitazone in a subgroup of 85 152 men with type 2 diabetes and benign prostatic hyperplasia. The hazard ratios (HR) for cancer of the bladder among the diabetic patients with benign prostatic hyperplasia for ever-users of pioglitazone (HR, 1.02; 95% CI, 0.75–1.39) and rosiglitazone (HR, 1.12; 95% CI, 0.92–1.37) were close to 1.0. [The study was not primarily aimed at analysing the risk of cancer of the bladder associated with use of pioglitazone or rosiglitazone, and therefore no dose–response relationship was assessed. Smoking and body mass index could not be adjusted for because of lack of such information in the databases. There was a concern over overlapping of the study population with that of Tseng (2012a).]

In a third study drawn from the entire database of the National Health Insurance of Taiwan, China, Tseng (2013b) evaluated the association between use of rosiglitazone and risk of cancer of the bladder after excluding patients who had ever been exposed to pioglitazone. A total of 885 236

patients with type 2 diabetes and receiving oral antidiabetic agents (except pioglitazone) and/or insulin were studied for incidence of cancer of the bladder from 1 January 2006 to 31 December 2009. Among these patients, 102 926 were ever-users and 782 310 were never-users of rosiglitazone, with 356 and 2753 incident cases of cancer of the bladder, respectively. The hazard ratio for cancer of the bladder for ever-users versus never-users of rosiglitazone was 0.98 (95% CI, 0.87–1.104) after adjustment for age, sex, diabetes duration, various comorbidities, and medications. Dose–response relationships were also evaluated, but neither the *P* values for the hazard ratios of the categories nor the *P* values for trends were significant. [This study evaluated use of rosiglitazone and risk of cancer of the bladder after excluding potential residual confounding from pioglitazone. The study used databases covering the whole nation and spanning the whole period since the start of rosiglitazone use in Taiwan, China. However, data on smoking and body mass index were not available for analyses. There was a concern regarding overlapping of the study population with that of Tseng (2012a). The follow-up duration of 4 years may also have been too short.]

Fujimoto *et al.* (2013) identified nine cases of cancer of the bladder in a cohort of 663 patients who had taken pioglitazone, in a database of 21 335 patients with type 2 diabetes from a single Japanese hospital between 2000 and 2011. They reported a hazard ratio of 1.75 (95% CI, 0.89–3.45) for cancer of the bladder among pioglitazone users compared with all patients with diabetes. [The Working Group noted that incident cases were defined as any bladder cancers diagnosed after onset of drug therapy. No information was given about total follow-up time. Duration and dose of drug were only given for identified cases, as were data about smoking status. No other details were given about confounders.]

2.1.3 Nested case–control studies

See Table 2.2

To determine whether the use of pioglitazone was associated with an increased risk of incident cancer of the bladder in people with type 2 diabetes, Azoulay *et al.* (2012) conducted a nested case–control analysis within a cohort of 115 727 people with type 2 diabetes in the United Kingdom General Practice Research Database. Participants were newly treated with oral hypoglycaemic agents between 1 January 1988 and 31 December 2009. All incident cases of cancer of the bladder occurring during follow-up (n = 470) were identified and 376 cases were matched to up to 20 controls (n = 6699) on year of birth, year of cohort entry, sex, and duration of follow-up. Exposure was defined as ever-use of pioglitazone and/or rosiglitazone (defined by the presence of at least one prescription between cohort entry and the year before the index date), along with measures of duration and cumulative dosage. Analyses were adjusted for smoking status, excessive alcohol use, obesity, HbA1c, previous bladder conditions, previous cancer (other than non-melanoma skin cancer), Charlson comorbidity score, and ever-use of other antidiabetic agents (metformin, sulfonylureas, insulin, and other oral hypoglycaemic agents). Overall, ever-use of pioglitazone was associated with an increased rate of cancer of the bladder (rate ratio, 1.83; 95% CI, 1.10–3.05), with a positive exposure–response trend (P = 0.030). The highest risk was observed in patients exposed for > 24 months (RR, 1.99; 95% CI, 1.14–3.45) and in those with a cumulative dosage > 28 000 mg (RR, 2.54; 95% CI, 1.05–6.14). [Enrolment of new users of diabetes medications, who may have had less severe disease, and adjustment for smoking were potential strengths of this study. However, there was a potential overlap in the study population with that of Wei *et al.* (2013).]

Table 2.2 Case–control studies of cancer and exposure to pioglitazone or rosiglitazone

Reference Study location and period	Total No. cases No. of controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Bladder cancer								
Azoulay *et al.* (2012)[a] United Kingdom, 1988–2009	376 6699	General Practice Research Database	Prescription records	Bladder (Read codes)	Never-use of any TZD	319	1.00 (ref.)	Up to 20 controls per case, matched on year of birth, year of cohort entry, sex, and duration of follow-up
					Exclusive ever-use of pioglitazone	19	1.83 (1.10–3.05)	
					Exclusive ever-use of rosiglitazone	36	1.14 (0.78–1.68)	
					Ever-use of both pioglitazone and rosiglitazone	2	0.78 (0.18–3.29)	
					Pioglitazone			
					Cumulative duration (mo):			
					≤ 12	1	0.56 (0.07–4.42)	
					13–24	2	3.03 (0.63–14.52)	
					> 24	16	1.99 (1.14–3.45)	
					P for trend		0.050	
					Cumulative dosage (mg):			
					≤ 10 500	7	1.58 (0.69–3.62)	
					10 501–28 000	6	1.66 (0.70–3.94)	
					> 28 000	6	2.54 (1.05–6.14)	
					P for trend		0.030	
					Rosiglitazone			
					Cumulative duration (d):			
					≤ 519	9	0.80 (0.40–1.62)	
					> 519–1022	13	1.33 (0.73–2.40)	
					> 1022	14	1.34 (0.75–2.40)	
					P for trend		0.32	
					Cumulative dosage (mg):			
					≤ 2464	8	0.71 (0.34–1.49)	
					> 2464–5152	15	1.50 (0.86–2.62)	
					> 5152	13	1.27 (0.69–2.32)	
					P for trend		0.49	

Table 2.2 (continued)

Reference Study location and period	Total No. cases No. of controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Chang *et al.* (2012)[b] Taiwan, China, 2000–7	1583 6308	Population	Reimbursement databases	Bladder (linkage through national cancer registry)	*Pioglitazone*			
					Never-user	NR	1.00 (ref)	
					Ever-user	NR	0.95 (0.70–1.29)	
					Cumulative dosage (DDD):			
					< 120	NR	0.83 (0.54–1.27)	
					≥ 120	NR	1.07 (0.72–1.57)	
					Cumulative duration (yr):			
					≤ 1	NR	0.87 (0.61–1.25)	
					1–2	NR	1.20 (0.65–2.22)	
					2–3	NR	0.84 (0.30–2.36)	
					≥ 3	NR	1.56 (0.51–4.74)	
					Cumulative dose duration:			
					High	NR	1.13 (0.69–1.83)	
					Intermediate	NR	1.06 (0.65–1.71)	
					Low	NR	0.77 (0.48–1.25)	
					Rosiglitazone			
					Never-user	NR	1.00 (ref.)	
					Ever-user	NR	1.05 (0.81–1.36)	
					Cumulative dosage (DDD):			
					< 120	NR	1.05 (0.77–1.45)	
					≥ 120	NR	1.05 (0.78–1.40)	
					Cumulative duration (yr):			
					≤ 1	NR	1.07 (0.80–1.42)	
					1–2	NR	1.26 (0.86–1.86)	
					2–3	NR	0.60 (0.33–1.08)	
					≥ 3	NR	1.14 (0.65–1.99)	
					Cumulative dose duration:			
					High	NR	1.09 (0.77–1.54)	
					Intermediate	NR	0.92 (0.65–1.29)	
					Low	NR	1.14 (0.82–1.58)	

Table 2.2 (continued)

Reference Study location and period	Total No. cases No. of controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Song *et al.* (2012) Republic of Korea, 2005–11	329 658	Hospital	Electronic medical records	Bladder (confirmed by cytology)	Pioglitazone (–) Pioglitazone (+)	308 21	1.00 (ref.) 2.09 (0.26–16.81)	Alcohol, smoking, coexisting cancer, haemoglobin and albumin Single centre (severance hospital), 1 : 2 age-sex-matched cases:controls; age, > 20 yr. No significant differences in diabetes duration, BMI, and renal function between cases and controls
Hassan *et al.* (2010) USA, 2000–8	122 86	Hospital	Interviewed with a structured and validated questionnaire	Liver (pathologically confirmed)	TZD (–) TZD (+)	116 6	1.00 (ref.) 0.3 (0.1–0.7)	Age, sex, race, education, cigarette smoking, alcohol drinking, HCV, HBV, and family history of cancer
Chang *et al.* (2012)[c] Taiwan, China, 2000–7	10 741 41 847	Population		Liver (linkage through national cancer registry)	*Pioglitazone* Never-user Ever-user Cumulative dosage (DDD): < 120 ≥ 120 Cumulative duration (yr): ≤ 1 1–2 2–3 ≥ 3	 10 267 (non-use) 474 225 249 352 79 30 13	 1.00 (ref.) 0.83 (0.72–0.95) 0.87 (0.72–1.05) 0.80 (0.67–0.95) 0.87 (0.74–1.02) 0.80 (0.59–1.07) 0.71 (0.45–1.14) 0.44 (0.23–0.86)	Age- and sex-matched controls (≥ 4 controls per case). A significantly lower risk of liver cancer was mainly observed in diabetic patients with chronic liver disease and with higher cumulative dosage or longer duration of use

Table 2.2 (continued)

Reference Study location and period	Total No. cases No. of controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Chang et al. (2012)[c] Taiwan, China, 2000–7 (cont.)					Rosiglitazone			Age- and sex-matched controls (≥ 4 controls per case). A significantly lower risk of liver cancer was mainly observed in diabetic patients with chronic liver disease and with higher cumulative dosage or longer duration of use
					Never-user of rosiglitazone	9154 (non-use)	1.00 (ref.)	
					Ever-user of rosiglitazone	1587	0.73 (0.65–0.81)	
					Cumulative dosage (DDD):			
					< 120	725	0.86 (0.75–0.98)	
					≥ 120	862	0.64 (0.56–0.72)	
					Cumulative duration (yr):			
					≤ 1	1034	0.78 (0.69–0.88)	
					1–2	330	0.66 (0.56–0.79)	
					2–3	135	0.59 (0.46–0.74)	
					≥ 3	88	0.64 (0.49–0.85)	
Colorectal cancer								
Chang *et al.* (2012)[d] Taiwan, China, 2000–7	7200 28 712	Population	Reimbursement databases	Colorectum (linkage through national cancer registry)	*Pioglitazone*			Age- and sex-matched controls (≥ 4 controls per case)
					Never-user	6822 (non-use)	1.00 (ref.)	
					Ever-user	378	1.04 (0.91–1.20)	
					Cumulative dosage (DDD):			
					< 120	170	1.15 (0.95–1.39)	
					≥ 120	280	0.97 (0.82–1.16)	
					Cumulative duration (yr):			
					≤ 1	278	1.15 (0.98–1.34)	
					1–2	60	0.82 (0.61–1.11)	
					2–3	26	0.86 (0.55–1.33)	
					≥ 3	14	0.77 (0.43–1.39)	
					Rosiglitazone			
					Never-user	6127	1.00 (ref.)	
					Ever-user	1073	0.86 (0.76–0.96)	
					Cumulative dosage (DDD):			
					< 120	434	0.89 (0.77–1.03)	
					≥ 120	639	0.83 (0.73–0.95)	

Table 2.2 (continued)

Reference Study location and period	Total No. cases No. of controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Chang et al. (2012)[d] Taiwan, China, 2000–7 (cont.)					Cumulative duration (yr):			
					≤ 1	678	0.91 (0.80–1.04)	
					1–2	220	0.78 (0.65–0.94)	
					2–3	99	0.69 (0.54–0.88)	
					≥ 3	76	0.83 (0.63–1.10)	
Lung cancer								
Chang et al. (2012)[e] Taiwan, China, 2000–7	5361 21 313	Population	Reimbursement databases	Lung (linkage through national cancer registry)	*Pioglitazone*			
					Never-user	NR	1.00 (ref.)	
					Ever-user	NR	1.14 (0.95–1.37)	
					Cumulative dosage (DDD):			
					< 120	NR	1.13 (0.89–1.44)	
					≥ 120	NR	1.20 (0.96–1.50)	
					Cumulative duration (yr)			
					≤ 1	NR	1.25 (1.01–1.53)	
					1–2	NR	1.09 (0.77–1.53)	
					2–3	NR	0.91 (0.51–1.61)	
					≥ 3	NR	0.20 (0.05–0.86)	
					Cumulative dose duration:			
					High	NR	1.00 (0.75–1.33)	
					Intermediate	NR	1.32 (1.01–1.73)	
					Low	NR	1.16 (0.88–1.51)	
					Rosiglitazone			
					Never-user	NR	1.00 (ref)	
					Ever-user	NR	1.12 (0.90–1.39)	
					Cumulative dosage (DDD):			
					< 120	NR	1.37 (1.08–1.74)	
					≥ 120	NR	1.00 (0.80–1.25)	

Table 2.2 (continued)

Reference Study location and period	Total No. cases No. of controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Chang et al. (2012)[e] Taiwan, China, 2000–7 (cont.)					Cumulative duration (yr):			
					≤ 1	NR	1.26 (1.01–1.58)	
					1–2	NR	0.83 (0.63–1.09)	
					2–3	NR	0.96 (0.70–1.33)	
					≥ 3 years	NR	1.17 (0.82–1.67)	
					Cumulative dose duration			
					High	NR	0.95 (0.74–1.22)	
					Intermediate	NR	1.13 (0.89–1.43)	
					Low	NR	1.34 (1.05–1.72)	

[a] Alcohol, obesity, smoking, HbA1c, previous bladder conditions, previous cancer (other than non-melanoma skin cancer), Charlson comorbidity score, and ever-use of other antidiabetic agents (metformin, sulfonylureas, insulin, and other oral hypoglycaemic agents).

[b] Multivariable model with stepwise selection of covariates, including pioglitazone, rosiglitazone, short-acting human insulin, metformin (mean daily dosage in quartiles), sulfonylurea (mean daily dosage in quartiles), number of oral antidiabetic agents, nephropathy, glinides, ACE inhibitors, chronic kidney disease, Ca-channel blockers, neuropathy.

[c] Multivariate model with stepwise selection of covariates, including pioglitazone, rosiglitazone, short-acting human insulin, metformin (mean daily dosage in quartiles), sulfonylurea (mean daily dosage in quartiles), number of oral antidiabetic agents, chronic liver disease, statins, aspirin, β-blockers, chronic kidney disease, glinides (oral antidiabetic agent), nephropathy, cerebrovascular disease, Ca-channel blockers, cardiovascular disease, chronic lung disease.

[d] Multivariate model with stepwise selection of covariates, including pioglitazone, rosiglitazone, short-acting human insulin, metformin (mean daily dosage in quartiles), sulfonylurea (mean daily dosage in quartiles), number of oral antidiabetic agents, glinides, nephropathy, neuropathy, chronic liver disease, statins, retinopathy, Ca-channel blockers, ACE inhibitors, peripheral vascular disease, depression, β-blockers, aspirin, chronic kidney disease, chronic lung disease, cerebrovascular disease.

[e] Multivariable model with stepwise selection of covariates, including pioglitazone, rosiglitazone, short-acting human insulin, metformin (mean daily dosage in quartiles), sulfonylurea (mean daily dosage in quartiles), number of oral antidiabetic agents, chronic lung disease, glinides, retinopathy, Ca-channel blockers, chronic kidney disease, statins, angiotensin receptor blockers, chronic liver disease, α-glucosidase inhibitors.

ACE, angiotensin-converting enzyme; BMI, body mass index; Ca, calcium; d, day; DDD, defined daily doses; HbA1c, glycated haemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; mo, month; NR, not reported; ref., reference; TZD, thiazolidinediones; yr, year

2.1.4 Case–control studies

See Table 2.2

Chang *et al.* (2012) conducted a nationwide case–control study to evaluate the risk of several malignancies in diabetic patients who received thiazolidinediones (pioglitazone or rosiglitazone). A total of 606 583 patients with type 2 diabetes, aged ≥ 30 years, without a history of cancer, were identified from the National Health Insurance claims database, Taiwan, China, between 1 January 2000 and 31 December 2000. As of 31 December 2007, patients with incident cancer of the liver, colorectum, lung, or urinary bladder were included as cases, and up to four age- and sex-matched controls were selected by risk-set sampling. Information was collected on prescribed drug types (according to the Anatomic Therapeutic Chemical classification system, A10BG02 for rosiglitazone and A10BG03 for pioglitazone), dosage, date of prescription, supply days, and total number of pills dispensed from the outpatient pharmacy-prescription database. Approximately 26.1% of patients had ever received rosiglitazone, and 14.1% had received pioglitazone. The mean cumulative duration was 522 days, and the mean daily dosage was 0.14 defined daily doses per day for rosiglitazone, compared with 375 days and 0.11 defined daily doses per day for pioglitazone.

With 1583 cases of cancer of the bladder and 70 559 diabetic controls, an increased risk of cancer of the bladder was associated with only ≥ 3 years of pioglitazone use (1.56; 95% CI, 0.51–4.74). The hazard ratio for the highest category of cumulative dose duration was 1.13 (95% CI, 0.69–1.83). [This study had a longer follow-up period than several others evaluating rosiglitazone and pioglitazone, but these drugs did not become available in Taiwan, China, until after follow-up began. The methods were not clearly described and it was not clear how cumulative dose was estimated. The investigators included chronic kidney disease and various drugs in the models. The important risk factor of tobacco smoking could not be adjusted for, but chronic lung disease, a proxy indicator of smoking, was included as a covariate. The study population potentially overlapped with that of Tseng (2012a, 2013a, b).]

In the Republic of Korea, Song *et al.* (2012) conducted a case–control study in diabetic patients with cancer of the bladder (n = 329) who presented at one hospital between November 2005 and June 2011. Cases exposed to pioglitazone were matched by sex and age to 658 control patients without cancer of the bladder who were listed on the hospital diabetes registry. The odds ratio for cancer of the bladder associated with a history of pioglitazone use was 2.09 (95% CI, 0.26–16.81) [but it was unclear whether this model was adjusted for potential confounders]. [The Working Group considered that the description of the methods and statistical analysis was inadequate, with conflicting information and some elements of the design (i.e. case–control study in a single tertiary hospital, history of pioglitazone or other medications prescribed before visiting this tertiary centre was not available, recall bias in information on confounders from the retrospective nature of the study, opposite association between pioglitazone use and cancer of the bladder in univariate and multivariate analyses) gave cause for concern.]

2.1.5 Other study designs

A study was conducted by Piccinni *et al.* (2011) using the FDA Adverse Event Reporting System, in the USA. Cases of cancer of the bladder were compared with all other reports of adverse effects within the system; a reporting odds ratio of 4.30 (95% CI, 2.82–6.52) was estimated for cancer of the bladder associated with pioglitazone compared with other antidiabetic drugs, and was elevated in each sex separately. [The Working Group noted that interpretation of these results was challenging because there

was no information about the population at risk: adverse event reports for drugs may not be a representative sample of the population. Analyses using the Adverse Events Reporting System may suffer from notoriety bias, but this study was conducted before concerns about pioglitazone were well known.]

2.1.6 Meta-analyses

Several meta-analyses have evaluated the association between the use of thiazolidinediones and cancer of the bladder (Zhu *et al.*, 2012; Colmers *et al.*, 2012b; Bosetti *et al.*, 2013; Ferwana *et al.*, 2013). [The Working Group found it difficult to compare meta-analyses since each included different, but partially overlapping, studies, some of which were unpublished.]

Zhu *et al.* (2012) conducted a meta-analysis on the association between pioglitazone and cancer of the bladder from five studies, including one prospective, randomized, controlled study (Dormandy *et al.*, 2005), three cohort studies (Lewis *et al.*, 2011; Neumann *et al.*, 2012; Tseng, 2012a), and one case–control study (Chang *et al.*, 2012). There was no evidence for the presence of significant heterogeneity between the five studies ($Q = 2.68$, $P = 0.61$; $I^2 = 0.0\%$). The meta-relative risk was 1.17 (95% CI, 1.03–1.32) for all studies. In patients with cumulative treatment exposure to pioglitazone for > 24 months, the meta-relative risk was 1.38 (95% CI, 1.12–1.70), and in those with a cumulative dose of > 28 000 mg, the meta-relative risk was 1.58 (95% CI, 1.12–2.06).

A meta-analysis by Colmers *et al.* (2012b) included unpublished results for cancer of the bladder associated with specific thiazolidinediones. The meta-relative risk for pioglitazone was 1.22 (95% CI, 1.07–1.39) and for rosiglitazone was 0.87 (95% CI, 0.34–2.23).

In a meta-analysis by Bosetti *et al.* (2013), the meta-relative risk was 1.20 (95% CI, 1.07–1.34) from six studies on pioglitazone, and 1.08 (95% CI, 0.95–1.23) from three studies on rosiglitazone. Longer duration of treatment (> 24 months) (1.42, 1.17–1.72) and higher cumulative dose (> 28 000 mg) of pioglitazone (1.64, 1.28–2.12) were associated with a significantly higher risk.

A meta-analysis by Ferwana *et al.* (2013) included six studies and reported a hazard ratio of 1.23 (95% CI, 1.09–1.39) associated with use of pioglitazone.

2.2 Cancer of the liver

There were no cohort studies evaluating the association between cancer of the liver and specific drugs of the thiazolidinedione class.

In a case–control study, Chang *et al.* (2012) (see Section 2.1.4 for description of study; see Table 2.2), reported a lower risk of cancer of the liver associated with both pioglitazone and rosiglitazone. The adjusted odds ratio (OR) for pioglitazone was 0.83 (95% CI, 0.72–0.95), and for rosiglitazone was 0.73 (95% CI, 0.65–0.81). Odds ratios decreased with increasing categories of duration of pioglitazone use (OR, 0.44; 95% CI, 0.23–0.86 for ≥ 3 years). [Although several risk factors for cancer of the liver were accounted for in the analysis, important potential confounders such as smoking, alcohol use, and hepatitis status, were not accounted for.]

2.3 Cancer of the colorectum

2.3.1 Case–control studies

See Table 2.2

In a multivariate analysis for risk of cancer of the colorectum associated with use of pioglitazone or rosiglitazone, Chang *et al.* (2012) (see Section 2.1.4 for description of study) reported odds ratios of 1.04 (95% CI, 0.91–1.20) associated with pioglitazone use, and 0.86 (95% CI, 0.76–0.96) associated with rosiglitazone use. The magnitude of the odds ratio for the highest exposure duration of ≥ 3 years was very similar for rosiglitazone (OR, 0.83; 95% CI, 0.63–1.10) and

for pioglitazone (OR, 0.77; 95% CI, 0.43–1.39). Furthermore, a trend of decreasing odds ratios with increasing cumulative duration of exposure was observed for both rosiglitazone and pioglitazone. [See Section 2.1.4 for the strengths and limitations of this study.]

2.3.2 Cohort studies

See Table 2.1

Ferrara *et al.* (2011) evaluated risk of cancer of the colorectum associated with pioglitazone use in a cohort of 252 467 male and female patients aged ≥ 40 years in the KPNC diabetes registry (see Section 2, introduction, for description). Data on use of diabetes medications were obtained from the pharmacy clinical database, and the filling of two prescriptions of pioglitazone within 6 months was defined as "ever used". Information was collected from electronic medical records about all confounders, except smoking, which was supplemented by postal-survey data. The hazard ratio for cancer of the colorectum associated with ever-use versus never-use of pioglitazone was 0.9 (95% CI, 0.7–1.1), after adjusting for a large number of potential confounders including current smoking, age, and ever-use of other diabetes medications. [This study was based on the same population as Lewis *et al.* (2012). The authors were only able to examine recently initiated therapy and short-term use (median, 1.6 years) of pioglitazone, although the latency period until development of cancer of the bladder may be longer.]

Neumann *et al.* (2012) (see Section 2.1.2 for description of study) investigated risk of cancer of the colorectum in users of pioglitazone or rosiglitazone compared with non-users, and reported hazard ratios of 0.97 (95% CI, 0.90–1.05) for pioglitazone, and 0.88 (95% CI, 0.82–0.95) for rosiglitazone.

Tseng (2012b) (see Section 2.1.2 for description of study) assessed risk of cancer of the colon in thiazolidinedione users versus non-users in a multivariable analysis. Hazard ratios of 0.78 (95% CI, 0.25–2.49) for pioglitazone users, and 1.22 (95% CI, 0.81–1.84) for rosiglitazone users were reported.

2.3.3 Meta-analyses

A meta-analysis derived from three observational studies (Colmers *et al.*, 2012a) calculated a meta-risk ratio of 0.97 (95% CI, 0.90–1.04) for cancer of the colorectum associated with pioglitazone use (see Section 2.1.6 for further description).

2.4 Cancer of the lung

2.4.1 Case–control studies

See Table 2.2

In a multivariable analysis for cancer of the lung, Chang *et al.* (2012) (see Section 2.1.4 for description of study) reported an odds ratio for pioglitazone use of 1.14 (95% CI, 0.95–1.37) and 1.12 (95% CI, 0.90–1.39) associated with rosiglitazone use. A higher risk was reported with a cumulative duration of ≥ 1 year for use of either pioglitazone or rosiglitazone, with adjusted odds ratios of 1.25 (95% CI, 1.01–1.53) and 1.26 (95% CI, 1.01–1.58), respectively. [The investigators included chronic kidney disease and various drugs in the models. The important risk factor of tobacco smoking could not be adjusted for, but chronic lung disease, a proxy indicator of smoking, was included as a covariate.]

2.4.2 Cohort studies

See Table 2.1

In a multivariable analysis in the study by Ferrara *et al.* (2011) (see Section 2.3.2 for description of study), no effect was found on the incidence of cancer of the lung or bronchus in pioglitazone users when compared with never-users (adjusted HR, 1.0; 95% CI, 0.8–1.3). [Adjustment for smoking was a strength of this study, but only

current smoking was considered, which may not have fully controlled for confounding.]

Similarly, in a cohort study of diabetic patients in France Neumann *et al.* (2012) (see Section 2.1.2 for description of study) reported no significant difference in the risk of cancer of the lung in users compared with non-exposed controls for pioglitazone (adjusted HR, 0.94; 95% CI, 0.87–1.02), or for rosiglitazone (adjusted HR, 0.91; 95% CI, 0.84–0.99). Hazard ratios were not adjusted for smoking.

2.4.3 Meta-analyses

A meta-analysis of "pulmonary malignancies" by Monami *et al.* (2008) showed that the meta-odds ratio for rosiglitazone versus comparators in clinical trials was 0.67 (95% CI, 0.30–1.51).

Colmers *et al.* (2012a) reported a meta-relative risk for ever-users of pioglitazone versus never-users of 0.95 (95% CI, 0.88–1.02) from two observational studies.

2.5 Cancer of the prostate

See Table 2.1

No case–control studies evaluated the association between use of pioglitazone or rosiglitazone and cancer of the prostate.

A cohort study by Ferrara *et al.* (2011) (see Section 2.3.2 for description of study) found no association when comparing ever-use of pioglitazone versus never-use (adjusted HR, 1.0; 95% CI, 0.8–1.2). Tseng (2011) (see Section 2 introduction for description of study) reported an inverse association for ever-use of pioglitazone versus never-use (adjusted HR, 0.77; 95% CI, 0.10–5.75) and for rosiglitazone (adjusted HR, 0.88; 95% CI, 0.43–1.80).

Two meta-analyses (Monami *et al.*, 2008; Colmers *et al.*, 2012a) reported meta-relative risks of around unity for cancer of the prostate associated with the use of pioglitazone or rosiglitazone.

2.6 Cancer of the breast

In the PROactive clinical trial (Dormandy *et al.*, 2005; see Section 2.1.1 for description of study), an imbalance in the number of cases of cancer of the breast was noted, with three cases in the pioglitazone group and eleven in the placebo group. [Insufficient data were provided to calculate a risk estimate. The PROactive trial was primarily aimed at evaluating macrovascular events associated with pioglitazone use.]

In a multivariable analysis in the study by Ferrara *et al.* (2011) (see Section 2.3.2 and 2.4.2 for comments and description of study; see Table 2.1), no effect was found in the incidence of cancer of the breast in pioglitazone users when compared with never-users (adjusted HR, 1.0; 95% CI, 0.8–1.3).

Similarly, a cohort study of diabetic patients in France (Neumann *et al.*, 2012; see Section 2.3.2 for comments and description of study; see Table 2.1) reported no significant difference in the risk of cancer of the breast in pioglitazone users when compared with non-exposed controls (adjusted HR, 0.91; 95% CI, 0.83–1.00). A significantly reduced risk of cancer of the breast was found in rosiglitazone users when compared with non-exposed controls (adjusted HR, 0.80; 95% CI, 0.73–0.88).

The meta-relative risk estimated by Colmers *et al.* (2012a) for cancer of the breast in ever-users of pioglitazone versus never-users from two observational studies was 0.93 (95% CI, 0.85–1.01).

2.7 Other site-specific cancers

See Table 2.1

Several of the studies described above also reported on other site-specific cancers. The study by Ferrara *et al.* (2011) reported relative risks of > 1 for some other cancers.

Neumann *et al.* (2012) reported a hazard ratio of near unity for cancer of the kidney among

users of pioglitazone or rosiglitazone, and hazard ratios of approximately 0.8 for both agents for cancer of the head and neck.

By using the National Health Insurance database of Taiwan, China, Tseng evaluated the association of pioglitazone and rosiglitazone with the risk of cancer of the thyroid (Tseng, 2012c), and cancer of the oral cavity, lip, and pharynx (Tseng, 2013c), respectively. The hazard ratio for cancer of the thyroid was 0.52 (95% CI, 0.07–3.93) for pioglitazone and 0.67 (95% CI, 0.23–1.95) for rosiglitazone (Tseng, 2012c); for cancer of the oral cavity, lip, and pharynx, the hazard ratio was 1.70 (95% CI, 0.22–13.20) for pioglitazone and 1.15 (95% CI, 0.44–3.04) for rosiglitazone (Tseng, 2013c).

In a meta-analysis by Colmers *et al.* (2012a), the meta-risk ratio for cancer of the kidney was 0.89 (95% CI, 0.76–1.04) for ever-users of pioglitazone versus never-users from two observational studies.

3. Cancer in Experimental Animals

3.1 Pioglitazone

3.1.1 Oral administration

(a) Mouse

See Table 3.1

As part of its pharmacology review of the New Drug Application package (NDA 21–073) for pioglitazone submitted by the Takeda America Research and Development Center, the FDA summarized the results of a 2-year study that was performed to evaluate the potential carcinogenicity of pioglitazone in mice (FDA, 1999a). In this study, groups of 60 male and 60 female CD-1 mice [age not reported] received pioglitazone by gavage at doses of 0 (vehicle), 0 (placebo suspension), 3, 10, 30, or 100 mg/kg body weight (bw) per day for 104 weeks. [Vehicle and placebo suspension were not specified.] There was a significant positive trend towards increased mortality with increasing dose in male mice. Increased incidences of benign pheochromocytoma of the adrenal gland were seen in exposed male mice, and increased incidences of leiomyosarcoma of the uterine cervix were seen in exposed female mice when compared with controls. [Although it was noted that the FDA identified these differences as being statistically significant, the Working Group could not confirm the statistical analyses because original study data (e.g. mortality) were not available (FDA, 1999a).]

(b) Genetically engineered mouse

Pino *et al.* (2004) reported increased incidence and multiplicity of adenoma of the large intestine in $Apc^{Min}/+$ mice (a genetically engineered mouse model that overexpresses the *Apc* gene, leading to rapid development of intestinal neoplasms) with dietary exposure to any of several peroxisome proliferator-activated receptor γ (PPARγ) agonists, including pioglitazone. In this study, male C57BL/6J-$Apc^{Min}/+$ mice (age, 6–7 weeks) were fed diets containing pioglitazone at a concentration selected to achieve a dose of 150 mg/kg bw per day for 8 weeks. All mice exposed to pioglitazone (15 out of 15, 100% [$P < 0.01$]) developed adenoma of the large intestine, compared with 60% (9 out of 15 mice) in the dietary control group. Pioglitazone increased the multiplicity of tumours of the large intestine, but not of the small intestine [data and statistics were provided in graphical form]. [The Working Group noted that, although used extensively as a model system for cancer chemoprevention, the predictive value of the $Apc^{Min}/+$ mouse in the identification of agents that may promote or otherwise stimulate carcinogenesis in the human colon was unknown.]

(c) Rat

See Table 3.2

Table 3.1 Studies of carcinogenicity in mice given pioglitazone orally

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD-1 (M, F) 104 wk FDA (1999a)	0 (vehicle control), 0 (placebo suspension), 3, 10, 30, 100 mg/kg bw per day by gavage in an unspecified vehicle or suspension. 60 M and 60 F/group	Benign pheochromocytoma of the adrenal gland: 0/60, 0/60, 0/60, 0/60, 2/60*, 1/60* (M) Leiomyosarcoma of the uterine cervix: 0/60, 0/60, 0/60, 0/60, 1/60*, 1/60* (F)	*$P < 0.05$ (Peto test)	The Working Group was unable to confirm the statistical analyses. Increase in mortality in exposed males.
C57BL6J-Apc^{Min}/+ (M) 8 wk Pino *et al.* (2004)	Pioglitazone mixed in feed and given to achieve daily doses of 0 or ~150 mg/kg bw 15/group	Large intestine adenoma : 9/15, 15/15*	*[$P < 0.01$] $P \leq 0.05$ for increased multiplicity of large intestine tumour [data and statistics read from graph]	Genetically engineered mouse sensitive to intestinal carcinogenesis. No increases in the multiplicity of small intestine tumours.

bw, body weight; F, female, M, male; wk, week

Table 3.2 Studies of carcinogenicity in rats given pioglitazone orally

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Sprague-Dawley (M, F) 104 wk FDA (1999a)	0 (vehicle control), 0 (placebo suspension), 1, 4, 8 (M only), 16, or 63 mg/kg bw per day by gavage in an unspecified vehicle or suspension 60 M and 60 F/group	Transitional cell carcinoma of urinary bladder: 0/60, 0/60, 0/60, 2/60, 3/60, 5/60, 4/60 (M) 0/60, 0/60, 0/60, 0/60, –, 0/60, 0/60 (F)	$P < 0.025$ (trend) (M)	Increase in mortality in exposed M and F
		Transitional cell papilloma of urinary bladder: 0/60, 0/60, 0/60, 0/60, 4/60, 2/60, 2/60 (M); 0/60, 0/60, 1/60, 1/60, –, 1/60, 0/60 (F)	NS	
		Fibrosarcoma of subcutis: 0/60, 0/60, 0/60, 0/60, 0/60, 2/60, 2/60 (M)	$P < 0.025$ (trend)	
		Subcutaneous lipoma: 0/60, 0/60, 1/60, 0/60, –, 1/60, 3/60 (F)	$P < 0.025$ (trend)	
CD (M) 104 wk Sato *et al.* (2011)	0 (control) or 16 mg/kg bw by gavage in citric acid granules for 85 wk 90/group	Urinary bladder Papilloma: 0/78, 7/82* Carcinoma: 0/78, 1/82	*$P \leq 0.05$ (Peto test)	

bw, body weight; F, female, M, male; NS, not significant; wk, week

As part of its pharmacology review of the New Drug Application package (NDA 21–073) for pioglitazone submitted by the Takeda America Research and Development Center, the FDA summarized the results of a 2-year study that was performed to evaluate the potential carcinogenicity of pioglitazone in rats (FDA, 1999a). In this study, groups of 60 male and 60 female Sprague-Dawley rats [age not reported] received pioglitazone by gavage at doses of 0 (vehicle), 0 (placebo suspension), 1, 4, 8 (males only), 16, or 63 mg/kg bw per day for 104 weeks. [Vehicle and placebo suspension were not specified.] There was a significant positive trend towards increased mortality with increasing dose in male and female rats.

Treatment with pioglitazone caused a significant positive trend in the incidence of transitional cell carcinoma of the urinary bladder in male rats. Although female rats did not demonstrate an increased incidence of transitional cell tumours of the urinary bladder, urothelial hyperplastic lesions were identified in male and female rats exposed to pioglitazone. In addition, pioglitazone induced a small but significant positive trend in the incidence of fibrosarcoma of the subcutis in male rats, and a significant positive trend in the incidence of subcutaneous lipoma in female rats (FDA, 1999a).

Sato *et al.* (2011) reported a study in which two groups of 90 male CD rats (age, 6 weeks) received pioglitazone (in citric acid granules) by gavage at a dose of 0 (control), or 16 mg/kg bw per day for 85 weeks, followed by a 19-week observation period. There was a significant increase in the incidence of papilloma of the urinary bladder in the exposed group (7 out of 82 rats) compared with the controls (0 out of 78 rats). There was also one carcinoma of the urinary bladder in the exposed group compared with none in the controls.

3.1.2 Coexposure with modifying agents

See Table 3.3

A group of 34 male and 35 female strain H Swiss mice was exposed by whole-body inhalation to mainstream cigarette smoke for 4 months, starting 12 hours after birth, and then kept in filtered air until the experiment was terminated at age 7 months. After weaning (at age 4–5 weeks), the mice also received diets containing pioglitazone at a concentration of 120 mg/kg. A control group of 34 male and 38 female mice was exposed to mainstream cigarette smoke only. In females, 5 out of 35 ($P < 0.01$) mice exposed to pioglitazone developed adenoma of the kidney versus 0 out of 38 controls. In males, 3 out of 32 mice developed kidney adenoma versus 0 out of 34 controls (La Maestra *et al.*, 2013).

3.2 Rosiglitazone

3.2.1 Oral administration

(a) Mouse

See Table 3.4

As part of its pharmacology review of the New Drug Application package (NDA 21–071) for rosiglitazone that was submitted by SmithKline Beecham Pharmaceuticals, the FDA summarized the results of a 2-year feeding study that was performed to evaluate the potential carcinogenicity of rosiglitazone in mice (FDA, 1999b). In this study, groups of 60 male and 60 female CD-1 mice [age not reported] received diet supplemented with rosiglitazone at concentrations that were selected to provide doses of 0 (control), 0.4, 1.5, or 6.0 mg/kg bw for 105 weeks. A significant positive trend towards increased mortality with increasing dose was seen in male and female mice. The reduction in survival of male mice in the group at the highest dose necessitated early termination of this dose group at week 95, instead of week 105. The only significant increase in the incidence of any neoplasm

Table 3.3 Studies of carcinogenicity involving exposure to pioglitazone or rosiglitazone with modifying agents

Species, strain (sex) Duration Reference	Modifying agent	Dosing regimen, Animals/group at start	Incidence of tumours	Significance
Mouse, H Swiss (M, F) 7 mo La Maestra *et al.* (2013)	Mainstream cigarette smoke by whole-body inhalation for 4 mo, starting 12 hours after birth, followed by filtered air for 3 mo	After weaning (at age, 4–5 wk), mice were also fed diets containing pioglitazone at a concentration of 0 (control) or 120 mg/kg. 34, 34/group (M) 38, 35/group (F)	Kidney adenoma: 0/34, 3/32 (M) 0/38, 5/35* (F)	*$P < 0.01$
Rat, F344 (F) 7 mo Lubet *et al.* (2008)	*N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (150 mg) by gavage in thanol : water, 2×/wk for 8 wk	After 2 wk, rats were given rosiglitazone by gavage at 0 (control), 50 mg/kg bw per day 35, 35/group	Urinary bladder carcinoma: 20/35, 34/34*	*$P < 0.01$
Rat, F344 (F) 8 mo Lubet *et al.* (2008)	*N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (150 mg) by gavage in ethanol : water, 2×/wk for 8 wk	2 wk later, rats were given rosiglitazone by gavage at 0 (control), 10 mg/kg bw per day 29, 30/group	Urinary bladder carcinoma: 8/29, 28/30*	*$P < 0.01$
Rat, F344 (F) 10 mo Lubet *et al.* (2008)	*N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine (150 mg) by gavage in ethanol : water, 2×/wk for 8 wk	2 wk later, rats were given rosiglitazone by gavage at 0 (control), 0.4, or 2 mg/kg bw per day 25, 29, 30/group	Urinary bladder carcinoma : 12/25, 19/29, 24/30*	*$P < 0.05$

bw, body weight; F, female, mo, month; M, male; wk, week

Table 3.4 Studies of carcinogenicity in mice given diets containing rosiglitazone

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD-1 (M, F) 105 wk FDA (1999b)	Rosiglitazone mixed in feed to achieve doses of 0 (control), 0.4, 1.5, or 6.0 mg/kg bw per day 60 M and 60 F/group	Liver haemangiosarcoma: 0/60, 4/60*, 0/60, 0/60 (M)	*$P = 0.013$	There was a significant trend for increasing mortality with increasing dose in M and F. High-dose males were killed at wk 95. No increase in the incidence of any type of neoplasm in females
C57BL6J-Apc^{Min}/+ (M) 8 wk Pino *et al.* (2004)	Rosiglitazone mixed in feed to achieve doses of 0 or ~20 mg/kg bw per day 15/group	Large intestine adenoma : 9/15, 14/14*	*[$P < 0.01$] $P \leq 0.05$ for increased multiplicity of large intestine tumours [data and statistics read from graph]	Genetically engineered mouse sensitive to intestinal carcinogenesis. No increases in multiplicity of small intestine tumours.

bw, body weight; F, female, M, male; wk, week; NR, not reported

in any dose group was an increased incidence of liver haemangiosarcoma in male mice at the lowest dose (FDA, 1999b). [Because no significant evidence of a dose–response relationship was seen, the Working Group concluded that there was no treatment-related positive trend in the incidence of liver haemangiosarcoma, or of any other tumour type in either sex.]

(b) Genetically engineered mouse

Pino *et al.* (2004) reported increased incidence and multiplicity of adenoma of the large intestine in Apc^{Min}/+ mice (a genetically engineered mouse model that overexpresses the *Apc* gene, leading to rapid development of intestinal neoplasms) with dietary exposure to rosiglitazone. In this study, male C57BL6J-Apc^{Min}/+ mice (age, 6–7 weeks) were fed rosiglitazone at a dietary concentration selected to achieve a dose of 20 mg/kg bw per day, for 8 weeks. All mice exposed to rosiglitazone (14 out of 14, 100% [$P < 0.01$]) developed adenoma of the large intestine, compared with 60% (9 out of 15 mice) in the dietary controls group. Rosiglitazone increased the multiplicity of tumours of the large intestine, but not of the small intestine [data and statistics were provided in in graphical form]. [The Working Group noted that, although used extensively as a model system for cancer chemoprevention, the predictive value of the Apc^{Min}/+ mouse in the identification of agents that may promote or otherwise stimulate carcinogenesis in the human colon was unknown.]

(c) Rat

See Table 3.5

As part of its pharmacology review of the New Drug Application package (NDA 21–071) for rosiglitazone submitted by SmithKline Beecham Pharmaceuticals, the FDA summarized the results of a 2-year study that was performed to evaluate the potential carcinogenicity of rosiglitazone in rats (FDA, 1999b). In this study, groups of 60 male and 60 female Sprague-Dawley rats [age not reported] received rosiglitazone by gavage at doses of 0 (control), 0.05, 0.3, or 2.0 mg/kg bw per day in 1% methylcellulose for 2 years. In comparison to vehicle controls, a significant increase in mortality was seen in males at the highest dose. Significant increases in the incidence of subcutaneous lipoma were seen in males at the intermediate dose, and in females at the highest dose. [Increases in the incidence of subcutaneous lipoma and adipocyte hyperplasia (a putative preneoplastic lesion that is linked to lipoma) in males and females, were considered to be treatment-related.]

3.2.2 Coexposure with modifying agents

See Table 3.3

In two studies evaluating the activity of rosiglitazone as a chemopreventive agent for cancer of the urinary bladder, groups of female F344 rats received *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) by gavage in 0.1 mL ethanol : water (25 : 75, v/v), twice per week, for 8 weeks. Beginning 2 weeks after the last dose of BBN, parallel groups of rats were given rosiglitazone at a dose of 0.4, 2, 10, or 50 mg/kg bw per day by gavage, or the vehicle (carboxymethylcellulose : polyethylene glycol 400; 50 : 50, v/v) only, for 7–10 months, followed by necropsy and histopathological examination of the urinary bladder (Lubet *et al.*, 2008).

In the first study, the incidence of carcinoma of the urinary bladder in rats exposed to BBN plus rosiglitazone (50 mg/kg bw per day) for 7 months was 100% (34 out of 34; $P < 0.01$), compared with 57% (20 out of 35) in the group exposed to BBN only. In the follow-up study with lower doses of rosiglitazone, the incidence of carcinoma of the urinary bladder at 8 months in the group receiving BBN plus rosiglitazone (10 mg/kg bw per day) was 93% (28 out of 30; $P < 0.01$), compared with 28% (8 out of 29) in the group exposed to BBN only. In the same study, the incidence of carcinoma of the urinary bladder at 10 months in groups treated

Table 3.5 Studies of carcinogenicity in rats given rosiglitazone by gavage

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Sprague-Dawley (M, F) 104 wk FDA (1999b)	Rosiglitazone given at doses of 0, 0 (second control group), 0.05, 0.3, or 2.0 mg/kg bw in 1% methylcellulose 60 M and 60 F/group	Subcutaneous lipoma: 3/60, 4/60, 5/59, 13/58*, 6/60 (M) 1/60, 2/60, 3/60, 1/59, 9/60** (F)	*P = 0.001 **P = 0.003	Significantly increased mortality in high-dose males Second control group reported, but no information on how it differed from the first control group

bw, body weight; F, female, M, male; wk, week

with BBN plus rosiglitazone (2 or 0.4 mg/kg bw per day) group was 80% (24 out of 30; $P < 0.05$) and 67% (19 out of 29), versus 48% (12 out of 25) in BBN-treated vehicle controls. When administered alone (without prior exposure to BNN), rosiglitazone (10 mg/kg bw) did not induce carcinoma of the urinary bladder during the 8-month observation period. [The Working Group noted that the predictive value of the BBN model for the identification of agents that can enhance or promote cancer of the human bladder had not been established.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion of pioglitazone

4.1.1 Humans

(a) Absorption, distribution, and excretion

In fasting individuals, pioglitazone was measurable in the serum within 30 minutes after oral administration, with peak concentrations observed within 2 hours. Administration with food slightly delayed the time to peak serum concentration (to 3–4 hours), but did not alter the extent of absorption. The mean serum half-life of pioglitazone ranged from 3 to 7 hours, while the mean serum half-life of the pharmacologically active metabolites M-III and M-IV ranged from 16 to 24 hours. Serum concentrations of total pioglitazone (pioglitazone plus active metabolites) remained elevated 24 hours after dosing (Takeda Pharmaceuticals, 2013).

The apparent oral clearance (CL/F) of pioglitazone has been calculated as 5–7 L per hour. The mean apparent volume of distribution (Vd/F) of pioglitazone after administration of a single oral dose was 0.63 ± 0.41 (mean ± standard deviation) L/kg bw. Pioglitazone binds extensively (> 99%) to protein in human serum, principally to serum albumin. Pioglitazone also binds other serum proteins, but with lower affinity. Metabolites M-III and M-IV also are extensively bound (> 98%) to serum albumin (Takeda Pharmaceuticals, 2013).

Steady-state serum concentrations of pioglitazone and total pioglitazone were achieved within 7 days. At steady state, M-III and M-IV reached serum concentrations equal to or greater than that of pioglitazone. In healthy volunteers and in patients with type 2 diabetes, pioglitazone comprised approximately 30–50% of the peak total pioglitazone serum concentrations and 20–25% of the total area under the curve (AUC) for serum concentration–time. Maximum serum concentration (C_{max}), AUC, and trough serum concentration (C_{min}) for pioglitazone and total pioglitazone increased proportionally at doses of 15 mg and 30 mg per day (Takeda Pharmaceuticals, 2013).

After oral administration, 15–30% of the administered dose of pioglitazone was recovered in the urine. Most of the oral dose was excreted into the bile either unchanged or as metabolites, and eliminated in the faeces. Renal elimination of pioglitazone was negligible (Takeda Pharmaceuticals, 2013).

There was no significant difference in the pharmacokinetic profile of pioglitazone in subjects with normal or with moderately impaired renal function. In patients with moderate and severe renal impairment, although mean serum concentrations of pioglitazone and its metabolites were increased, no dose adjustment is needed. After repeated oral doses of pioglitazone, mean AUC values were decreased in patients with severe renal impairment compared with healthy subjects with normal renal function for pioglitazone (Budde *et al.*, 2003).

In a multi-dosing study, pioglitazone was rapidly absorbed, with median time to maximal serum concentration (C_{max}) occurring within 2 hours. Serum concentrations of pioglitazone and its active metabolites remained elevated 24 hours after exposure (Christensen *et al.*, 2005).

(b) Metabolism

Pioglitazone is extensively metabolized by hydroxylation and oxidation to its active metabolites, which are keto and hydroxy derivatives. The active metabolites include M-II active (hydroxy), M-III active (keto), and M-IV active (hydroxy) (Fig. 4.1; Takeda Pharmaceuticals, 2013).

In-vitro data have demonstrated that multiple isoforms of cytochrome P450 (CYP) are involved in the metabolism of pioglitazone, including CYP2C8 and, to a lesser degree, CYP3A4 (Kirchheiner *et al.*, 2005). CYP2C9 is not significantly involved in the elimination of pioglitazone (Jaakkola *et al.*, 2006). Pioglitazone is not a strong inducer of CYP3A4, and pioglitazone was not shown to induce CYPs (Nowak *et al.*, 2002).

4.1.2 Experimental systems

In pharmacokinetic studies with male rats, peak plasma concentrations of pioglitazone were reported at 1 hour, and the plasma terminal half-life of pioglitazone was 7.5 hours. The distribution of pioglitazone was not extensive; the tissue/plasma ratio was low (< 0.5), except for the gastrointestinal tract (Krieter *et al.*, 1994).

The AUCs for pioglitazone metabolites M-III and M-IV were higher in female rats than in males, while levels of M-II were similar in both sexes (Fujita *et al.*, 2003).

4.2 Absorption, distribution, metabolism, and excretion of rosiglitazone

4.2.1 Humans

(a) Absorption, distribution, and excretion

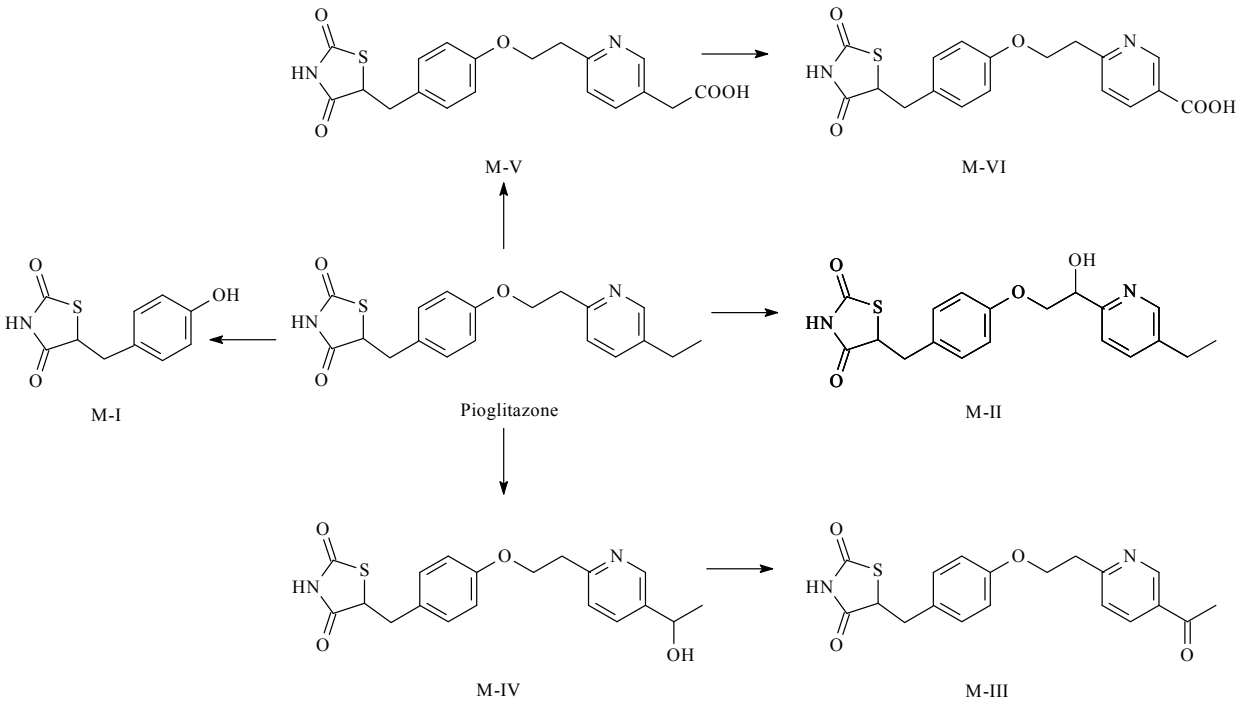
In a study in healthy volunteers, the absorption of rosiglitazone was relatively rapid, with 99% oral bioavailability after oral absorption (Cox *et al.*, 2000).

Peak plasma concentrations were observed about 1 hour after single oral doses. Maximum plasma concentration (C_{max}) and the AUC of rosiglitazone increased in a dose-proportional manner over the therapeutic dose range (National Library of Medicine, 2010).

The mean oral volume of distribution of rosiglitazone was approximately 17.6 L, based on a population pharmacokinetic analysis. Rosiglitazone is approximately 99.8% bound to plasma proteins, primarily albumin (National Library of Medicine, 2010).

The elimination half-life of rosiglitazone was 3–4 hours and was independent of dose. The time to C_{max} and the elimination half-life for two metabolites in plasma were significantly longer than for rosiglitazone itself (4–6 hours versus 0.5–1 hours, and about 5 days versus 3–7 hours) (Cox *et al.*, 2000).

Fig. 4.1 Pioglitazone and metabolites



Compiled by the Working Group

After oral or intravenous administration of rosiglitazone maleate, approximately 64% and 23% of the administered dose was eliminated in the urine and in the faeces, respectively (National Library of Medicine, 2010). No unchanged drug was eliminated in the urine.

In a pharmacokinetics study of administration of rosiglitazone with food, absorption measured via T_{max} was delayed by 1.75 hours. The C_{max} was reduced by approximately 20%, but the geometric mean ratio of AUC for the fed/fasted state was 0.94. No dose adjustment is required for administration of rosiglitazone with food (Freed *et al.*, 1999).

The half-life values of rosiglitazone are similar in fasted and fed subjects (Bulliman *et al.*, 1995).

Ethnicity had no impact on the pharmacokinetics of rosiglitazone among healthy subjects (Chu *et al.*, 2007).

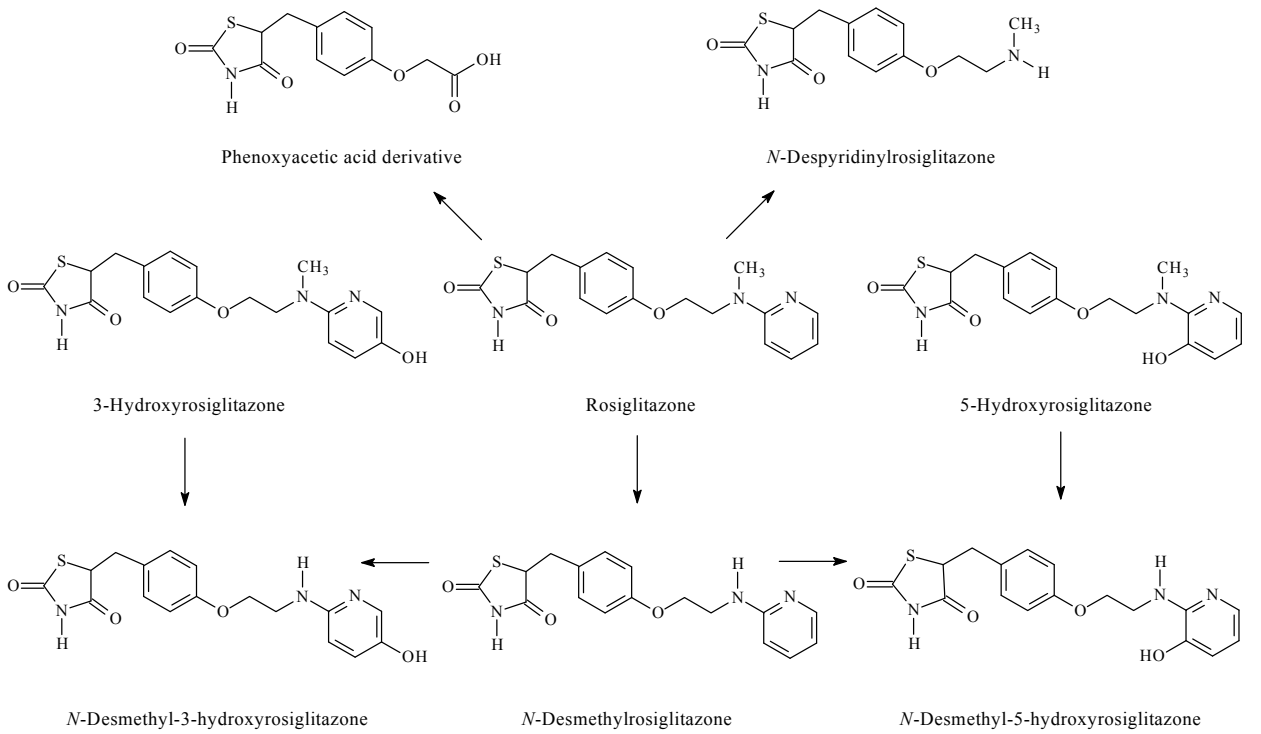
In patients with mild, moderate, or severe renal insufficiency there are slight increases in AUC for rosiglitazone (10–20%), which were not deemed to be clinically relevant (Chapelsky *et al.*, 2003).

In a placental transfer study, the risk of placental transfer of rosiglitazone was higher after 10 weeks of gestation (Chan *et al.*, 2005).

(b) Metabolism

Rosiglitazone is extensively metabolized by CYP2C9 and CYP2C8, with no unchanged drug excreted in the urine (Kirchheiner *et al.*, 2005). The major routes of metabolism were *N*-demethylation and hydroxylation, leading to *N*-desmethyl-rosiglitazone and 3-hydroxy-rosiglitazone, followed by conjugation with sulfate and glucuronic acid. All the circulating metabolites were considerably less potent than the parent compound and, therefore, are not expected to contribute to the activity of rosiglitazone (National Library of Medicine, 2010; see Fig. 4.2).

Fig. 4.2 Rosiglitazone and metabolites



Compiled by the Working Group

4.2.2 Experimental systems

Rosigilitazone was extensively metabolized after oral administration in mice. Mean bioavailability was found to be 100%, 60%, and 95% in rats, dogs, and humans, respectively (EMEA, 2005).

The main metabolites observed in humans are also observed in rats; however, the clearance in rats was almost ten times higher than in humans, probably due to the higher levels of CYP2C in rat microsomes (EMEA, 2005; Calixto *et al.*, 2011).

4.3 Genetic and related effects

4.3.1 Humans

DNA damage

Incubating pioglitazone (100 μM) with human peripheral blood lymphocytes significantly increased the frequency of chromosomal aberrations, sister chromatid exchanges, and increased levels of 8-oxodeoxyguanosine (Table 4.1; Alzoubi *et al.*, 2012).

4.3.2 Experimental systems

(a) DNA damage

Male Sprague-Dawley rats treated with pioglitazone by gavage had a dose-dependent increase in the frequency of DNA damage in peripheral blood lymphoctyes and liver cells, as measured by comet assays. The addition of an enzyme mixture containing endonuclease III and formamidopyrimidine glycosylase significantly increased the frequency of DNA damage, suggesting that DNA damage was due to oxidation of DNA bases (Table 4.1; Bedir *et al.*, 2008).

Pioglitazone did not increase the frequency of chromosomal aberrations in Chinese hamster lung cells. Pioglitazone did not induce unscheduled DNA synthesis in primary rat hepatocytes,

Table 4.1 Genetic and related effects of pioglitazone

Test system	Results		Dose or concentration (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
In vitro				
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, with 20-minute pre-incubation, reverse mutation	–	–	5000 µg/plate	FDA Drug Approval Package (1999a)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA1538, reverse mutation	–	–	2000 µg/plate	FDA Drug Approval Package (1999a)
Escherichia coli WP2 *uvrA*, with 20-minute pre-incubation, mutation	–	–	5000 µg/plate	FDA Drug Approval Package (1999a)
Gene mutation, Chinese hamster ovary cells, *Hprt* gene	–	–	200 µg/mL – S9; 500 µg/mL + S9	FDA Drug Approval Package (1999a)
Gene mutation, AS52 Chinese hamster cells, *Xprt* gene	–	–	200 µg/mL – S9; 200 µg/mL + S9	FDA Drug Approval Package (1999a)
Unscheduled DNA synthesis, male F344 rat primary hepatocytes	–	NT	100 µg/mL	FDA Drug Approval Package (1999a)
Chromosomal aberration, Chinese hamster lung cells	–	–	5 mM	FDA Drug Approval Package (1999a)
Chromosomal aberration, human peripheral blood lymphocytes	+	NT	100 µM	Alzoubi *et al.* (2012)
Sister chromatid exchange, human peripheral blood lymphocytes	+	NT	100 µM	Alzoubi *et al.* (2012)
8-Oxodeoxyguanosine, human peripheral blood lymphocytes	+	NT	100 µM	Alzoubi *et al.* (2012)
In vivo				
Micronucleus formation, CD-1 mice	–		5000 mg/kg bw per day, single intraperitoneal injection, up to 72 hours	FDA Drug Approval Package (1999a)
Comet assay, male Sprague-Dawley rat peripheral blood lymphocytes	+		10 mg/kg bw per day, by gavage, for 14 days	Bedir *et al.* (2008)
Comet assay, male Sprague-Dawley rat liver cells	+		10 mg/kg bw per day, by gavage, for 14 days	Bedir *et al.* (2008)

+, positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NR, not reported; NT, not tested

or micronucleus formation in CD-1 mice (Table 4.1; FDA Drug Approval Package, 1999a).

Male Sprague-Dawley rats treated with rosiglitazone by gavage showed a dose-dependent increase in the frequency of DNA damage in peripheral blood lymphoctyes and liver cells, as measured by comet assays (Table 4.2; Bedir *et al.*, 2006).

In-vitro assays for chromosomal aberration and unscheduled DNA synthesis, and in-vivo assays for micronucleus formation gave negative results with rosiglitazone (Table 4.2; FDA Drug Approval Package, 1999b).

Rosiglitazone gave negative results in the Growth Arrest and DNA Damage gene 45α-Green Fluorescent Protein (GADD45α-GFP) GreenScreen Human Cell genotoxicity assay in the presence or absence of metabolic activation (Table 4.2; Luzy *et al.*, 2012).

(b) Gene mutations

Pioglitazone and its metabolites M-I, M-IV, M-V, and M-VI were not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, or in *Escherichia coli* strain WP2 *uvrA*, in either the presence or absence of metabolic activation. Pioglitazone was not mutagenic at the *Hprt* gene of Chinese hamster ovary cells, or at the *Xprt* gene of AS52 Chinese hamster cells. Pioglitazone metabolites M-I and M-VI were mutagenic in mouse lymphoma L5178Y cells in the presence of metabolic activation; metabolites M-IV and M-V gave negative results, and the assay conducted with pioglitazone was considered inadequate (Table 4.1; Table 4.3; FDA Drug Approval Package, 1999a).

Rosiglitazone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538, or in *E. coli* strain WP2 *uvrA*, in either the presence or absence of an exogenous metabolic activation system. Rosiglitazone was mutagenic in mouse lymphoma L5178Y cells in the presence of metabolic activation (Table 4.2; FDA Drug Approval Package, 1999b).

4.4 Other mechanistic data

Pioglitazone selectively stimulates PPARγ, and to a lesser extent PPARα (Smith, 2001). Acidification of the urine, as a result of ammonium chloride administration in male rats, did not alter PPARα, PPARβ (PPARδ), or PPARγ mRNA or protein expression, PPARα- or PPARγ-regulated gene expression, total or phosphorylated epidermal growth factor receptor (Egfr) protein, *Egfr* or *Akt2* gene expression, or urothelial-cell proliferation. These results suggested that the suppression of bladder tumorigenesis by acidifying the urine of rats exposed to PPARγ agonists, such as pioglitazone, was not due to alterations in PPARα, PPARβ, or Egfr expression or PPAR signalling in the bladder epithelium of rats (Achanzar *et al.*, 2007; Sato *et al.*, 2011).

Roglitazone significantly increased the incidence of tumours of the bladder induced BBN in female F344 rats. The mechanism for the induction of these tumours was not known (Lubet *et al.*, 2008).

Strain H Swiss mice exposed to mainstream cigarette smoke since birth for 4 months, and subsequently exposed to pioglitazone, had lower levels of DNA damage in exfoliated bladder cells, as measured by comet assays, than mice exposed to mainstream cigarette smoke and fed control diet. However, the mice exposed to mainstream cigarette smoke and then pioglitazone had an increased incidence of kidney tubular epithelium hyperplasia, kidney adenoma, kidney lesions, and/or urinary tract lesions, compared with mice exposed to mainstream cigarette smoke only, or sham-treated mice. These data suggested that pioglitazone can act as a promoter of tumours of the kidney in mice. Mice exposed to mainstream cigarette smoke and pioglitazone had more acidic urine than sham-exposed mice (La Maestra *et al.*, 2013). [There was not a group that received pioglitazone only.]

In male C57BL/6J-Apc^{Min}/+ mice, a heterozygous mouse strain susceptible to intestinal

Table 4.2 Genetic and related effects of rosiglitazone

Test system	Results		Dose or concentration (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
In vitro				
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, TA1538, reverse mutation	–	–	5000 µg/plate	FDA Drug Approval Package (1999b)
Escherichia coli WP2 *uvrA*, mutation	–	–	5000 µg/plate	FDA Drug Approval Package (1999b)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	?	+	100 µg/mL – S9; 200 µg/mL + S9	FDA Drug Approval Package (1999b)
Chromosomal aberration, human lymphocytes	–	–	240 µg/mL	FDA Drug Approval Package (1999b)
GADD45α-GFP GreenScreen Human Cell, genotoxicity assay	–	–	NR	Luzy *et al.* (2012)
In vivo				
Micronucleus formation, CD-1 mice	–		700 mg/kg bw, single intraperitoneal injection, up to 72 hours	FDA Drug Approval Package (1999b)
Unscheduled DNA synthesis, male Sprague-Dawley rat primary hepatocytes	–		2000 µg/mL	FDA Drug Approval Package (1999b)
Comet assay, male Sprague-Dawley rat peripheral blood lymphocytes	+		1.0 mg/kg bw per day, for 14 days	Bedir *et al.* (2006)
Comet assay, male Sprague-Dawley rat liver cells	+		0.5 mg/kg bw per day, for 14 days	Bedir *et al.* (2006)

+, positive; –, negative; ?, inconclusive; GADD45, growth arrest and DNA damage gene; GFP, green fluorescent protein; HID, highest ineffective dose; LED, lowest effective dose; S9, supernatant fraction of liver homogenate × 9000 *g*

Table 4.3 Genetic and related effects of metabolites of pioglitazone

Test system	Results		Dose or concentration (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
M-I				
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, reverse mutation	–	–	NR	FDA Drug Approval Package (1999a)
Escherichia coli WP2 *uvrA* mutations	–	–	NR	FDA Drug Approval Package (1999a)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	?	+	NR	FDA Drug Approval Package (1999a)
M-IV				
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, reverse mutation	–	–	NR	FDA Drug Approval Package (1999a)
Escherichia coli WP2 *uvrA*, mutation	–	–	NR	FDA Drug Approval Package (1999a)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	–	–	NR	FDA Drug Approval Package (1999a)
M-V				
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, reverse mutation	–	–	2000 µg/plate	FDA Drug Approval Package (1999a)
Escherichia coli WP2 *uvrA*, mutation	–	–	2000 µg/plate	FDA Drug Approval Package (1999a)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	–	–	NR	FDA Drug Approval Package (1999a)
M-VI				
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, reverse mutation	–	–	2000 µg/plate	FDA Drug Approval Package (1999a)
Escherichia coli WP2 *uvrA*, mutation	–	–	2000 µg/plate	FDA Drug Approval Package (1999a)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	?	+	NR	FDA Drug Approval Package (1999a)

+, positive; –, negative;?, inconclusive; LED, lowest effective dose; HID, highest ineffective dose; NR, not reported; NT, not tested

neoplasms, PPARγ agonists (troglitazone, 150 mg/kg bw per day; rosiglitazone, 20 mg/kg bw per day; or NID525, 150 mg/kg bw per day) significantly increased the multiplicity of neoplasms (primarily adenomas) in the large intestine. The mechanism was not explored (Pino *et al.*, 2004).

The administration of rosiglitazone (20 mg/kg bw per day for 8 weeks) to male C57BL/6J-Apc^{Min}/+ mice significantly increased the multiplicity of tumours of the large intestine, and increased expression of PPARγ in the large intestine, and increased expression of β-catenin. A similar increase in tumour multiplicity, and β-catenin expression, was observed with troglitazone, another PPARγ agonist (150 mg/kg bw per day) (Lefebvre *et al.*, 1998).

Male Sprague-Dawley rats were treated with rosiglitazone at 8 mg/kg bw per day by gavage for up to 16 days. Treatment with rosiglitazone had modest effects on levels of the transcription factor Egr-1 in the bladder urothelium. Likewise, there was minimal effect with fenofibrate, a PPARα agonist. In contrast, ragaglitazar, a dual-action agonist of PPARα and γ, and a rat bladder carcinogen, caused a substantial increase in the level of Egr-1. These data suggested that the co-activation of both PPARα and γ is required for the increased expression of Egr-1 (Egerod *et al.*, 2005).

The relationship between nuclear EGR-1 protein levels and stage of human bladder tumour was investigated using tissue microarrays. The extent of nuclear EGR-1 immunostaining was associated with a higher risk of progression to stage T2–T4 cancer of the bladder (Egerod *et al.*, 2009a).

Male Sprague-Dawley rats given rosiglitazone plus fenofibrate expressed Egr-1 protein on both the dorsal and ventral regions of the urinary bladder (Egerod *et al.*, 2009b).

In male Sprague-Dawley rats, rosiglitazone (given by gavage at 8 or 20 mg/kg bw per day for 7 days) had minimal effects on the levels of the transcription factor Egr-1 in the bladder urothelium, heart, or liver. In contrast, fenofibrate, a PPARα agonist, increased the levels of Egr-1 in the liver and heart (Egerod *et al.*, 2010).

4.5 Susceptibility

No data were available to the Working Group.

4.6 Mechanistic considerations

Pioglitazone, a PPARγ agonist, has been implicated in cancer of the urinary bladder in rats, and rosiglitazone, which is also a PPARγ agonist, promotes cancer of the urinary bladder in rats, and possibly tumours of the kidney in mice. Four mechanisms of carcinogenicity have been considered in rats treated with PPARγ agonists: (i) genotoxicity of metabolites formed from the agonists; (ii) cytotoxicity of the agonists or their metabolites in the urothelium, causing cancer due to a proliferation-driven chronic "wound-healing response"; (iii) formation of urinary solids (urolithiasis), due to urinary changes induced by the agonists or their metabolites, which results in chronic irritation of the urothelium; and (iv) a receptor-mediated effect of the agonists, with carcinogenesis caused by activation of PPARγ transcription factors in the urothelium. These mechanisms may not be mutually exclusive.

4.6.1 Genotoxicity

When assessed using a standard battery of assays for genotoxicity, pioglitazone and rosiglitazone have typically given negative results; nonetheless, there are exceptions. Certain metabolites of pioglitazone and rosiglitazone have given positive results in the assay for gene mutation in mouse lymphoma cells and, more recently, pioglitazone has been reported to increase the frequency of chromosomal aberration, sister chromatid exchange, and formation of 8-oxodeoxyguanosine in human peripheral blood lymphocytes, and both pioglitazone and

rosiglitazone gave positive results in comet assays in liver cells and peripheral blood lymphocytes from rats. Thus, while perhaps not the primary mechanism, the contribution of genotoxicity to the carcinogenic activity of pioglitazone or rosiglitazone in the urothelium of rats cannot presently be excluded.

4.6.2 Agonist cytotoxicity

Pioglitazone and rosiglitazone are lipophilic drugs that are excreted to a limited extent in the urine of rats. Since urothelial carcinogenesis is typically considered to be mediated by direct urinary exposure to the drugs or their metabolites, rather than through systemic distribution in the blood, a mechanism involving cytotoxicity as a result of direct exposure to the agonists or their metabolites appeared unlikely.

4.6.3 Urolithiasis

The induction of tumours of the urinary bladder in rodents as a consequence of the formation of urinary solids has been documented for several compounds, including carbonic anhydrase inhibitors, HIV protease inhibitors, and sulfonamides. PPARγ agonists are known to cause fluid accumulation, oedema, cardiac enlargement, and heart failure, effects that can lead to significant changes in urine composition. The administration of pioglitazone to rats results in the formation of urinary solids. This occurs to a greater extent in rats than in mice, and in male rats than in female rats; these trends correspond to the greater susceptibility of rats compared with mice, and of male rats compared with female rats, to the induction of urothelial tumours upon the administration of pioglitazone. Further support for a urolithiasis-based mechanism comes from the observation that tumours arising from pioglitazone occur predominantly on the ventral surface of the bladder, the region where urinary solids would settle in rat bladders, and that acidifying the urine through the administration of ammonium chloride in the diet decreases the amount of urinary solids and the extent of tumorigenesis in the urinary bladder. Urinary acidification did not alter the expression of PPARα, PPARγ, or epidermal growth factor receptor in the rat bladder urothelium, which suggests that a receptor-mediated mechanism is not involved in the tumorigenic response. A similar urolithiasis-based mechanism has been proposed for muraglitazar, a dual-action PPARα and PPARγ agonist (Achanzar *et al.*, 2007).

4.6.4 Receptor-mediated effect

Although a urolithiasis-based mechanism appears plausible for induction of tumours of the bladder in rats treated with pioglitazone, such a mechanism cannot explain the tumours of the bladder observed in rats given rosiglitazone after initiation with BBN (because urinary solids were not observed; Lubet *et al.*, 2008), the promotion of intestinal neoplasms in susceptible mouse strains given pioglitazone, rosiglitazone, or other PPARγ agonists such as troglitazone or NID525 (Lefebvre *et al.*, 1998; Pino *et al.*, 2004), the induction of tumours of the kidney in mice exposed to mainstream cigarette smoke and then pioglitazone (La Maestra *et al.*, 2013), or the induction of tumours of the urinary bladder in rats treated with naveglitazar, a γ-dominant PPARα and PPARγ agonist (Long *et al.*, 2008).

The promoting activity of rosiglitazone in the rat bladder has been attributed to an increased expression of Egr-1, ribosomal S6 protein phosphorylation, and c-Jun transcription factor phosphorylation, which can lead to hypertrophy, hyperplasia, and subsequently urothelial-cancer progression. While these responses appear to be greater with the dual-acting PPARα and PPARγ agonist ragaglitazar, a modest response does occur with rosiglitazone (Egerod *et al.*, 2005, 2009b, 2010). Furthermore, pioglitazone also shows modest PPARα agonist activity that may

contribute to the mechanism of induction of tumours of the bladder (Sakamoto *et al.*, 2000). The induction of intestinal neoplasms in susceptible mouse strains treated with PPARγ agonists may be a consequence of increased expression of β-catenin protein, which activates transcription factors associated with colon tumorigenesis (Lefebvre *et al.*, 1998; Pino *et al.*, 2004).

5. Summary of Data Reported

5.1 Exposure data

Thiazolidinediones are a unique class of synthetic oral drug that exert direct effects on the mechanisms of insulin resistance, and result in improved insulin action and reduced hyperglycaemia. Two thiazolidinediones, rosiglitazone and pioglitazone, initially showed great promise as receptor-mediated oral therapy for type 2 diabetes mellitus.

Pioglitazone hydrochloride is approved in some countries for the treatment of type 2 diabetes mellitus. It is available both as a single agent and in combination with other oral medications for diabetes. Until 2009, pioglitazone was among the most widely used oral drugs for the treatment of type 2 diabetes mellitus. Use of pioglitazone hydrochloride has declined following studies suggesting links to cancer of the bladder, heart failure, and bone fractures. While regulatory agencies in France and Germany banned pioglitazone in 2011, global sales remained substantial at US$ 3.3 billion in 2012.

Rosiglitazone maleate is approved in some countries for the treatment of type 2 diabetes mellitus. It is available both as a single agent and in combination with other oral medications for diabetes. Until 2007, rosiglitazone was among the most widely used oral drugs for treatment of type 2 diabetes. Use of rosiglitazone has declined over the last few years following studies suggesting links to heart attack, heart failure, and bone fractures. While this agent is banned in Europe and restricted in the USA, substantial use continues in some countries, including China (global sales of US$ 41 million).

5.2 Human carcinogenicity data

5.2.1 Cancer of the bladder

The risk of cancer of the bladder associated with the use of pioglitazone and rosiglitazone was assessed in several studies, some with overlapping populations, from Europe, North America and Asia. Some subjects may have received both drugs (in sequence) at some time during treatment for diabetes.

Information for pioglitazone was evaluated in one large randomized controlled trial, four cohort studies, and three case–control studies, some with overlapping study populations. Ever-use of pioglitazone was associated with an increased risk of cancer of the bladder in all studies except one case–control study from Taiwan, China, across all study designs and geographical regions, with risk ratios that ranged from 1.2 in the observational studies to a nearly threefold statistically significant increase in the randomized controlled trial. In this trial, the Working Group noted the excess occurrence of these cancers (14 in the treatment group versus 5 in the placebo group) within a short follow-up time (11 of the bladder cancers occurred within 1 year of randomization), and the large number of patients enrolled, and double-blind experimental design resulting in the balance of confounding factors at baseline.

Dose–response relationships were assessed in five studies, three of which were high-quality population-based studies (which adjusted for smoking or chronic obstructive pulmonary disease in the absence of data on smoking) conducted within the large health insurance databases from the USA, United Kingdom, and Taiwan, China. Greater risks were reported with

higher dosage or longer use in the case–control study in the United Kingdom, and in the cohort study in the USA. Observation of a dose–response relationship helped to mitigate concerns about potential confounding by most risk factors; nevertheless, the magnitude of the excess risks observed was modest and some estimates were imprecise.

Among ever-users of rosiglitazone, with data available from two case–control studies and two cohort studies, risk ratios for cancer of the bladder were close to the null in all except one study from the United Kingdom.

The Working Group was unable to consistently rule out confounding, selection bias, detection bias, and bias related to indication or severity of disease in the populations studied as potential explanations for positive associations with pioglitazone. Most of the studies were based on medical databases, which allowed for adjustment for potential confounding by medical factors, but did not permit direct control for cigarette smoking and other risk factors. However, for pioglitazone, increased risks were consistently seen in the studies that adjusted for smoking (one cohort study from the USA, two studies from the United Kingdom, and one study from Taiwan, China that adjusted for chronic obstructive pulmonary disease), as well as those that did not. The potential for confounding by smoking is also mitigated by the fact that, in the same studies, there was no consistent evidence of cancer of the lung and elevated risks were not found among rosigilitazone users in the same studies. Furthermore, an excess of cancer of the bladder among pioglitozone users, and not cancer of the lung, was observed in the trial that randomized for potential confounders including smoking.

5.2.2 Other cancer sites

The risk of cancers at several other sites, including the liver, kidney, colorectum, lung, prostate, and breast, among patients using pioglitazone and rosiglitazone has also been evaluated in studies using cohort and case–control designs. No consistent pattern of increased risk was reported for any other specific cancer site, or for all cancers combined for either drug.

5.3 Animal carcinogenicity data

5.3.1 Pioglitazone

In a 2-year study in mice treated by gavage, pioglitazone produced increases in the incidence of benign pheochromocytoma of the adrenal gland in males and increases in the incidence of leiomyosarcoma of the uterine cervix in females. Administration of pioglitazone in the feed caused a significant increase in the incidence of large intestine adenoma in one study in genetically engineered male mice sensitive to intestinal carcinogenesis. In a study in male and female neonatal mice, pioglitazone in the feed promoted mainstream cigarette smoke-induced kidney adenoma in females.

In a first 2-year study in rats treated by gavage, pioglitazone induced a significant positive trend in the incidence of transitional cell carcinoma of the urinary bladder and a significant positive trend in the incidence of subcutaneous fibrosarcoma of the subcutis in males. It also caused a significant positive trend in the incidence of subcutaneous lipoma in females. In a second 2-year study in male rats treated by gavage, pioglitazone caused a significant increase in the incidence of transitional cell papilloma of the urinary bladder.

5.3.2 Rosiglitazone

Administration of diets containing rosiglitazone caused a significant increase in the incidence of large intestine adenoma in one study in genetically engineered male mice sensitive to intestinal carcinogenesis. In a 2-year study in male and female mice treated by gavage, a

significant increase in the incidence of liver haemangiosarcoma was observed in males, but this was not treatment-related.

In a 2-year study in rats treated by gavage, rosiglitazone induced significant increases in the incidence of subcutaneous lipoma in males and females.

In two studies in female mice, coexposure to *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine plus rosiglitazone significantly increased the incidences of *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine-induced carcinoma of the urinary bladder.

5.4 Mechanistic and other relevant data

Pioglitazone and rosiglitazone undergo extensive phase I metabolism. Although pioglitazone and rosiglitazone have typically given negative results when assessed in standard batteries of genotoxicity assays, exceptions have been noted. Certain pioglitazone metabolites and rosiglitazone have given positive results in assays in the mouse lymphoma cells; pioglitazone increased the levels of chromosomal aberration, sister chromatid exchange, and 8-oxodeoxyguanosine in human peripheral blood lymphocytes; and both pioglitazone and rosiglitazone gave positive results in comet assays in liver cells and peripheral blood lymphocytes from rats. Four mechanisms have been considered for the induction of bladder tumours in rats administered pioglitazone (genotoxicity of pioglitazone metabolites; cytotoxicity, urolithiasis, and PPARγ and α receptor-mediated effects). While not mutually exclusive, data supporting urolithiasis and receptor-mediated mechanisms appear to be the strongest. Likewise, receptor-mediated effects may play a role in the tumorigenic response observed in other experimental models (e.g. intestinal neoplasia in mice).

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of pioglitazone. A positive association has been observed between pioglitazone and cancer of the bladder.

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

6.2 Cancer in experimental animals

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

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

6.3 Overall evaluation

Pioglitazone is *probably carcinogenic to humans (Group 2A).*

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

References

Achanzar WE, Moyer CF, Marthaler LT, Gullo R, Chen SJ, French MH *et al.* (2007). Urine acidification has no effect on peroxisome proliferator-activated receptor (PPAR) signalling or epidermal growth factor (EGF) expression in rat urinary bladder urothelium. *Toxicol Appl Pharmacol*, 223(3):246–56. doi:10.1016/j.taap.2007.06.015 PMID:17663016

AFFSAPS (2013). Use of medications containing pioglitazone (Actos, Competact) suspended June 9th 2011. Press release. Available from: http://ansm.sante.fr/var/ansm_site/storage/original/application/4e293bcd0814c025b94d46d7502a0958.pdf. Accessed 10 July 2014.

Al-Ghamdi AF, Hefnawy MM (2012). Electrochemical determination of rosiglitazone by square-wave adsorptive stripping voltammetry method. *Arab J Chem*, 5:383–389. doi:10.1016/j.arabjc.2011.07.011

Alzoubi K, Khabour O, Hussain N, Al-Azzam S, Mhaidat N (2012). Evaluation of vitamin B12 effects on DNA damage induced by pioglitazone. *Mutat Res*,

748(1–2):48–51. doi:10.1016/j.mrgentox.2012.06.009 PMID:22790087

Avandia (2010). Rosiglitazone maleate. Tablet, film coated. Anonymous. Total physicians care Inc. 1–27. Available from: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=ec682aec-e98f-41a1-9d21-eb7580ea3a8a. Accessed 1 October 2014.

Azoulay L, Yin H, Filion KB, Assayag J, Majdan A, Pollak MN *et al.* (2012). The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. *BMJ*, 344(may30 3):e3645 doi:10.1136/bmj.e3645 PMID:22653981

BDdrugs (2013). Online drug index of Bangladesh. Available from: http://www.bddrugs.com/

Bedir A, Aliyazicioglu Y, Bilgici B, Yurdakul Z, Uysal M, Suvaci DE *et al.* (2008). Assessment of genotoxicity in rats treated with the antidiabetic agent, pioglitazone. *Environ Mol Mutagen*, 49(3):185–91. doi:10.1002/em.20365 PMID:18213655

Bedir A, Aliyazicioglu Y, Kahraman H, Yurdakul Z, Uysal M, Suvaci DE *et al.* (2006). Genotoxicity in rats treated with the antidiabetic agent, rosiglitazone. *Environ Mol Mutagen*, 47(9):718–24. doi:10.1002/em.20261 PMID:17078099

Bosetti C, Rosato V, Buniato D, Zambon A, La Vecchia C, Corrao G (2013). Cancer risk for patients using thiazolidinediones for type 2 diabetes: a meta-analysis. *Oncologist*, 18(2):148–56. doi:10.1634/theoncologist.2012-0302 PMID:23345544

BrFAM (2011). Important safety information on the use of medicinal products containing pioglitazone. Press release: Available from: http://www.bfarm.de/SharedDocs/Risikoinformationen/Pharmakovigilanz/EN/RHB/2011/rhb-pioglitazon.pdf?__blob=publicationFile&v=3. Accessed 8 July 2014.

Budde K, Neumayer HH, Fritsche L, Sulowicz W, Stompôr T, Eckland D (2003). The pharmacokinetics of pioglitazone in patients with impaired renal function. *Br J Clin Pharmacol*, 55(4):368–74. doi:10.1046/j.1365-2125.2003.01785.x PMID:12680885

Bulliman S, Allen A, Harris AM *et al.* (1995). The influence of food on the pharmacokinetics of BRL 49653 in healthy male volunteers. *Br J Clin Pharmacol*, 40:517

Calixto LA, de Oliveira AR, Jabor VA, Bonato PS (2011). In vitro characterization of rosiglitazone metabolites and determination of the kinetic parameters employing rat liver microsomal fraction. *Eur J Drug Metab Pharmacokinet*, 36(3):159–66. doi:10.1007/s13318-011-0039-8 PMID:21499911

Cantello BC, Cawthorne MA, Cottam GP *et al.* (1994). [[omega-(Heterocyclylamino)alkoxy]benzyl]-2,4-thiazolidinediones as potent antihyperglycemic agents. *J Med Chem*, 37:3977–3985. doi:10.1021/jm00049a017 PMID:7966158

Cayman SDS (2013). Safety data sheet. Cayman Chemical Company.

Chan LY, Yeung JH, Lau TK (2005). Placental transfer of rosiglitazone in the first trimester of human pregnancy. *Fertil Steril*, 83(4):955–8. doi:10.1016/j.fertnstert.2004.10.045 PMID:15820806

Chang CH, Lin JW, Wu LC, Lai MS, Chuang LM, Chan KA (2012). Association of thiazolidinediones with liver cancer and colorectal cancer in type 2 diabetes mellitus. *Hepatology*, 55(5):1462–72. doi:10.1002/hep.25509 PMID:22135104

Chapelsky MC, Thompson-Culkin K, Miller AK, Sack M, Blum R, Freed MI (2003). Pharmacokinetics of rosiglitazone in patients with varying degrees of renal insufficiency. *J Clin Pharmacol*, 43(3):252–9. doi:10.1177/0091270002250602 PMID:12638393

ChemicalBook (2013). Available from: http://www.chemicalbook.com/ProductMSDSDetailCB9257580_EN.htm. Accessed 08 July 2014.

ChemSpider (2013). ChemSpider: The free chemical database. Royal Society of Chemistry. Available from: http://www.chemspider.com. Accessed 10 July 2014.

Christensen ML, Meibohm B, Capparelli EV, Velasquez-Mieyer P, Burghen GA, Tamborlane WV (2005). Single- and multiple-dose pharmacokinetics of pioglitazone in adolescents with type 2 diabetes. *J Clin Pharmacol*, 45(10):1137–44. doi:10.1177/0091270005279578 PMID:16172178

Chu KM, Hu OY, Pao LH, Hsiong CH (2007). Pharmacokinetics of oral rosiglitazone in Taiwanese and post hoc comparisons with Caucasian, Japanese, Korean, and mainland Chinese subjects. *J Pharm Pharm Sci*, 10(4):411–9. PMID:18261363

Colmers IN, Bowker SL, Johnson JA (2012a). Thiazolidinedione use and cancer incidence in type 2 diabetes: a systematic review and meta-analysis. *Diabetes Metab*, 38(6):475–84. doi:10.1016/j.diabet.2012.06.003 PMID:23041441

Colmers IN, Bowker SL, Majumdar SR, Johnson JA (2012b). Use of thiazolidinediones and the risk of bladder cancer among people with type 2 diabetes: a meta-analysis. *CMAJ*, 184(12):E675–83. doi:10.1503/cmaj.112102 PMID:22761478

Cox PJ, Ryan DA, Hollis FJ, Harris AM, Miller AK, Vousden M *et al.* (2000). Absorption, disposition, and metabolism of rosiglitazone, a potent thiazolidinedione insulin sensitizer, in humans. *Drug Metab Dispos*, 28(7):772–80. PMID:10859151

DHHS/FDA (2007). Electronic Orange Book-Approved Drug Products with Therapeutic Equivalence Evaluations. Available from: http://www.fda.gov/cder/ob/

Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK *et al.*; PROactive investigators (2005). Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive

Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet*, 366(9493):1279–89. doi:10.1016/S0140-6736(05)67528-9 PMID:16214598

Drugbank (2013). Drug Bank: Open Data Drug & Drug Target Database. Available from: http://www.drugbank.ca/

Egerod FL, Bartels A, Fristrup N, Borre M, Ørntoft TF, Oleksiewicz MB *et al.* (2009a). High frequency of tumor cells with nuclear Egr-1 protein expression in human bladder cancer is associated with disease progression. *BMC Cancer*, 9(1):385 doi:10.1186/1471-2407-9-385 PMID:19878561

Egerod FL, Brünner N, Svendsen JE, Oleksiewicz MB (2010). PPARalpha and PPARgamma are co-expressed, functional and show positive interactions in the rat urinary bladder urothelium. *J Appl Toxicol*, 30(2):151–62. PMID:19757489

Egerod FL, Nielsen HS, Iversen L, Thorup I, Storgaard T, Oleksiewicz MB (2005). Biomarkers for early effects of carcinogenic dual-acting PPAR agonists in rat urinary bladder urothelium in vivo. *Biomarkers*, 10(4):295–309. doi:10.1080/13547500500218682 PMID:16240504

Egerod FL, Svendsen JE, Hinley J, Southgate J, Bartels A, Brünner N *et al.* (2009b). PPAR α and PPAR γ coactivation rapidly induces Egr-1 in the nuclei of the dorsal and ventral urinary bladder and kidney pelvis urothelium of rats. *Toxicol Pathol*, 37(7):947–58. doi:10.1177/0192623309351723 PMID:20008548

EMA; European Medicines Agency (2010). European Medicines Agency recommends suspension of Avandia, Avandamet and Avaglim. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2010/09/news_detail_001119.jsp&mid=WC0b01ac058004d5c1

EMA CHMP (2012). Assessment report Pioglitazone Accord. PM: EPA (2011). Estimation Program Interface (EPI). United States, Environmental Protection Agency.

EMEA; European Medecines Agency (2005). Rosiglitazone. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000522/WC500028918.pdf. Accessed 20 June 2014.

EMEA; European Medicines Agency (2012). European Public Assessment Report. Pioglitazone. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002277/WC500126045.pdf. Accessed 10 July 2014.

Enzo PDS (2012). Product data sheet: Enzo life sciences.

FDA (1999a). Review and evaluation of pharmacology and toxicology data: Pioglitazone. Pharmacology Reviews Application Number: 021073. Center for Drug Evaluation and Research. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/99/021073A_Actos.cfm. Accessed 1 July 2014.

FDA (1999b). Review and evaluation of pharmacology and toxicology data: Rosiglitazone. Pharmacology Reviews Application Number: 021071. Center for Drug Evaluation and Research. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/99/21071_Avandia.cfm. Accessed 1 July 2014.

FDA (2013a). Actos Product Information. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021073s043s044lbl.pdf. Accessed 10 July 2014.

FDA (2013b). Actos Risk Evaluation and Mitigation Strategy. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2012/021073s045,021842s016,022024s009,021925s012ltr.pdf. Accessed 10 July 2014.

FDA (2013c). Joint Meeting of the Endocrinologic and Metabolic Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee Meeting Announcement Committee. Available from: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/ucm331504.htm. Accessed 11 June 2013.

FDA Drug Approval Package (1999a). Actos (Pioglitazone Hydrochloride) Tablets, Application No. 021073.

FDA Drug Approval Package (1999b). Avandia (Rosiglitazone Maleate) Tablets, Application No. 21-071.

Ferrara A, Lewis JD, Quesenberry CP Jr, Peng T, Strom BL, Van Den Eeden SK *et al.* (2011). Cohort study of pioglitazone and cancer incidence in patients with diabetes. *Diabetes Care*, 34(4):923–9. doi:10.2337/dc10-1067 PMID:21447664

Ferwana M, Firwana B, Hasan R, Al-Mallah MH, Kim S, Montori VM *et al.* (2013). Pioglitazone and risk of bladder cancer: a meta-analysis of controlled studies. *Diabet Med*, 30(9):1026–32. doi:10.1111/dme.12144 PMID:23350856

Freed MI, Allen A, Jorkasky DK, DiCicco RA (1999). Systemic exposure to rosiglitazone is unaltered by food. *Eur J Clin Pharmacol*, 55(1):53–6. doi:10.1007/s002280050592 PMID:10206085

Fujimoto K, Hamamoto Y, Honjo S, Kawasaki Y, Mori K, Tatsuoka H *et al.* (2013). Possible link of pioglitazone with bladder cancer in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract*, 99(2):e21–3. doi:10.1016/j.diabres.2012.11.013 PMID:23228390

Fujita Y, Yamada Y, Kusama M, Yamauchi T, Kamon J, Kadowaki T *et al.* (2003). Sex differences in the pharmacokinetics of pioglitazone in rats. *Comp Biochem Physiol C Toxicol Pharmacol*, 136(1):85–94. doi:10.1016/S1532-0456(03)00194-7 PMID:14522601

Giaginis C, Theocharis S, Tsantili-Kakoulidou A (2007). Investigation of the lipophilic behaviour of some thiazolidinediones. Relationships with PPAR-γ activity. *J Chromatogr B Analyt Technol Biomed Life*

Sci, 857:181–187. doi:10.1016/j.jchromb.2007.07.013 PMID:17660053

Gowramma B, Babu B, Meyyanathan SN *et al.* (2012a). New chiral normal phase HPLC method for determination of pioglitazone enantiomers on its marketed formulation. *J Pharm Res*, 5:72

Gowramma B, Babu B, Meyyanathan SN *et al.* (2012b). Chiral HPLC Method for the Enantioselective Analysis of Rosiglitazone Maleate in Formulations. *J Pharm Res*, 5(1):79–81.

GSK (2012). AVANDIA product monograph: GlaxoSmithKline Inc.

Hassan MM, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK *et al.* (2010). Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer*, 116(8):1938–46. doi:10.1002/cncr.24982 PMID:20166205

Hillaire-Buys D, Faillie JL, Montastruc JL (2011). Pioglitazone and bladder cancer. *Lancet*, 378(9802):1543–4, author reply 1544–5. doi:10.1016/S0140-6736(11)61662-0 PMID:22035550

IMS Health (2012a). Multinational Integrated Data Analysis (MIDAS). Plymouth Meeting, Pennsylvania: IMS Health.

IMS Health (2012b). National Disease and Therapeutic Index (NDTI) 2004–2012. Plymouth Meeting, Pennsylvania: IMS Health.

Jaakkola T, Laitila J, Neuvonen PJ, Backman JT (2006). Pioglitazone is metabolised by CYP2C8 and CYP3A4 in vitro: potential for interactions with CYP2C8 inhibitors. *Basic Clin Pharmacol Toxicol*, 99(1):44–51. doi:10.1111/j.1742-7843.2006.pto_437.x PMID:16867170

Jain D, Jain S, Jain D, Amin M (2008). Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. *J Chromatogr Sci*, 46(6):501–4. doi:10.1093/chromsci/46.6.501 PMID:18647470

Jedlicka A, Klimes J, Grafnetterová T (2004). Reversed-phase HPLC methods for purity test and assay of pioglitazone hydrochloride in tablets. *Pharmazie*, 59(3):178–82. PMID:15074587

Julie NL, Julie IM, Kende AI, Wilson GL (2008). Mitochondrial dysfunction and delayed hepatotoxicity: another lesson from troglitazone. *Diabetologia*, 51(11):2108–16. doi:10.1007/s00125-008-1133-6 PMID:18726085

Kirchheiner J, Roots I, Goldammer M, Rosenkranz B, Brockmöller J (2005). Effect of genetic polymorphisms in cytochrome p450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: clinical relevance. *Clin Pharmacokinet*, 44(12):1209–25. doi:10.2165/00003088-200544120-00002 PMID:16372821

Krieter PA, Colletti AE, Doss GA, Miller RR (1994). Disposition and metabolism of the hypoglycemic agent pioglitazone in rats. *Drug Metab Dispos*, 22(4):625–30. PMID:7956739

Krishna SR, Rao B *et al.* (2008). Isolation, synthesis and characterization of rosiglitazone maleate impurities. *E-Journal of Chemistry*, 5:562–566. doi:10.1155/2008/308724

Kumar YR, Reddy AR, Eswaraiah S, Mukkanti K, Reddy MS, Suryanarayana MV (2004). Structural characterization of impurities in pioglitazone. *Pharmazie*, 59(11):836–9. PMID:15587582

La Maestra S, Micale RT, De Flora S, D'Agostini F, Ganchev G, Iltcheva M *et al.* (2013). DNA damage in exfoliated cells and histopathological alterations in the urinary tract of mice exposed to cigarette smoke and treated with chemopreventive agents. *Carcinogenesis*, 34(1):183–9. doi:10.1093/carcin/bgs314 PMID:23042096

Langchem (2013). Langchem material safety data sheet. China Langchem Inc.

Lefebvre A-M, Chen I, Desreumaux P, Najib J, Fruchart JC, Geboes K *et al.* (1998). Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-APCMin/+ mice. *Nat Med*, 4(9):1053–7. doi:10.1038/2036 PMID:9734399

Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP Jr *et al.* (2011). Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care*, 34(4):916–22. doi:10.2337/dc10-1068 PMID:21447663

Lewis JD, Strom BL, Bilker W, Nessel L, Vaughn DJ, Ferrara A, *et al.* (2012). Pioglitazone HCl (ACTOS) Clinical Study No. 01–03-TL-OPI-524. Cohort Study of Pioglitazone and Bladder Cancer in Patients with Diabetes Fourth Interim Analysis (8-Year). Report with Data from 1 January 1997 to 31 December 2010. Available from: http://general.takedapharm.com/Trial-Disclosure/01-03-TL-OPI-524-8-year-Interim-Report.pdf. Accessed 2 March 2012.

Lin ZJ, Ji W, Desai-Krieger D, Shum L (2003). Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-MS/MS. *J Pharm Biomed Anal*, 33(1):101–8. doi:10.1016/S0731-7085(03)00344-3 PMID:12946536

Loke YK, Singh S, Furberg CD (2009). Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. *CMAJ*, 180(1):32–9. doi:10.1503/cmaj.080486 PMID:19073651

Long GG, Reynolds VL, Lopez-Martinez A, Ryan TE, White SL, Eldridge SR (2008). Urothelial carcinogenesis in the urinary bladder of rats treated with naveglitazar, a γ-dominant PPAR α/γ agonist: lack of evidence for urolithiasis as an inciting event. *Toxicol Pathol*, 36(2):218–31. doi:10.1177/0192623307311757 PMID:18474944

Lu YC, Li YC (2013). How doctors practice evidence-based medicine. *J Eval Clin Pract*, 19:44–49. doi:10.1111/j.1365-2753.2011.01765.x PMID:21883721

Lubet RA, Fischer SM, Steele VE, Juliana MM, Desmond R, Grubbs CJ (2008). Rosiglitazone, a PPAR gamma agonist: potent promoter of hydroxybutyl(butyl) nitrosamine-induced urinary bladder cancers. *Int J Cancer*, 123(10):2254–9. doi:10.1002/ijc.23765 PMID:18712722

Luzy A-P, Orsini N, Linget J-M, Bouvier G (2012). Evaluation of the GADD45α-GFP GreenScreen HC assay for rapid and reliable in vitro early genotoxicity screening. *J Appl Toxicol*, n/a doi:10.1002/jat.2793 PMID:22806210

Madivada LR, Anumala RR, Gilla G, Alla S, Charagondla K, Kagga M *et al.* (2009). An improved process for pioglitazone and its pharmaceutically acceptable salt. *Org Process Res Dev*, 13(6):1190–4. doi:10.1021/op900131m

Martín J, Buchberger W, Santos JL, Alonso E, Aparicio I (2012). High-performance liquid chromatography quadrupole time-of-flight mass spectrometry method for the analysis of antidiabetic drugs in aqueous environmental samples. *J Chromatogr B Analyt Technol Biomed Life Sci*, 895–896:94–101. doi:10.1016/j.jchromb.2012.03.023 PMID:22483984

Milne GWA (2000). Drugs: Synonyms & Properties. Ashgate Publ Co.

Monami M, Lamanna C, Marchionni N, Mannucci E (2008). Rosiglitazone and risk of cancer: a meta-analysis of randomized clinical trials. *Diabetes Care*, 31(7):1455–60. doi:10.2337/dc07-2308 PMID:18375416

Mostafa GA, Al-Majed A (2008). Characteristics of new composite- and classical potentiometric sensors for the determination of pioglitazone in some pharmaceutical formulations. *J Pharm Biomed Anal*, 48(1):57–61. doi:10.1016/j.jpba.2008.04.029 PMID:18555633

National Library of Medicine (2010). Avandia (rosiglitazone maleate) tablet, film coated. Bethesda (MD): National Institutes of Health, Health & Human Services. Available from: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=ef14122b-7fff-45fa-b13b-0ea9e48bd57d. Accessed 20 June 2014.

Neumann A, Weill A, Ricordeau P, Fagot JP, Alla F, Allemand H (2012). Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. *Diabetologia*, 55(7):1953–62. doi:10.1007/s00125-012-2538-9 PMID:22460763

Nissen SE, Wolski K (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med*, 356:2457–2471. doi:10.1056/NEJMoa072761 PMID:17517853

NLM (2013). Hazardous Substances Data Bank: National library of medicine.

Nowak SN, Edwards DJ, Clarke A, Anderson GD, Jaber LA (2002). Pioglitazone: effect on CYP3A4 activity. *J Clin Pharmacol*, 42(12):1299–302. doi:10.1177/009127000204201209 PMID:12463723

O'Maille G, Pai SM, Tao X, Douglas GT Jr, Jenkins RG (2008). An improved LC-ESI-MS-MS method for simultaneous quantitation of rosiglitazone and N-desmethyl rosiglitazone in human plasma. *J Pharm Biomed Anal*, 48(3):934–9. doi:10.1016/j.jpba.2008.08.001 PMID:18818043

O'Neil (2006). Pioglithazone. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station (NJ): Merck & Co., Inc.

Palem CR, Gannu R, Yamsani SK, Yamsani VV, Yamsani MR (2011). Development of a high-performance liquid chromatography method for simultaneous determination of pioglitazone and felodipine in pig serum: application to pharmacokinetic study. *Biomed Chromatogr*, 25(8):952–8. doi:10.1002/bmc.1553 PMID:21058416

Pang W, Yang H, Wu Z *et al.* (2009). LC-MS-MS in MRM mode for detection and structural identification of synthetic hypoglycemic drugs added illegally to 'natural' anti-diabetic herbal products. *Chromatographia*, 70:1353–1359. doi:10.1365/s10337-009-1344-0

Perez AT (2013). Pioglitazone and risk of bladder cancer: clarification of the design of the French study. *Diabetologia*, 56(1):227 doi:10.1007/s00125-012-2767-y PMID:23135221

Physicians Desk Reference (2012) Pioglitazone. 66th ed. Montvale (NJ): PDR Network.

Piccinni C, Motola D, Marchesini G, Poluzzi E (2011). Assessing the association of pioglitazone use and bladder cancer through drug adverse event reporting. *Diabetes Care*, 34(6):1369–71. doi:10.2337/dc10-2412 PMID:21515844

Pino MV, Kelley MF, Jayyosi Z (2004). Promotion of colon tumors in C57BL/6J-APC(min)/+ mice by thiazolidinedione PPARgamma agonists and a structurally unrelated PPARgamma agonist. *Toxicol Pathol*, 32(1):58–63. doi:10.1080/01926230490261320 PMID:14713549

Pubchem (2013). Pubchem Databases: National Center for Biotechnology, Pioglitazone. In CID.4829 U.S. National Library of Medicine. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?q=all&cid=4829#ec. Accessed 10 July 2014.

Pubchem Substance (2013). Rosiglitazone (SID 46504556). Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=46504556

Radhakrishna T, Satyanarayana J, Satyanarayana A (2002b). LC determination of rosiglitazone in bulk and pharmaceutical formulation. *J Pharm Biomed Anal*, 29:873–880. doi:10.1016/S0731-7085(02)00209-1 PMID:12093521

Radhakrishna T, Sreenivas Rao D, Om Reddy G (2002a). Determination of pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC methods. *J Pharm Biomed Anal*, 29(4):593–607. doi:10.1016/S0731-7085(02)00036-5 PMID:12093488

Ravikanth CH, Kumar AA, Kiran VU (2011). Sensitive and rapid HPLC method for the determination of pioglitazone in rat serum. *Int J Pharmaceutical Sciences Drug Research*, 3(1):38–41.

Richter J, Jirman J, Havlícek J, Hrdina R (2007). Pioglitazone impurities. *Pharmazie*, 62(8):580–4. doi:10.1691/ph.2007.8.6720 PMID:17867551

Ruiter R, Visser LE, van Herk-Sukel MP, Geelhoed-Duijvestijn PH, de Bie S, Straus SM *et al.* (2012). Prescribing of rosiglitazone and pioglitazone following safety signals: analysis of trends in dispensing patterns in the Netherlands from 1998 to 2008. *Drug Saf*, 35:471–480. doi:10.2165/11596950-000000000-00000 PMID:22540371

Rx List (2013). RxList - The internet drug index. Available from: http://www.rxlist.com

Sakamoto J, Kimura H, Moriyama S, Odaka H, Momose Y, Sugiyama Y *et al.* (2000). Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone. *Biochem Biophys Res Commun*, 278(3):704–11. doi:10.1006/bbrc.2000.3868 PMID:11095972

Sato K, Awasaki Y, Kandori H, Tanakamaru ZY, Nagai H, Baron D *et al.* (2011). Suppressive effects of acid-forming diet against the tumorigenic potential of pioglitazone hydrochloride in the urinary bladder of male rats. *Toxicol Appl Pharmacol*, 251(3):234–44. doi:10.1016/j.taap.2011.01.006 PMID:21255596

SciFinder (2013). SciFinder Databases. Registry, Chemcats. American Chemical Society.

Seedher N, Kanojia M (2008). Micellar solubilization of some poorly soluble antidiabetic drugs: a technical note. *AAPS PharmSciTech*, 9:431–436. doi:10.1208/s12249-008-9057-5 PMID:18431666

Seedher N, Kanojia M (2009). Co-solvent solubilization of some poorly-soluble antidiabetic drugs. *Pharm Dev Technol*, 14:185–192. doi:10.1080/10837450802498894 PMID:19519190

Singh S, Loke YK, Furberg CD (2007a). Thiazolidinediones and heart failure: a teleo-analysis. *Diabetes Care*, 30(8):2148–53. doi:10.2337/dc07-0141 PMID:17536074

Singh S, Loke YK, Furberg CD (2007b). Long-term risk of cardiovascular events with rosiglitazone: A meta-analysis. *JAMA*, 298:1189–1195. doi:10.1001/jama.298.10.1189 PMID:17848653

Sireesha MR Chandan S, Gurupadayya BM, Aswani Kumar CH (2011). Selective and validated spectrophotometric methods for determination of rosiglitazone and pioglitazone with 2, 4-DNP. Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS University, Mysore, India. Available from: http://www.ijptonline.com/wp-content/uploads/2009/10/1554-1564.pdf. Accessed 1 October 2014.

Smith U (2001). Pioglitazone: mechanism of action. *Int J Clin Pract Suppl*, (121):13–8. PMID:11594239

Song SO, Kim KJ, Lee BW, Kang ES, Cha BS, Lee HC (2012). The risk of bladder cancer in korean diabetic subjects treated with pioglitazone. *Diabetes Metab J*, 36(5):371–8. doi:10.4093/dmj.2012.36.5.371 PMID:23130322

Souri E, Jalalizadeh H, Saremi S (2008). Development and validation of a simple and rapid HPLC method for determination of pioglitazone in human plasma and its application to a pharmacokinetic study. *J Chromatogr Sci*, 46(9):809–12. doi:10.1093/chromsci/46.9.809 PMID:19007483

Sripalakit P, Neamhom P, Saraphanchotiwitthaya A (2006). High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci*, 843(2):164–9. doi:10.1016/j.jchromb.2006.05.032 PMID:16815107

Sultana N, Arayne MS, Shafi N, Siddiqui FA, Hussain A (2011). Development and validation of new assay method for the simultaneous analysis of diltiazem, metformin, pioglitazone and rosiglitazone by RP-HPLC and its applications in pharmaceuticals and human serum. *J Chromatogr Sci*, 49(10):774–9. doi:10.1093/chrsci/49.10.774 PMID:22080805

Sweetman SC (2011) Monographs on drugs and ancillary substances. In: Martindale: The Complete Drug Reference. 37th ed. Vol. A. London, UK: Pharmaceutical Press, pp.494.

Takeda Pharmaceuticals (2013). Actos Product Information. Deerfield (Il): Takeda Pharmaceuticals America, Inc. Available from: http://www.takeda.us/products. Accessed 19 June 2014.

Thevis M, Geyer H, Schänzer W (2005). Identification of oral antidiabetics and their metabolites in human urine by liquid chromatography/tandem mass spectrometry–a matter for doping control analysis. *Rapid Commun Mass Spectrom*, 19(7):928–36. doi:10.1002/rcm.1875 PMID:15747323

Tseng CH (2011). Diabetes and risk of prostate cancer: a study using the National Health Insurance. *Diabetes Care*, 34(3):616–21. doi:10.2337/dc10-1640 PMID:21273499

Tseng CH (2012a). Pioglitazone and bladder cancer: a population-based study of Taiwanese. *Diabetes Care*, 35(2):278–80. doi:10.2337/dc11-1449 PMID:22210574

Tseng CH (2012b). Diabetes, metformin use, and colon cancer: a population-based cohort study in Taiwan. *Eur J Endocrinol*, 167(3):409–16. doi:10.1530/EJE-12-0369 PMID:22778198

Tseng CH (2012c). Thyroid cancer risk is not increased in diabetic patients. *PLoS ONE*, 7(12):e53096 doi:10.1371/journal.pone.0053096 PMID:23300866

Tseng CH (2012d). Pioglitazone and bladder cancer in human studies: is it diabetes itself, diabetes drugs, flawed analyses or different ethnicities? *J Formos Med*

Assoc, 111(3):123–31. doi:10.1016/j.jfma.2011.10.003 PMID:22423665

Tseng CH (2013a). Rosiglitazone is not associated with an increased risk of bladder cancer. *Cancer Epidemiol*, 37(4):385–9. doi:10.1016/j.canep.2013.03.013 PMID:23619142

Tseng CH (2013b). Benign prostatic hyperplasia is a significant risk factor for bladder cancer in diabetic patients: a population-based cohort study using the National Health Insurance in Taiwan. *BMC Cancer*, 13(1):7 doi:10.1186/1471-2407-13-7 PMID:23286275

Tseng CH (2013c). Oral cancer in Taiwan: is diabetes a risk factor? *Clin Oral Investig*, 17(5):1357–64. doi:10.1007/s00784-012-0820-3 PMID:22895832

Venkatesh P, Harisudhan T, Choudhury H *et al.* (2006). Simultaneous estimation of six anti-diabetic drugs–glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay. *Biomed Chromatogr*, 20:1043–1048. doi:10.1002/bmc.635 PMID:16506282

Wang M, Miksa IR (2007). Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 856:318–327. doi:10.1016/j.jchromb.2007.06.037 PMID:17689303

Wei L, MacDonald TM, Mackenzie IS (2013). Pioglitazone and bladder cancer: a propensity score matched cohort study. *Br J Clin Pharmacol*, 75(1):254–9. doi:10.1111/j.1365-2125.2012.04325.x PMID:22574756

WHO (2007). Cumulative List No. 14 of INN in Latin, English, French, Spanish, Arabic, Chinese and Russian (in alphabetical order of Latin name), with supllementary information. World Health Organisation.

Woodcock J, Sharfstein JM, Hamburg M (2010). Regulatory action on rosiglitazone by the U.S. Food and Drug Administration. *N Engl J Med*, 363(16):1489–91. doi:10.1056/NEJMp1010788 PMID:20942663

Xavier CM, Basavaiah K (2012). Implementation of Quality by Design for the Development and Validation of Pioglitazone Hydrochloride by RP-UPLC with Application to Formulated Forms *Int School Res Network*, 1–11.

Xue YJ, Turner KC, Meeker JB, Pursley J, Arnold M, Unger S (2003). Quantitative determination of pioglitazone in human serum by direct-injection high-performance liquid chromatography mass spectrometry and its application to a bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci*, 795(2):215–26. doi:10.1016/S1570-0232(03)00575-0 PMID:14522026

Yamashita K, Murakami H, Okuda T, Motohashi M (1996). High-performance liquid chromatographic determination of pioglitazone and its metabolites in human serum and urine. *J Chromatogr B Biomed Appl*, 677(1):141–6. doi:10.1016/0378-4347(95)00440-8 PMID:8925086

Yardımcı C, Ozaltın N, Gürlek A (2007). Simultaneous determination of rosiglitazone and metformin in plasma by gradient liquid chromatography with UV detection. *Talanta*, 72(4):1416–22. doi:10.1016/j.talanta.2007.01.042 PMID:19071778

Zhong WZ, Lakings DB (1989). Determination of pioglitazone in dog serum using solid-phase extraction and high-performance liquid chromatography with ultraviolet (229 nm) detection. *J Chromatogr A*, 490(2):377–85. doi:10.1016/S0378-4347(00)82795-4 PMID:2768410

Zhu Z, Shen Z, Lu Y, Zhong S, Xu C (2012). Increased risk of bladder cancer with pioglitazone therapy in patients with diabetes: a meta-analysis. *Diabetes Res Clin Pract*, 98(1):159–63. doi:10.1016/j.diabres.2012.05.006 PMID:22705039