DIGOXIN

1. Exposure Data

Digoxin is a cardiac glycoside isolated from plants of the genus *Digitalis*. The use of preparations of cardiac glycoside (synonyms: digitalis, cardiac steroids) dates back to 1785, when William Withering published his monograph "An account of the foxglove and some of its medical uses" (Withering, 1785; Albrecht & Geiss, 2000). Isolated digoxin has been used since the early 20th century (Cheng & Rybak, 2010).

The Working Group noted that only four of the many digitalis glycosides present in the plant remain important in the marketplace. These are digoxin, digitoxin, β-acetyldigoxin and methyldigoxin (Kleemann, 2012). Furthermore, the term "digitalis use" found in many reports probably refers not to the use of plant material, which is not commercially available as a medicinal product, but to the use of the isolated compounds. Of the four medicinally available compounds, digoxin is the most important and is exclusively available in some countries, such as the USA (see Section 1.3). The Working Group estimated that digoxin represents at least 90% of the world market for digitalis glycosides.

While use of digitoxin worldwide is much less than that of digoxin, it may be significant in individual countries. Thus, studies reporting use of "digitalis" should be carefully scrutinized since the agent to which people were actually exposed could have been any one of the four digitalis glycosides.

The Working Group noted that most of what has been used under the term "digitalis" in North America and Europe has been digoxin; however, there may be parts of the world where crude extract of the digitalis plant is still in use. No data on the use of digitalis extract were available to the Working Group.

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 20830-75-5 (SciFinder, 2013)

Chem. Abstr. Serv. Name: Card-20(22)-enolide, 3-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12,14-dihydroxy-, (3β,5β,12β)- (SciFinder, 2013)

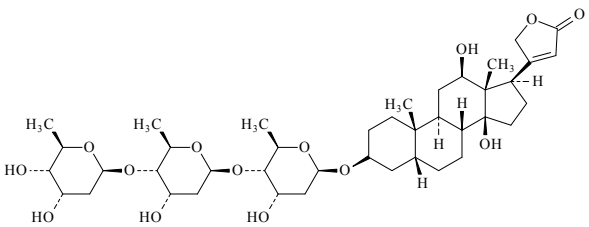
IUPAC Systematic Name: 3-[(3*S*,5*R*,8*R*,9*S*,10*S*,12*R*,13*S*,14*S*,17*R*)-3-[(2*R*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-5-[(2*S*,4*S*,5*S*,6*R*)-4,5-dihydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-12,14-dihydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[*a*]phenanthren-17-yl]-2*H*-furan-5-one (PubChem, 2013)

Synonyms: 12β-hydroxydigitoxin

Proprietary names for digoxin: Cardigox; Cardiogoxin; Cardioxin; Cardixin; Cardoxin; Chloroformic digitalin; Coragoxine; Cordioxil; Davoxin; Digacin; Digicor; Digitek; Digomal; Digon; Digosin; Digoxin Nativelle; Dilanacin; Dixina; Dokim; Dynamos; Eudigox; Fargoxin; Grexin; Homolle's digitalin; Lanacordin; Lanacrist; Lanicor; Lanikor; Lanocardin; Lanorale; Lanoxicaps; Lanoxin; Lanoxin PG; Lenoxicaps; Lenoxin; Longdigox; Mapluxin; NSC 95 100; Natigoxin; NeoDioxanin; Novodigal-Amp.; Purgoxin; Rougoxin; Stillacor; Toloxin; Vanoxin (from SciFinder, 2013).

1.1.2 Structural and molecular formulae and relative molecular mass

From USP (2007)



$C_{41}H_{64}O_{14}$

Relative molecular mass: 780.94

1.1.3 Chemical and physical properties of the pure substance

Description: Odourless, colourless to white crystals, or white crystalline powder, radially arranged four- and five-sided triclinic plates from dilute alcohol or pyridine (British Pharmacopoeia, 2009; PubChem, 2013)

Melting point: Digoxin melts and decomposes between 230 °C and 265 °C (Foss & Benezra, 1980; ChemicalBook, 2013)

Density: 1.36 ± 0.1 g/cm^3 (temperature, 20 °C; pressure, 760 Torr) (SciFinder, 2013)

Spectroscopy data: Specific optical rotation, ultraviolet, infrared, nuclear magnetic resonance, and mass spectral data were reported in the literature (Foss & Benezra, 1980; British Pharmacopoeia, 2009; HSDB, 2013)

Solubility: In water, 64.8 mg/L at 25 °C; soluble in dilute alcohol, pyridine, or mixture of chloroform and alcohol; almost insoluble in ether, acetone, ethyl acetate, chloroform; slightly soluble in diluted alcohol, and very slightly soluble in 40% propylene glycol; (PubChem, 2013)

Stability data: Digoxin is indefinitely stable when kept in the dark in a tightly closed container. No degradation is noted in tablets after 5 years when stored in tightly closed containers. A solution of digoxin hydrolyses in the presence of acid, yielding digoxigenin bis-digitoxoside, digoxigenin mono-digitoxoside and digoxigenin. A neutral solution in ethanol and propylene glycol is stable for up to 5 years. Digoxin solutions are relatively stable to light, except when stored under intense light for long periods of time (Foss & Benezra, 1980)

Storage: Digoxin preparations should be protected from light and stored at 15–25 °C (HSDB, 2013)

Octanol/water partition coefficient (log P): 1.26 (HSDB, 2013)

Dissociation constant: pK_a, basic = –3; pK_a, acidic = 7.15 (DrugBank, 2013)

Vapour pressure: 3.3 × 10^{-30} mm Hg at 25 °C (PubChem, 2013)

Flash point: 278.5 ± 27.8 °C (SciFinder, 2013)

1.1.4 Technical products and impurities

Since digoxin is isolated from plant materials, at least 21 other cardiac glycosides, including digitoxin, may occur as impurities (British Pharmacopoeia, 2009). The purity of digoxin is

typically at least 95% (see Section 1.5). According to the European Pharmacopoeia (2008), not more than 0.5% digitoxin in relation to digoxin may be present as impurity.

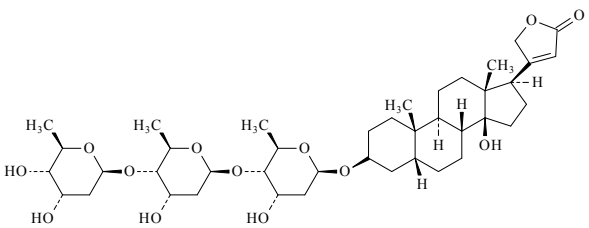
(a) Nomenclature for digitoxin

Chem. Abstr. Serv. Reg. No.: 71-63-6 (SciFinder, 2013)

Chem. Abstr. Serv. Name: 3*β*-[(*O*-2,6-dideoxy-*β*-D-ribo-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-*β*-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-*β*-D-ribo-hexopyranosyl)oxy]-14-hydroxy-4*β*,14*β*-card-20(22)-enolide.

Proprietary names for digitoxin: Crystodigin, Digimed, Digimerck.

(b) Structural and molecular formulae and relative molecular mass of digitoxin



$C_{41}H_{64}O_{13}$

Relative molecular mass: 764.94

1.2 Analysis

Compendial methods to determine digoxin and digitoxin in pharmaceutical preparations are typically based on liquid chromatography with ultraviolet detection. For detection in human plasma or urine, liquid chromatography with mass spectrometric detection is required to achieve the necessary lower detection limits. The analytical methods are summarized in Table 1.1.

1.3 Production and use

1.3.1 Production

Digoxin is isolated from *Digitalis lanata* Ehrh., the woolly foxglove, from the *Scrophulariaceae* family. For the isolation of the therapeutically important secondary glycosides, the finely ground material is moisturized and exposed to glucosidase enzymes at 30–37 °C until glucose is completely removed. Extraction procedures, usually followed by precipitation of tannic acid and related phenolic products with lead salts, afford a crude mixture of cardioactive compounds, which is further purified by chromatography and/or crystallization. Originally, mixtures of glycosides or crude plant extracts were used in therapy; these have been replaced by chemically pure drugs today, which allow better control of therapy. Total syntheses of cardiac steroids and their corresponding glycosides have been accomplished but are not used commercially (Albrecht & Geiss, 2000).

Digitoxin is isolated by extraction of the leaves and seeds of *Digitalis purpurea* L. (purple foxglove) with 50% ethanol and subsequent treatment with the enzyme digilanidase, which effects cleavage of the β-D-glucose moiety at the chain end of the main glycoside, purpureaglycoside A (Kleemann, 2012).

β-Acetyldigoxin is prepared from digoxin by acetylation with acetic acid. Methyldigoxin can be prepared by methylation of digoxin, e.g. with dimethyl sulfate (Kleemann, 2012).

1.3.2 Use

(a) Indications

Digoxin and digitoxin are therapeutically the most widely used digitalis glycosides. Table 1.2 lists the most commonly reported clinical indications for digoxin in the USA. While digoxin was once regarded as the drug of choice for congestive heart failure with reduced left ventricular

Table 1.1 Analytical methods for digoxin

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Compendial methods				
Digoxin injection, digoxin Tablet and digoxin oral solution	–	LC-UV Column: Packing L1 Mobile phase: water and acetonitrile Flow rate: 3 mL/min Wavelength: 218 nm	NR	USP (2007)
Digoxin injection, paediatric digoxin injection, paediatric digoxin oral solution, and digoxin tablets	–	LC-UV Column: C_{18} Mobile phase: acetonitrile:water (10:90) and water:acetonitrile (10:90) Flow rate: 1.5 mL/min Wavelength : 220 nm	NR	BP (2009)
Non-compendial methods				
Human plasma, rat plasma and rat brain	Addition of DMA, addition of NaCl saturated 0.1 mol/L NaOH, collection of organic layer, centrifugation	LC-MS-MS Column: C_{18} Mobile phases: ammonium carbonate, and methanol pH 9.0 Flow rate: 0.7 mL/min SRM: 779.4 *m/z*, 649.4 *m/z*	0.1 ng/mL (LLOQ)	Hirabayashi *et al.* (2011)
Human plasma	Deproteinization with perchloric acid in water, mixing and centrifugation	LC-ESI-MS Column: C_{18} Mobile phase: mixture of methanol and formic acid in sodium acetate Flow rate: 1 mL/min SIM: 803.5 *m/z*	0.5 ng/mL (LLOQ)	Vlase *et al.* (2009)
Human blood and tissues	Mixing with sodium acetate buffer pH 7, homogenization, centrifugation, loaded on SPE column conditioned with methanol, water, and sodium acetate buffer, washing with sodium acetate buffer, dried under vacuum, second wash with 20% isopropyl alcohol, drying, addition of acetone, vacuum drying, elution with acetone	LC-ESI-MS Column: C_8 Mobile phase: 0.1% formic acid in a mixture of 55% methanol and 45% water Flow rate: 0.2 mL/min SIM: 803.4 *m/z*	0.2 ng/g (LLOQ)	Frommherz *et al.* (2008)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Human serum	Addition of methyl *tert*-butyl ether, centrifugation, evaporation, and reconstitution in methanol	LC-ESI-MS Column: C_{18} Mobile phase: 10 mM ammonium acetate/0.1% formic acid in water and 0.1% formic acid in acetonitrile Flow rate: 0.3 mL/min SRM transition: 798.6 *m/z*, 651.5 *m/z*	0.1 ng/mL (LLOQ)	Li *et al.* (2010)
Human blood	Mixing with ammonium carbonate buffer, extraction (ethyl acetate/hepatene/dichloromethane = 3 : 1 : 1), centrifugation, collection of organic layer, evaporation, reconstitution in acetonitrile:water	LC-ESI-MS Column: C_{18} Mobile phase: 10 mM ammonium formate and acetonitrile pH 3.1 Flow rate: 0.3 mL/min MRM transitions: 798.4 *m/z*, 651.3 *m/z*; 798.4 *m/z*, 633.3 *m/z*	0.08 ng/mL (LLOQ) 0.032 ng/mL (LOD)	Oiestad *et al.* (2009)
Human plasma	Mixing with 10% ammonium hydroxide, addition of chloroform, centrifugation, evaporation, reconstitution in 1 mM trifluoroacetic acid and acetonitrile (7 : 3).	LC-ESI-MS Column: UPLC® AQUITY® Mobile phase: 30% 1mM ammonium trifluoroacetate in acetonitrile and 100% water Flow rate: 0.1 mL/min SIM transition: 780.94 *m/z*, 893.5 *m/z*	0.1 ng/mL (LLOQ)	Grabowski *et al.* (2009)
Human plasma	Addition of concentrated NaOH and methyl *t*-butyl ether, shaking, centrifugation, evaporation, reconstitution in mobile phase	LC-ESI-MS Column: C_8 Mobile phase: 0.25 mM sodium acetate in water and 0.25 mM sodium acetate in methanol Flow rate: 0.25 mL/min SIM: 803.4 *m/z* (positive mode)	0.05 ng/mL (LLOQ) 0.025 ng/mL (LOD)	Kirby *et al.* (2008)
Human plasma	Addition of buffer solution pH 6.0, loading into oasis HLB30 mg 96-well plate preconditioned with methanol:water (40:60), elution of analyte with pure methanol, evaporation and reconstitution in methanol	LC-ESI-MS Column: C_{18} Mobile phase: 10 mmol/L ammonium hydrogen carbonate/methanol (1 : 9) and 10 mmol/L ammonium hydrogen carbonate/methanol (9 : 1) Flow rate: 0.6 mL/min SRM transition: 798.5 *m/z*, 651 *m/z*	0.04 ng/mL (LLOQ)	Hashimoto *et al.* (2008)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Human plasma and urine	Addition of buffer solution pH 6, loading into oasis HLB30 mg 96-well plate preconditioned with methanol:water (40:60), elution of analyte with pure methanol, evaporation, reconstitution in methanol	LC-ESI-MS Column: C_{18} Mobile phase: 5 mM ammonium acetate and acetonitrile Flow rate: 250 µL/min SRM transition: 798.5 *m/z*, 651.4 *m/z* (positive mode)	0.2 ng/mL (LLOQ) 1 ng/mL (LLOQ)	Salvador *et al.* (2006)
Drinking-water, ground water, surface water, and waste water	SPE by using oasis HLB cartridge	LC-MS-TOF Column: C_8 Mobile phase: acetonitrile, water with 0.1% formic acid	1–1000 ng/L (LOD)	Ferrer & Thurman (2012)
Water, soil, sediment, and biosolids	Extraction with solvents, and SPE	LC-MS-MS	50 ng/L in water (LOD)	EPA (2007)
Plant extract	Extracted from herbaceous plants of the genus *Digitalis*	LC-ESI-MS Column: C_{18} Mobile phase: aqueous ammonium formate/methanol (40/60% v/v), pure methanol Flow rate: 0.3 mL/min SRM transition: 798.5 *m/z*, 780.4 *m/z*	38–936 pg/g in solution (LOD)	Josephs *et al.* (2010)
Rat plasma	Addition of ammonium chloride buffer, acetonitrile and methylene chloride, vortexing, centrifugation, evaporation of organic layer, reconstitution	LC-ESI-MS Column: C_{18} Mobile phase: acetonitrile/ammonium formate Flow rate: 0.2 mL/min SRM transition: 798.60 *m/z*, 651.6 *m/z*	0.1 ng/L (LOQ)	Yao *et al.* (2003)
Human serum	Incubation, centrifugation, supernatant loaded into a vial and frozen	IC Colloidal gold mAb probe-colloidal gold conjugate with IgG	Visual detection limit, 2 ng/mL Detection time, 2–5 min	Omidfar *et al.* (2010)
Human blood and urine	Addition of water and ammonium acetate buffer (2 M, pH 9.5), centrifugation, collection of supernatant, clean-up by SPE	LC-ESI-MS Column: C_{18} Mobile phase: 20% acetonitrile in 80% 2 mM ammonium formate and 80% acetonitrile in 20% 2 mM ammonium formate Flow rate: 0.2 mL/min SRM transition: 799.4 *m/z*, 651.4 *m/z*	0.05 ng/mL (LLOQ)	Guan *et al.* (1999)

Table 1.1 (continued)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Rat intestinal perfusion samples	NR	LC-UV Column: C_{18} Mobile phase: 10 mM ammonium acetate, methanol, acetonitrile (50 : 25 : 25) Flow rate: 0.5 mL/min pH 3.0 Wavelength: 220 nm	25 ng/mL (LOQ)	Varma *et al.* (2004)
Human plasma	NR	LC-ESI-MS Column: C_{18} Mobile phase: acetonitrile and 2 mM ammonium acetate pH 3.0 Flow rate: 0.2 mL/min SRM transition:7 99 *m/z*	NR	Tracqui *et al.* (1997)

DMA, *N,N*-dimethylacetamide; IC, immunochromatography; IgG, immunoglobulin G; LC-ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LC-MS-MS, liquid chromatography tandem mass spectrometry; LC-TOF-MS, liquid chromatography time of flight mass spectrometry; LC-UV, liquid chromatography ultraviolet spectroscopy; LLOQ, lower limit of quantification; LOD, limit of detection; LOQ, limit of quantification; mAb, monoclonal antibody; min, minute; MRM, multiple reaction monitoring; *m/z*, mass/charge; NaCl, sodium chloride; NaOH, sodium hydroxide; NR, not reported; SIM, selected ion monitoring; SPE, solid-phase extraction; SRM, selected reaction monitoring

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

Diagnosis	ICD-9 code[a]	Drug uses (in 1000s)	Percentage of total
Atrial fibrillation	427.301	1595	42.3
Hypertensive heart disease, other	402.901	621	16.5
Congestive heart failure	428.001	501	13.3
Other primary cardiomyopathy, NOS	425.402	113	3.0
Chronic ischaemic disease, unspecified	414.901	81	2.1
Essential hypertension, NOS	401.901	65	1.7
Surgery after heart disease treatment	V67.038	53	1.4
Medical follow-up after atherosclerotic heart disease	V67.533	50	1.3
Paroxysmal supraventricular tachycardia	427.001	50	1.3
Chronic ischaemic disease, unspecified, with hypertension	414.501	50	1.3
All other diagnoses	–	593	15.7
Total with reported diagnoses	–	3771	100.0

[a] ICD-9 codes are a more detailed, proprietary version developed by IMS Health.
Prepared by the Working Group on the basis of data from IMS Health (2012b)
ICD-9, International Classification of Diseases Revision Nine; NOS, not otherwise specified

ejection fraction and for atrial fibrillation, it has been largely supplanted by other medications (Sleeswijk *et al.*, 2007). Digitoxin is useful for maintenance therapy because its long half-life (5 – 9 days) provides a sustained therapeutic effect even if a dose is missed. For the same reason toxic reactions are not easy to manage. Elimination is independent of renal function (Albrecht & Geiss, 2000).

For congestive heart failure, use of digoxin fails to improve survival (Digitalis Investigation Group, 1997) when compared with placebo, unlike other leading therapies. It does, however, provide symptomatic benefits in some cases and is associated with reduced risk of hospitalization. USA guidelines suggest its use in situations where recommended therapies (diuretics, angiotensin-converting-enzyme inhibitors and β-blockers) fail to produce adequate symptom relief (Hunt *et al.*, 2009). European guidelines continue to recommend digoxin as one of several therapies used in combination for the management of congestive heart failure (Dickstein *et al.*, 2008).

As for congestive heart failure, use of digoxin for atrial fibrillation has also declined in preference for other medications, particularly β-blockers and non-dihydropyridine calcium-channel blockers. Digoxin is generally less effective than other drugs in producing consistent reduction of heart rate, particularly during exertion (McNamara *et al.*, 2003). Joint USA/European Union guidelines recommend against use of digoxin as a first-line agent in most cases of atrial fibrillation (Fuster *et al.*, 2006).

(b) Dosage

Administration is typically oral, although preparations for intravenous administration exist. Typically, digoxin is used orally for months to years, while intravenous use requires careful medical monitoring and is given only in the short-term. The absorption ratio was found to be 70%, the decay ratio is 20%, the effective dose level is 2 mg, and the maintenance dose is 0.5 mg (Albrecht & Geiss, 2000).

For the treatment of heart failure, atrial fibrillation, the loading-dose regimen for intravenous administration is a single dose of 0.4–0.6 mg, with additional doses of 0.1–0.3 mg every 6–8 hours to be given with caution until there is clinical evidence of adequate effect, and the

total dose should not exceed 0.008–0.015 mg/kg bw. The oral dosage for this indication is a single dose of 0.5–0.75 mg, then additional doses of 0.125–0.375 mg may be given cautiously every 6–8 hours until clinical evidence of adequate effect, up to a total dose of 0.75–1.25 mg (for a patient weighing 70 kg). The maintenance dose is 0.125–0.5 mg/day, intravenous or oral (Medscape (2013).

Most generic tablet preparations of digoxin average 70–80% oral bioavailability, with 90–100% oral bioavailability for digoxin elixir and the encapsulated gel preparation. Parenteral digoxin is available for intravenous administration, and is of value in patients who are unable to take oral formulations. Caution to avoid overdosing is necessary in elderly patients or those with renal impairment (Li-Saw-Hee & Lip, 1998). In general, the therapeutic index for digoxin is narrow (Ehle *et al.*, 2011).

When digoxin is indicated, suggested therapeutic ranges of serum concentrations of digoxin are lower now than in the past (Hunt *et al.*, 2009), particularly given the report that mortality among digoxin users was associated with higher serum concentrations of this drug (Rathore *et al.*, 2003). In a study of post-mortem cases, the range of serum digoxin concentrations in cases of overdose was 2.7–6.8 nmol/L (mean, 4.7 nmol/L) [2.1–5.3 ng/L (mean, 3.7 ng/L)] (Eriksson *et al.*, 1984).

Country-dependent differences in formulations may be correlated to the range of available tablet strengths. For example, the dosage was significantly higher in some hospitals in the USA and France than in the United Kingdom, and significantly higher in France than in the USA (Saunders *et al.*, 1997).

(c) Trends in use

Use of digoxin in the USA has declined substantially for treatment of congestive heart failure (Banerjee & Stafford, 2010) and of atrial fibrillation (Stafford *et al.*, 1998; Fang *et al.*, 2004). Trends in the European Union may have lagged behind those in the USA, but use for both conditions has declined (Sturm *et al.*, 2007). Use of digoxin may have been reduced between 1991 and 2004 in the USA, but not in the United Kingdom (Haynes *et al.*, 2008).

The Food and Drug Administration (FDA) reported that digitoxin and acetyldigitoxin are no longer manufactured in the USA (FDA, 2013).

Globally, there are 160 licensed products containing digoxin, while there are only seven licensed products containing digitoxin in Germany, Austria, Hungary, and Norway (Index Nominum, 2013).

Despite the introduction of new therapeutic strategies, cardiac glycosides are still widely used, and digoxin belongs to the 10 most frequently prescribed drugs in the USA (Albrecht & Geiss, 2000). In Estonia, the consumption of digoxin was very high in the times of the former Soviet Union and decreased in the first years of independence. When problems with drug availability were overcome, the use of digoxin increased by 35% in 1994–97 (Pähkla *et al.*, 1999).

While a rare event, the homicidal use of digoxin has been described. Suicide by digoxin may have been more frequent in continental Europe, but has also occurred in the USA and England (Burchell, 1983).

Total worldwide sales of digoxin were US$ 142 million in 2012, with 33% occurring in the USA (US$ 47 million). Other nations reporting appreciable use of digoxin included Japan (US$ 14 million), Canada (US$ 11 million), and the United Kingdom (US$ 9 million) (IMS Health, 2012a).

In the USA in 2012, digoxin was reported by office-based physicians in 1.85 million drug uses, and was being taken by approximately 700 000 patients (IMS Health, 2012b). The trend in use of digoxin in the USA is shown in Fig. 1.1. According to the IMS Health National Prescription Audit Plus, there were a total of 9.6 million prescriptions

for digoxin in 2012, down from 14.6 million prescriptions in 2008 (IMS Health, 2012c).

1.4 Occurrence and exposure

1.4.1 Natural occurrence

The principal natural occurrence of digoxin is in the leaves of *Digitalis lanata* Ehrh., but it may also occur in some other *Digitalis* species (Hollman, 1985). After leaf-tissue damage or plant harvest, the primary glycoside lanatoside C is converted to the secondary glycoside digoxin by the endogenous enzyme, digilanidase, present in the leaves, and by subsequent deacetylation. *D. lanata* leaves were found to contain digoxin at 8.6–13.2 µg/100 mg and its precursor, lanatoside C, at 55.8–153.2 µg/100 mg, depending on the health of the plant material (Pellati *et al.*, 2009). Environmental factors that influence the digoxin content in *D. lanata* are carbon-dioxide enrichment and water stress (Stuhlfauth *et al.*, 1987).

1.4.2 Occupational exposure

No data were available to the Working Group.

1.5 Regulations and guidelines

Digoxin has been assigned classification as a "water hazard" in Germany and as an "environmental hazard" in several USA states (SciFinder (2013). The United States Environmental Protection Agency (EPA) assigned it to the list of "extremely hazardous substances" mandated by Section 302 of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA), for which the reportable quantity is 10 lbs [~4.5 kg] and the threshold planning quantity is 10/10 000 lbs [4.5/4536 kg].

Digoxin is specified in several official pharmacopoeias (Table 1.3).

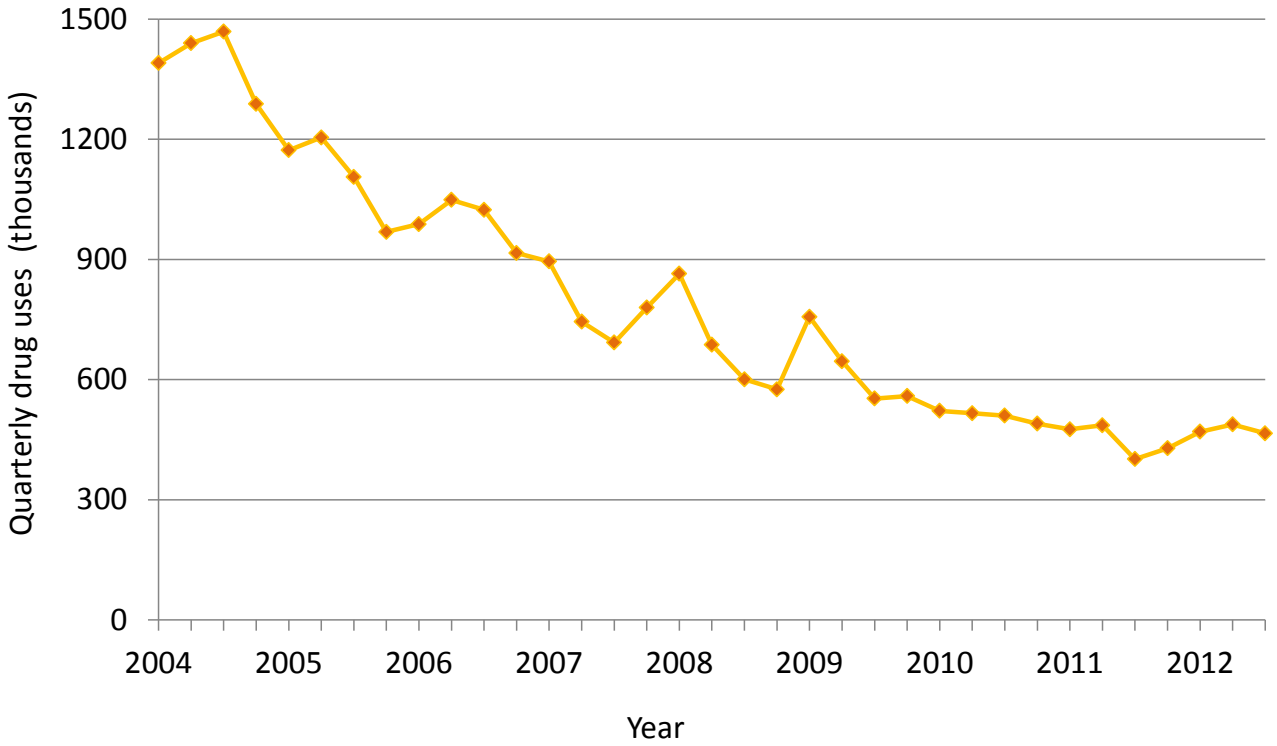
2. Cancer in Humans

Beginning in the late 1970s, several small studies based on case series or chart reviews reported a lower risk of cancer of the breast in women using "digitalis" (see introduction to Section 1) (Stenkvist *et al.*, 1979, 1982; Goldin & Safa, 1984). These reports, mostly in brief correspondence, have been cited as supporting the consideration of digitalis as a possible therapy for cancer of the breast (Stenkvist, 1999; Haux, 1999); however, because so little information was provided and larger studies with stronger designs were available, these early studies were judged to be uninformative and were not considered further.

The studies reviewed by the Working Group included a measure of relative risk, such as odds ratios, hazard ratios, and incidence rate ratios. Varied designs were used in these studies. Some studies evaluated associations between risks of cancers of all types and exposures to a wide range of pharmaceuticals, or to a more restricted range of cardiovascular drugs. Others examined risk factors for specific cancers, typically including prescription drugs together with evaluation of other demographic and health parameters. In recent years, national registries of prescription drug use have yielded large data sets in which follow-up can be linked to cancer outcomes in cohort studies.

Many reports described only "digitalis" exposure, and therefore may refer to either digoxin (much more commonly used, especially in recent years) or digitoxin. Even when some epidemiological studies specified "digoxin," the subjects who were enrolled during years when digitoxin was more widely used might have also used digitoxin (e.g. because of renal failure). The studies describing "digitalis" use are therefore included, with the exposure type digoxin, digitoxin, or digitalis, indicated in the tables. Most

Fig. 1.1 Trends in use of digoxin as a drug in the USA



Prepared by the Working Group on the basis of data from IMS Health National Disease and Therapeutic Index, 2004–12 (IMS Health, 2012b).

of what has been used under the term "digitalis" in North America and Europe has been digoxin.

2.1 Cancer of the breast

2.1.1 Case–control studies

See Table 2.1

Studies of the association of risk of cancer with use of digoxin and related drugs have focused mainly on cancer of the breast. Aromaa *et al.* (1976) reported a register-based case–control study in which use of "digitalis" (and many other cardiovascular drugs) in the year before diagnosis was compared in 109 hypertensive women with cancer of the breast and in 109 matched hypertensive women without cancer of the breast. Hypertensive women with cancer of the breast were more likely to be using digitalis than were women without cancer of the breast (relative risk, RR, 2.67; 95% CI, 0.99–8.33; in the subset restricted to 65 pairs with similar follow-up time.). [Both cases and controls were hypertensive and both were therefore at a high risk of cardiovascular disease. This comparability enhanced internal validity, but it may have reduced generalizability.]

Lenfant-Pejovic *et al.* (1990) described risk factors for cancer of the breast in men in France and Switzerland, comparing 91 cases with 255 controls recruited from hospital cancer clinics in France and a cancer registry in Switzerland, and matched for age and area of residence. Data on risk factors were limited to information available in physician interviews by mail or telephone, and clinical record reviews. Of all prescribed drugs, only use of digitalis for at least 3 months before

Table 1.3 Regulations in pharmacopoeial monographs on digoxin

Regulation	WHO International Pharmacopoeia, 4th edition	United States Pharmacopeial Convention 30	European Pharmacopoeia 7.0	Japanese Pharmacopoeia XVI
Content $C_{41}H_{64}O_{14}$ (dried substance)	95.0–103.0%	95.0–101.0%	96.0–102.0%	96.0–106.0%
Identity tests	Tests ABD or BCD may be applied: A. IR B. TLC C. Colour reaction with dinitrobenzene/ethanol D. Colour reaction with ferric chloride/glacial acetic acid/sulfuric acid	A. IR B. HPLC C. TLC	IR	1. Colour reaction with ferric chloride hexahydrate/acetic acid/sulfuric acid 2. IR
Specific optical rotation	+13.6° to +14.2° (0.10 g/mL in pyridine)	–	+13.9° to 15.9° (0.50 g in 25 mL methanol/methylene chloride 50 : 50)	+10.0 to + 13.0° (0.20 g in 10 mL pyridine)
Sulfated ash	Max. 1.0 mg/g	–	Max. 0.1%	–
Loss on drying	Max. 10 mg/g	Max. 1.0%	Max. 1.0%	Max. 1.0%
Residue on ignition	–	Max. 0.5%	–	Max. 0.5%
Gitoxin	Absorbance at 352 nm, max. 0.22 (about 40 mg/g)	–	–	–
Related substances/purity	TLC test, absence of spots that are more intense than standard solution at 0.25 mg/mL	TLC test, no spot that is more intensive than gitoxin standard solution (not more than 3% of any related glycoside as gitoxin)	HPLC: specific limits for about 12 related substances are specified	HPLC: total area of peaks of impurities is max. 3%
Organic volatile impurities	–	General requirements, except limits for methylene chloride and chloroform are 2000 µg/g	–	–
Bacterial endotoxins	Max. 200.0 IU of endotoxin per mg	–	–	–

HPLC, high-performance liquid chromatography; IR, infrared; IU, international units; TLC, thin-layer chromatography

Adapted from The United States Pharmacopoeial Convention (2006), European Pharmacopoeia (2008), The International Pharmacopoeia (2011), Pharmaceuticals and Medical Devices Agency (2011)

diagnosis was associated with increased risk (11 users among cases; odds ratio, OR, 4.1; 95% CI, 1.4–12.4). [The data from France and Switzerland were collected in different ways and the Working Group questioned the quality of the data obtained from medical records and physician interviews.]

In another study of risk factors for cancer of the breast in men, Ewertz *et al.* (2001) compared 156 incident cases in men in Norway, Sweden, and Denmark with 468 men matched for year of birth, and country. Many variables were evaluated using self-administered questionnaires, including use of prescribed drugs. Among all drugs assessed, digoxin stood out most strongly, with odds ratios for digoxin of 1.8 (95% CI, 0.7–4.4) in men with < 5 years use and 2.0 (0.9–4.4) for ≥ 5 years use. After adjustment for body mass index determined from self-estimated weight and height 10 years before diagnosis, the association between cancer of the breast and digoxin use was still 1.8 ($P = 0.08$). [Recalculated by the Working Group from observed/expected data to be 1.9 (95% CI, 1.05–3.48).]

Ahern *et al.* (2008) identified 5565 postmenopausal women with incident cancer of the breast who used digoxin with a 10 : 1 birth year- and residence area-matched population-control group in Denmark in 1991–2007. Use of digoxin was ascertained by county-level prescription registry data, and by design, all subjects were required to have used digoxin for ≥ 2 years before diagnosis (and use was likely to be current). Adjustments included age, past use of hormone replacement therapy, nonsteroidal anti-inflammatory drugs (NSAIDs), and anticoagulants including aspirin. Among the cases of cancer of the breast, 324 used digoxin compared with 2546 controls, yielding an adjusted odds ratio of 1.30 (95% CI, 1.14–1.48). Relative to non-users, the odds ratios increased with duration of use from 1.25 (95% CI, 1.03–1.52) with 1–3 years of use to 1.30 (95% CI, 1.05–1.61) with 4–6 years of use to 1.39 (95% CI, 1.10–1.74) with > 6 years of use. The findings persisted after adjustment for exposure to estrogen, use of other drugs, confounding by indication, and frequency of mammography. [This large study was regarded as being of high quality. However, the Working Group noted that some important risk factors of cancer of the breast, notably parity, obesity, and alcohol drinking, were not controlled in the analysis.]

2.1.2 Cohort studies

See Table 2.2

Using data from persons enrolled in the Kaiser Permanente Medical Care Programme, Friedman & Ury (1980) linked prescription-drug use for 95 drugs and drug classes between 1969 and 1973 to subsequent cancer outcomes (56 types) registered within this health-care system until 1976. The drugs evaluated included "digitalis" as a group. A more detailed presentation of digitalis-related associations used cancer-outcome data for 143 594 subjects updated to 1980 (Friedman, 1984) (results provided in Table 2.2). The age–sex standardized morbidity ratio for cancer of the breast and ever-use of digitalis was 1.2 [95% CI, 0.74–1.87]. [This study was large and was able to examine the association of cancer with many different drugs; however, the precision of specific drug–cancer associations was limited and there was some concern about the large number of comparisons.]

Haux *et al.* (2001) used a database of plasma concentrations of digitoxin for 9271 women and men in Trondheim, Norway, who were undergoing their first treatment with digitoxin between 1986 and 1996. The risk of developing cancer in people receiving their first treatment with digitoxin was compared with the incidence of cancers with at least 30 expected cases (all sites, breast, prostrate, colorectum, lung, kidney/urinary, melanoma, lymphoid/leukaemia) in the national population. Standardized incidence ratios (SIR) for most cancers, including cancer of the breast, were higher (typically by about 25%) among digitoxin users. In an analysis of cancer

Table 2.1 Case–control studies on use of digoxin and cancer of the breast

Reference, study location and period	Subjects	Exposure assessment	Organ site	Exposed cases	Exposure category	Relative risk (95% CI)	Adjustments for potential confounders	Comments
Aromaa *et al.* (1976), Finland, cases reported in 1973	Women with breast cancers and hypertension (n = 109) compared with matched women with hypertension only (n = 109)	Prescription-acquired cardiovascular drugs	Breast	28 16	Any digitalis use vs no use Any digitalis use vs no use (case–control pairs with comparable treatment duration)	1.33 (0.73–2.48) 2.67 (0.99–8.33)	Age, geographical area	Digitalis use was a secondary outcome, but the strongest association seen among prescription drug used; probably included some digitoxin users.
Lenfant-Pejovic *et al.* (1990), Switzerland, 1970–86, and France, 1975–88	Men with breast cancer (n = 91) identified in hospital or by tumour registries compared with men with colorectal, haematolymphatic, or skin cancers (n = 255)	Hospital chart abstracts and physician interview; digitalis specified	Breast, adeno-carcinoma	11	Any digitalis use vs no use	4.1 (1.4–12.4)	Controls matched by age and hospital	Digitalis was the only one of many therapeutic drugs for which an association was found. Probably included some digitoxin users.
Ewertz *et al.* (2001), Norway, Sweden, Denmark, 1987–91	Men with breast cancer (n = 156) compared with men in population registry (n = 468)	Self-reported questionnaires including prescription-drug use and other demographic and health data	Breast	20	Never digoxin Digoxin < 5 yr Digoxin ≥ 5 yr	1.0 (ref.) 1.8 (0.7–4.4) 2.0 (0.9–4.4).	Matched for sex, age; overall analysis adjusted for BMI	Multiple comparisons to diverse demographic, health, and drug-use variables, but association for digoxin appeared to be the strongest among drugs; probably included some digitoxin users. P = 0.08 for overall association between digoxin use and breast cancer

Table 2.1 (continued)

Reference, study location and period	Subjects	Exposure assessment	Organ site	Exposed cases	Exposure category	Relative risk (95% CI)	Adjustments for potential confounders	Comments
Ahern *et al.* (2008), North Jutland and Aarhus Counties, Denmark, 1991–2007	Postmenopausal women with breast cancer (n = 5565) compared with matched women from population registry (n = 55 650)	County-based pharmacy registries	Breast	5241	Never-user	1.0 (ref.)	Age, location; use of anti-inflammatory drugs, anticoagulants or HRT	Tumour ER status not examined. Association not greatly changed by adjustments; Suggestion of increased risk with longer duration of use. May have included some digitoxin users in early years, although described as digoxin users. Adjusted for age, county of residence, and past receipt of HRT, anticoagulants, high- and low-dose aspirin, and NSAIDs.
				324	Ever used digoxin (restricted to case–control pairs with comparable treatment duration)	1.30 (1.14–1.48)		
				128	1–3 yr	1.25 (1.03–1.52)		
				103	4–6 yr	1.30 (1.05–1.61)		
				93	7–18 yr	1.39 (1.10–1.74)		

BMI, body mass index; ER, estrogen receptor; HRT, hormone replacement therapy; NSAIDs; non-steroidal anti-inflammatory drugs; ref., reference; vs, versus; yr, year

incidence in people before their first use of digitoxin, odds ratios for most cancers were similarly increased. An analysis of the relationship between risk of cancer and serum concentration of digitoxin did not show a coherent relationship for cancer of the breast. [The Working Group noted that the national population used as comparison group was external to the study population and may differ in its underlying disease risk or in the quality of cancer ascertainment. Elevated risk of cancer in the study population before beginning treatment may be attributable to underlying increases in the frequency of common risk factors for cancer and for cardiovascular disease requiring digitoxin, rather than the use of digitoxin itself. In addition, estimates of digitoxin dose were based on a single measurement at the start of treatment and there was no information about ongoing exposure.]

Biggar *et al.* (2011) reported a nationwide cohort study in Denmark, evaluating incidence of cancer of the breast in women prescribed digoxin. Data were obtained by linking the national Danish prescription-drug database (available since 1995) and the nationwide Danish cancer registry until 2008. Among 104 648 women using digoxin, 2144 developed cancer of the breast. Risks associated with current and former use, and duration of current use among new users only were analysed, with incidence rate ratios for cancer of the breast adjusted for attained age at diagnosis and calendar year. The relative risk (RR) for current use was 1.39 (95% CI, 1.32–1.46), with higher risk for developing estrogen receptor-positive tumours (RR, 1.35; 95% CI, 1.26–1.45) than estrogen receptor-negative tumours (RR, 1.20; 95% CI, 1.03–1.40) among digoxin users. Incidence was not increased in women who had used digoxin in the past (SIR, 0.91; 95% CI, 0.83–1.00). Increased incidence was not associated with duration of use, but declined to baseline within 1 year after use of digoxin had ceased. [This was regarded as a high-quality study, with the capacity to examine risk by estrogen-receptor status being a particular strength. The study did not examine the effect of menopausal status; however, most women included were postmenopausal (median age, 79 years). Information on other covariates was limited. While there are many risk factors for cancer of the breast, the inability to control for alcohol drinking and obesity was likely to be of greatest concern.]

Biggar *et al.* (2013) examined features of cancer of the breast in a case–case comparison of cancers developed in 369 women who were using digoxin at the time of diagnosis with 34 085 cancers in women not using digoxin. Tumours in users were significantly more likely ($P = 0.002$) to be estrogen receptor-positive (85%) than estrogen receptor-negative (79%), and to have low versus high histological grades, features suggesting better prognosis. [The prognostic factors for cancer of the breast in women receiving digoxin and in women receiving estrogen were similar and more favourable, e.g. estrogen receptor-positive tumours, than in women not receiving treatment (IARC, 2012).]

2.2 Cancers of the uterus and ovary

Cohort study

See Table 2.3

In a cohort study in Denmark, Biggar *et al.* (2012) evaluated the risk of cancer of the uterus. The methods and data sources were identical to those in the study of cancer of the breast described in Section 2.1.2 (Biggar *et al.*, 2011). As with cancer of the breast, the incidence of cancer of the uterus (n = 461 cases in digoxin users) was increased among current users (RR, 1.48; 95% CI, 1.32–1.65). In addition, this study also evaluated cancers of the ovary (n = 277) and cervix (n = 117) as "control cancers," finding no increase in the incidence of either cancer (RR for cancer of the ovary, 1.06; 95% CI, 0.92–1.22; RR for cancer of the cervix, 1.00; 95% CI, 0.79–1.25)

Table 2.2 Cohort studies on use of digoxin and cancer of the breast

Reference, location, and period	Subjects	Exposure assessment	Organ site	Exposed cases	Exposure category	Relative risk (95% CI)	Adjustments for potential confounders Comments
Friedman (1984), Kaiser Permanente Medical Care Program (USA), 1969–80	Members of a private health-care insurance programme (n = 143 594)	Pharmacy database from Health Plan	Lung Colon Breast Prostate	48 35 20 34	Digitalis ever-use (digoxin, digitoxin, digitalis)	1.7 [1.22–2.20] 1.5 [1.02–2.04] 1.2 [0.74–1.87] 1.4 [1.00–2.01]	Age, sex Main summary for all drug-cancer relationships reported by Friedman & Ury (1980). Updated to 1980: Friedman (1984). Multiple comparisons. No association found for other cancers.
Haux *et al.* (2001), Trondheim, Norway, 1986–96	People (n = 9 271) undergoing their first digitoxin treatment	Digitoxin in plasma measured in a central laboratory	All sites Female breast Prostate Colorectum Lung Kidney/urinary Melanoma Leukaemia/ lymphoma (C81–C 85/C88/92)	641 57 108 127 63 59 61 53	Digitoxin use	1.27 (1.18–1.37) 1.25 (0.95–1.62) 1.25 (1.03–1.50) 1.29 (1.06–1.51) 1.35 (1.04–1.74) 1.14 (0.87–1.47) 1.23 (0.94–1.58) 1.41 (1.06–1.85)	Age, year of birth, sex Incidence compared to population incidence when > 30 cases were expected Use based on single assessment of digitoxin. A high risk of cancer diagnosed before digitoxin measurement (not shown) suggested high cancer risk preceded use. Expected numbers of cancers obtained from national registry rates.
			Breast		Digitoxin concentration (ng/mL): < 16 16–22 > 22	 1.00 (ref.) 1.04 (0.59–1.84) 0.90 (0.48–1.67)	 Dose–response on the cohort on digitoxin users by different levels of digitoxin plasma concentration at first measurement divided in tertiles
Biggar *et al.* (2011), Denmark, 1995–2008	Women aged ≥ 20 yr (n = 2 011 381)	Nationwide pharmacy registry for drug exposure	Breast	46 872 2144 454 1690	Never Ever Former Current	1.0 1.24 (1.18–1.30) 0.91 (0.83–1.00) 1.39 (1.32–1.46)	Attained age, calendar-year Association found only with current use of digoxin and stronger when restricted to women with ER-positive tumours. Duration results apply to all breast cancers, regardless of ER status.

Table 2.2 (continued)

Reference, location, and period	Subjects	Exposure assessment	Organ site	Exposed cases	Exposure category	Relative risk (95% CI)	Adjustments for potential confounders Comments
Biggar et al. (2011), Denmark, 1995–2008 (cont.)					Duration of use in new users only (mo):		
				306	0–12	1.65 (1.47–1.86)	
				147	13–24	1.31 (1.12–1.55)	
				92	25–36	1.13 (0.92–1.38)	
				265	37+	1.31 (1.16–1.48)	

ER, estrogen receptor; mo, month; ref., reference; vs, versus; yr, year

among current users. Patterns of risk with duration of digoxin use were not consistent by cancer type. For cancer of the uterus, stronger associations were observed for digoxin use of 0–12 months (RR, 1.60; 95% CI, 1.23–2.07) and > 37 months (RR, 1.91; 95% CI, 1.51–2.41) among current users, while for cancer of the ovary the strongest association was for digoxin use of 0–12 months among current users (RR, 1.37; 95% CI, 1.01–1.86) among current users. [The strengths and limitations of this study were the same as for the study of cancer of the breast based on the same cohort (Biggar *et al.*, 2011).]

2.3 Cancer of the prostate

Cohort studies

See Table 2.4

Platz *et al.* (2011) examined the association between incidence of cancer of the prostate and use of digoxin in the USA-based Health Professionals Follow-up Study, following 47 884 men from 1986 until 2006. Data on use of digoxin were obtained by self-administered questionnaire at baseline and at 2-year intervals during follow-up. Ever-users of digoxin had lower incidence of cancer of the prostate compared with never-users, after adjustment for multiple risk factors, including race, body mass index, exercise, and smoking (RR, 0.83; 95% CI, 0.72–0.94), which was not changed by adjustment for other cardiovascular drugs (cholesterol-lowering agents, aspirin). The inverse association was seen regardless of indication for digoxin use (heart failure or arrhythmia), present when digoxin was the only cardiac medication used (other than aspirin), apparent at all stages of cancer of the prostate, and stronger in current than former users. The adjusted risk ratio for cancer of the prostate decreased with duration of use from 0.87 (0.73–1.04) for those with < 5 years of use to 0.54 (0.37–0.79) for those with ≥ 10 years of use (P for trend < 0.001). [This was regarded as a high-quality study with robust findings adjusted for an extensive array of covariates. Although exposure data were self-reported, reports by the health professionals were assumed to be of relatively high quality. Cancer outcomes were also self-reported, but validated by pathology-record review in 95% of cases.]

The association between cancer of the prostate and ever-use of drugs in the digitalis group was examined in the cohort study by Friedman & Ury (1980) and Friedman (1984), described in Section 2.1.2. The standardized morbidity ratio was 1.4 [95% CI, 1.00–2.01; 34 cases].

An increased risk of cancer of the prostate was also reported in the Norwegian cohort study by Haux *et al.*, (2001). The relative risk was 1.25 (95% CI, 1.03–1.50). [As noted in Section 2.1.2, relative risks were elevated for most of the cancers examined, leading to doubts about the appropriateness of the comparison group.]

2.4 Non-Hodgkin lymphoma

Case–control study

See Table 2.5

To determine whether the development of non-Hodgkin lymphoma is associated with medication use, Bernstein & Ross (1992) reviewed prescription-medication use in 619 cases of non-Hodgkin lymphoma in Los Angeles, USA, between 1979 and 1982, that were matched to 619 age, race, sex, and neighbourhood controls. Among 49 medications evaluated (along with many other health conditions and immunizations), the odds ratios for use of digitalis were 1.55 (95% CI, 0.99–2.43) for men and women combined, 2.4 (95% CI, 1.31–4.38) for women and 0.75 (95% CI, 0.36–1.59) for men. A trend with duration of use was found in women, but not in men. [Multiple comparisons were made with many drug- and non-drug-related variables, and the association with digitalis, seen only

Table 2.3 Cohort study on use of digoxin and cancer of the corpus uteri, cervix, and ovary

Reference, location, and period	Subjects	Exposure assessment	Organ sites	Exposed cases	Exposure categories	Relative risk (95% CI)	Adjustments for potential confounders Comments
Biggar *et al.* (2012), Denmark, 1995–2008	See Table 2.2 and Biggar *et al.* (2011)	Nationwide pharmacy registry	Corpus uteri	111	Former	1.20 (0.99–1.45)	Attained age, calendar year Association to digoxin found only for uterine cancer and statistically significant only in current users; marginal association for former users. For uterine cancer, increase greatest with prolonged use; For all, a higher incidence was noted in the first year after diagnosis, which could suggest confounding by indication
				350	Current	1.48 (1.32–1.65)	
					Duration of use (mo):		
				59	0–12	1.60 (1.23–2.07)	
				26	13–24	1.19 (0.81–1.75)	
				11	25–36	0.70 (0.39–1.27)	
				71	37+	1.91 (1.51–2.41)	
			Ovary	70	Former	0.95 (0.75–1.21)	
				207	Current	1.06 (0.92–1.22)	
					Duration of use (mo):		
				42	0–12	1.37 (1.01–1.86)	
				20	13–24	1.11 (0.71–1.72)	
				13	25–36	1.01 (0.58–1.74)	
				30	37+	1.02 (0.71–1.46)	
			Cervix uteri	36	Former	1.18 (0.85–1.65)	
				81	Current	1.00 (0.79–1.25)	
					Duration of use (mo):		
				18	0–12	1.44 (0.91–2.30)	
				8	13–24	1.10 (0.55–2.20)	
				5	25–36	0.96 (0.40–2.31)	
				8	37+	0.66 (0.33–1.32)	

mo, month

Table 2.4 Cohort study on use of digoxin and cancer of the prostate

Reference, location, and period	Subjects	Exposure assessment	Organ sites	Exposed cases	Exposure categories	Relative risk (95% CI)	Adjustments for potential confounders Comments
Platz *et al.* (2011), Health Professionals Follow-up Study, USA, 1985–2006	Men aged 40–75 years (*n* = 47 884)	Self-reported questionnaire data about current use of digoxin	Prostate, invasive cancer	4923	Never	1.0	Age, race, calendar year, BMI, height, smoking, diabetes, diet, exercise, vitamin E supplement Cohort analysis undertaken to assess effects observed in vitro (see Section 4). Cancer self-report supplemented with death-certificate data; pathology-record review: 94.5% complete.
				243	Ever	0.83 (0.72–0.94)	
				175	Current	0.78 (0.67–0.90)	
					Duration of use (yr):		
					Never	1.0	
				125	< 5	0.87 (0.73–1.04)	
				90	5–9.9	0.87 (0.70–1.07)	
				28	≥ 10	0.54 (0.37–0.79)	

BMI, body mass index; yr, year

Table 2.5 Case–control study on use of digitalis and non-Hodgkin lymphoma

Reference, location, and period	Subjects	Exposure assessment	Organ sites	Exposure categories	Exposed cases	Relative risk (95% CI)	Adjustments for potential confounders
Bernstein & Ross (1992), Los Angeles County (USA), 1979–82	Cases, 619 Controls, 619 (neighbourhood)	Personal interview and questionnaire including ever-use of "digitalis"	Non-Hodgkin lymphoma	No digitalis	35	1.00	Matched on age, sex, race, and neighbourhood
				Digitalis (all)	52	1.55 (0.99–2.43)	
				Men	12	0.75 (0.36–1.59)	
				Women	40	2.40 (1.31–4.38)	
				All (men and women)			
				No digitalis		1.00	
				Digitalis 1–12 mo	23	1.35 (0.99–2.43)	
				Digitalis ≥ 13 mo	28	1.68 (0.92–3.08)	
				P for trend		0.063	
				Men			
				No digitalis		1.00	
				Digitalis 1–12 mo	7	1.00 (0.35–2.85)	
				Digitalis ≥ 13 mo		0.56 (0.19–1.66)	
				P for trend		0.34	
				Women			
				No digitalis		1.00	
				Digitalis 1–12 mo	16	1.72 (0.76–3.91)	
				Digitalis ≥ 13 mo	23	3.05 (1.35–6.87)	
				P for trend		0.042	

mo, month

in women and not in men, could have been a chance finding.]

2.5 Other cancer sites

See Table 2.2

Elevated relative risks of cancers of the lung and colorectum were observed in the cohort study by Friedman & Ury (1980) and Friedman (1984), and in the cohort study by Haux *et al.* (2001) described in Section 2.1.2. The relative risk of cancer of the lung was 1.7 [95% CI, 1.22–2.20] in the former study, and 1.35 (95% CI, 1.04–1.74) in the latter. For cancer of the colorectum, the relative risks were 1.5 [95% CI, 1.02–2.04] and 1.29 (95% CI, 1.06–1.51) for the same studies, respectively. Haux *et al.* (2001) also reported an increased risk of leukaemia and lymphoma combined (RR. 1.41; 95% CI, 1.06–1.85). [The Working Group considered that the study by Haux *et al.* (2001) may have used an inappropriate comparison group, as noted in Section 2.1.2, and had limited confidence in the results. The elevated relative risk of cancer of the lung could be due to an association between smoking and cardiovascular disease for which digitalis was prescribed.]

3. Cancer in Experimental Animals

No data were available to the Working Group.

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Absorption and distribution

Digoxin exhibits first-order kinetics (Ehle *et al.*, 2011). In six healthy volunteers (average age, 20 ± 2.5 years) given a single infusion of digoxin of 750 µg for 20 minutes (Finch *et al.*, 1984), digoxin had a half-life of 37.2 ± 12 hours, an area under the curve (AUC) of concentration–time of 147.7 ± 78.6 ng/mL per hour, a large volume of distribution (311.4 ± 94.0 L) and clearance rate of 108.6 ± 59.1 mL/minute. In a study in four healthy men given 1 mg of tritium-labelled digoxin by intravenous injection (Marcus *et al.*, 1964), the drug disappeared very rapidly from the circulation; 3 minutes and 1 hour after the injection, only 15.9% and 2.8%, of the administered dose, respectively, was detected in the blood. The onset of pharmacological action, after intravenous administration, is detected within 15–30 minutes, and maximum effect within 1–4 hours (Ehle *et al.*, 2011).

The distribution of digoxin follows a two-compartment model (Reuning *et al.*, 1973), comprising plasma and rapidly equilibrating tissues (compartment one [small volume]), and the more slowly equilibrating tissues (compartment two [large volume]) (Currie *et al.*, 2011). Equilibrium between compartments is achieved after a minimum of 6 hours, distribution half-life is 35 minutes, onset of action (oral) approximately 30–120 minutes, and time to peak action (oral) is 6–8 hours (Currie *et al.*, 2011), or 2–6 hours, as reported by Ehle *et al.* (2011). Digoxin is 20–25% bound to plasma proteins (Ehle *et al.*, 2011).

After oral administration of digoxin, half-life and time to steady state vary significantly

between individuals, and are also dependent on renal function (Ehle *et al.*, 2011). In healthy subjects, the half-life is 1.5–2 days (Currie *et al.*, 2011; Ehle *et al.*, 2011), and steady state is reached in 5–7 days (Ehle *et al.*, 2011). In anuric patients, half-life is prolonged to 3.5–5 days (Currie *et al.*, 2011; Ehle *et al.*, 2011), and steady state is reached in up to 15–20 days (Ehle *et al.*, 2011). The volume of distribution is 4–7 L/kg in healthy subjects (Ehle *et al.*, 2011), but is decreased in people with renal disease and hypothyroidism, and increased in people with hyperthyroidism (Currie *et al.*, 2011). A study of 32 men and 35 women receiving long-term therapy with digoxin (in doses individualized according to body weight), showed no sex-based differences in serum concentration of digoxin (Lee & Chan 2006).

Oral bioavailability (F) of digoxin varies with formulation, and between individuals. Bioavailability from digoxin capsules, elixirs, or tablets are 90%, 80%, and 70%, respectively (Ehle *et al.*, 2011), and almost 100% from gelatine capsules (Currie *et al.*, 2011). Bioavailability of digoxin is physiologically controlled by the transmembrane transporter, P-glycoprotein, which has efflux pump function (Riganti *et al.*, 2011). P-glycoprotein controls bioavailability from its location on apical (or luminal) membranes of enterocytes of the small intestine, by active extrusion of digoxin, back into the lumen of gastrointestinal tract. A critical factor in intestinal absorption is the rate of apical efflux (Riganti *et al.*, 2011).

(i) *Studies supporting an effect of* MDR1 *polymorphism*

A study in 21 Caucasian individuals given a single oral dose of digoxin of 0.25 mg showed a correlation between polymorphism of the *MDR1* gene [the gene encoding P-glycoprotein, standard nomenclature, *ABCB1*] at exon 26 (C3435T) and significantly lower levels of duodenal expression and function of *MDR1*. Polymorphic individuals had higher plasma concentrations of digoxin compared with those with wildtype (C3435C) alleles (Hoffmeyer *et al.*, 2000).

In eight volunteers, pre-treatment with rifampicin, an inducer of P-glycoprotein, altered absorption of digoxin. The rifampicin-induced mean concentration of digoxin in people carrying the T-allele single-nucleotide polymorphism was higher than that of the wildtype (CC) population (Hoffmeyer *et al.*, 2000).

In healthy volunteers (with the TT and CC genotypes [$n = 7$ in each group]) given multiple oral doses of digoxin (0.25 mg per day) to achieve steady-state conditions, a statistically significant difference (mean, 38%) was found in maximum serum concentration of digoxin (C_{max}) between the two groups [read from Figure: CC, ~1.60 µg/L; TT, ~2.15 µg/L]. This may reflect the importance of genotype in determining absorption after oral administration of digoxin (Hoffmeyer *et al.*, 2000).

In 24 healthy Caucasian men who were homozygous carriers of the wildtype exon 26 C3435T (CC), or heterozygous (CT), or homozygous mutant (TT) [$n = 8$ in each group], AUC_{0-4h} ($P = 0.042$) and C_{max} ($P = 0.043$) differed significantly, with higher serum concentrations of digoxin in men with the 3435TT genotype than in those with wildtype C3435T (CC). No influence on digoxin parameters was detected for other single-nucleotide polymorphisms (Johne *et al.*, 2002).

Genotypes deduced from single-nucleotide polymorphism 2677G-T (exon 21) and 3435C-T, substantiated by haplotype analysis, also showed significant differences in AUC_{0-4h} and C_{max}. These analyses indicated that haplotype 12 (2677G/3435T) was associated with high values of AUC_{0-4h} and C_{max} for orally administered digoxin (Johne *et al.*, 2002).

In homozygous carriers of TT, kinetic parameters indicated a faster and more complete absorption of digoxin than in carriers of the wildtype. The digoxin plasma time course was evidenced by a 24% higher C_{max} and by a 22% higher AUC_{0-4h},

considered to result from increased rate (indicated by the steeper ascending phase of the curve in TT individuals) and extent of absorption (and not primarily of distribution) (Johne *et al.*, 2002).

High doses of digoxin are thought to saturate P-glycoprotein transport, triggering additional mechanisms. Thus, it is likely that at low doses, the pharmacokinetics of digoxin will be influenced by P-glycoprotein transport only, and thus would be more greatly perturbed by genetic differences in P-glycoprotein activity (Johne *et al.*, 2002).

A study of elderly patients in the Netherlands (n = 195; mean age, 79.4 years) who were taking digoxin regularly also showed that the common *MDR1* variants, 1236C-T, 2677G-T, and 3435C-T and the associated TTT haplotype were correlated with higher serum concentrations of digoxin (Aarnoudse *et al.*, 2008).

To understand the relative contribution of environmental and genetic factors to the pharmacokinetic variability of oral and intravenous digoxin, Birkenfeld *et al.* (2009) conducted a pilot study in 11 pairs of monozygotic twins (whose genes are almost identical), and 4 pairs of dizygotic twins (control). Measures of peak plasma concentration and T_{max} of digoxin, and calculated AUC, bioavailability, and renal clearance, after oral or intravenous administration, demonstrated strong correlation between monozygotic twins, findings explained largely by inheritance of P-glycoprotein function (Birkenfeld *et al.*, 2009).

(ii) Studies not supporting an effect of MDR1 *polymorphism*

Other studies have not shown an association between polymorphism in the *MDR1* gene and increased plasma concentrations of digoxin. A study in 114 healthy Japanese people given a single oral dose of digoxin of 0.25 mg (Sakaeda *et al.*, 2001) showed the serum concentration to be lower in those with a mutant allele (C3435T) at exon 26 of the *MDR1* gene. For the wildtype allele (CC), heterozygotes with a mutant T allele (C3435T) (CT), and homozygotes for the mutant allele (TT), values for AUC_{0-4h} (± standard deviation) were 4.11 ± 0.57, 3.20 ± 0.49, and 3.27 ± 0.58 ng/hour per mL, respectively. There was a significant difference between CC and CT or TT.

In a study in 39 Caucasian patients with congestive heart failure given digoxin at 0.25 mg per day for at least 7 days to reach steady state, Kurzawski *et al.* (2007) evaluated the effects of *MDR1* gene polymorphism on serum concentrations of digoxin, and in 24 patients, the effects of coadministration of digoxin with P-glycoprotein inhibitors. Significantly higher (approximately 1.5-fold) ($P < 0.002$) minimum serum concentrations of digoxin at steady state ($C_{min\,ss}$) were shown in patients given inhibitors of P-glycoprotein (0.868 ± 0.348 ng/mL), compared with those not given inhibitors (0.524 ± 0.281 ng/mL); however, in contrast to other studies, no association was found between 3435C > T and 2677G > A,T *MDR1* single-nucleotide polymorphisms and steady-state serum concentrations of digoxin (Kurzawski *et al.*, 2007).

A higher (1 mg) single oral dose of digoxin, without drug pre-treatment, in 50 healthy white men (aged 18–40 years) showed no differences in the AUC_{0-4h}, C_{max}, or t_{max} (as indices of digoxin absorption) among the genotype groups tested (Gerloff *et al.*, 2002). In contrast to previous reports (Hoffmeyer *et al.*, 2000), no differences were seen between homozygous carriers of the C and T allele in exon 26 3435 (AUC_{0-4h}, 9.24 and 9.38 mg/hour; C_{max}, 4.73 and 3.81 µg/L; t_{max}, 0.83 and 1.14 hours, respectively). The *MDR1* single-nucleotide polymorphisms studied, including that in exon 26, did not affect the absorption of a single oral dose of 1 mg of digoxin, and it was suggested that the higher dose (1 mg) of digoxin may have caused saturation of the transport capacity of intestinal P-glycoprotein. The pharmacokinetics of digoxin showed substantial variation within each genotypic group, indicating that factors additional to

P-glycoprotein may influence the absorption of digoxin (Gerloff *et al.*, 2002).

It is likely that passive diffusion (Gerloff *et al.*, 2002) or other transporters (Johne *et al.*, 2002), in addition to P-glycoprotein, contribute to variations in the pharmacokinetics of digoxin. Digoxin is a substrate for OATP8 (a member of the organic anion-transporting polypeptide group), for which genetic variants have been identified (Johne *et al.*, 2002), the effects of which, have not yet been elucidated. In addition, genetic variation in regulatory proteins, for example, the pregnane X receptor, involved in regulation of P-glycoprotein, may also affect digoxin disposition (Birkenfeld *et al.*, 2009). The absorption of digoxin may also be influenced by environmental factors (such as diet) by induction or inhibition of P-glycoprotein activity (Johne *et al.*, 2002; Gerloff *et al.*, 2002), or by genetic variants governing its distribution and elimination (Gerloff *et al.*, 2002).

(b) Metabolism

Gault *et al.* (1984) demonstrated a major metabolic sequence of digoxin hydrolysis, oxidation, and conjugation, leading to polar end-metabolites. In this study, 10 patients with end-stage renal failure (who were dependent on dialysis), and 5 patients with comparatively normal renal function were given digoxin (as an oral dose of 150 μCi of [^{3}H]digoxin-12α) and the metabolites were analysed by high-performance liquid chromatography (HPLC). Of these patients, 13 were receiving maintenance therapy with digoxin and were at steady state. The extent and time course of metabolism of digoxin varied between subjects, but variation was not significant between the two groups with different renal function. For all 15 patients, at 6 hours after drug administration, 26% (range, 7–76%) of the radiolabel was in the form of polar metabolites (quantitatively the most abundant metabolites), and 60% (range, 11–88%) was unchanged digoxin. Metabolites usually found albeit in small amounts were 3β-digoxigenin and its mono- and bis-digitoxosides, and 3-keto and 3α(epi)-digoxigenin.

This metabolic route comprised initial hydrolysis to 3β-digoxigenin with release of sugars in the stomach or liver, followed rapidly by oxidation to 3-keto-digoxigenin, epimerization to 3α(epi)-digoxigenin and finally glucuronide conjugation to polar species, 3-epi-glucuronide and 3-epi-sulfate. Results also indicated that conjugation of the mono-digitoxoside may occur, with steroid-ring hydroxylation, producing two isomers. In individuals demonstrating extensive metabolism, the lactone ring may be opened (possibly by a lactonase), forming a highly polar metabolite, or reduced, forming dihydro-metabolites (Gault *et al.*, 1984).

In studies using suspensions of freshly isolated human hepatocytes in vitro, metabolism of [^{3}H]digoxin-12α has been shown to be very low (Lacarelle *et al.*, 1991); after a 2-hour incubation, extracellular radiolabel represented largely unchanged digoxin (up to 93%), with a minor (5% of the total extracellular radiolabel) unidentified polar metabolite. Similar results were obtained over a 24-hour exposure time in cultured human hepatocytes, and also in human liver microsomal fractions, indicating that cleavage of digoxin sugars is not dependent on the cytochrome P450 (CYP) system that requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Lacarelle *et al.*, 1991; also see Fig. 4.1).

Digoxigenin mono-digitoxoside was extensively metabolized by human cultured hepatocytes to a single, more polar metabolite, which was subsequently completely hydrolysed by β-D-glucuronidase, and thus identified as the glucuronide of digoxigenin mono-digitoxoside. The extent of glucuronidation analysed in human liver microsomal fractions prepared from 13 different subjects was shown to vary among individuals by a factor of 3 (Lacarelle *et al.*, 1991).

Digoxigenin was also extensively biotransformed by cultured human hepatocytes. HPLC peaks were shown for one or more glucuronides,

3-epi-digoxigenin, unchanged digoxigenin, and possibly for unidentified metabolites. The intracellular concentration of 3-epi-digoxigenin decreased, due to conversion to polar compounds, which effluxed from the cells as formed. In human liver microsomes, no metabolites were observed in the absence of cofactor (NADPH or uridine 5′-diphospho-glucuronic acid, UDPGA); however, with NADPH present, "pre-digoxigenin" was detected. Formation of "pre-digoxigenin" therefore appeared to be CYP-dependent, with a large variability observed among individuals (Lacarelle *et al.*, 1991; also see Fig. 4.1).

In contrast, formation of 3-epi-digoxigenin did not depend on microsomal enzymes; it was only observed after incubation of digoxigenin with hepatocytes, and not with microsomes. In the presence of both NADPH and UDPGA, only small quantities of polar compounds were observed. These findings confirmed that 3-epi-digoxigenin is formed before synthesis of polar compounds. Thus, the main metabolic route for digoxigenin in vitro is the formation of 3-epi-digoxigenin, which is conjugated to a glucuronide (Lacarelle *et al.*, 1991; also see Fig. 4.1).

(c) Elimination

Recovery of digoxin in the urine was reported as 70–85% (Currie *et al.*, 2011) and 50–70% (Ehle *et al.*, 2011). Drug recovery in the faeces was, on average, 14.8% of the administered dose, of which 14% comprised metabolic products (Marcus *et al.*, 1964).

In a study of the mechanisms of intestinal and biliary transport of digoxin, eight healthy men (aged 21–37 years), were given segmental intestinal perfusion of a P-glycoprotein inhibitor (quinidine) or inducer (rifampin), with intravenous administration of digoxin (1 mg). Results showed that intestinal P-glycoprotein mediates the elimination of intravenously administered digoxin from the systemic circulation into the gut lumen, as well as the control of absorption of orally administered digoxin from the gastrointestinal tract. These data also demonstrated a non-renal mechanism of elimination of digoxin, entailing direct secretion into the small intestine from the systemic circulation, which had greater importance than elimination via bile (Drescher *et al.*, 2003).

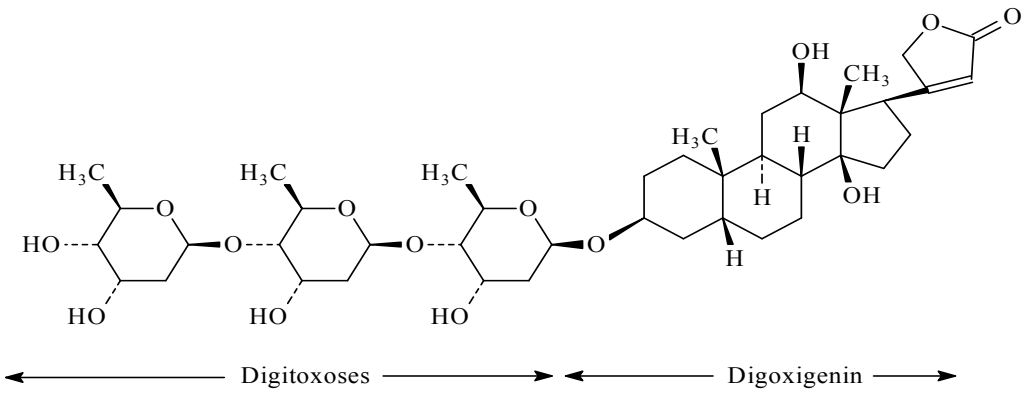
The organic anion transporter in human kidney (OATP4C1) may have an initial role in the transport of digoxin to the kidney. These transporters have been isolated, and shown by immunohistochemical analysis to be localized at the basolateral membrane of the proximal tubule cell in the kidney. Both human OATP4C1 and rat OATP4C1 transport digoxin in a sodium-independent manner (Mikkaichi *et al.*, 2004).

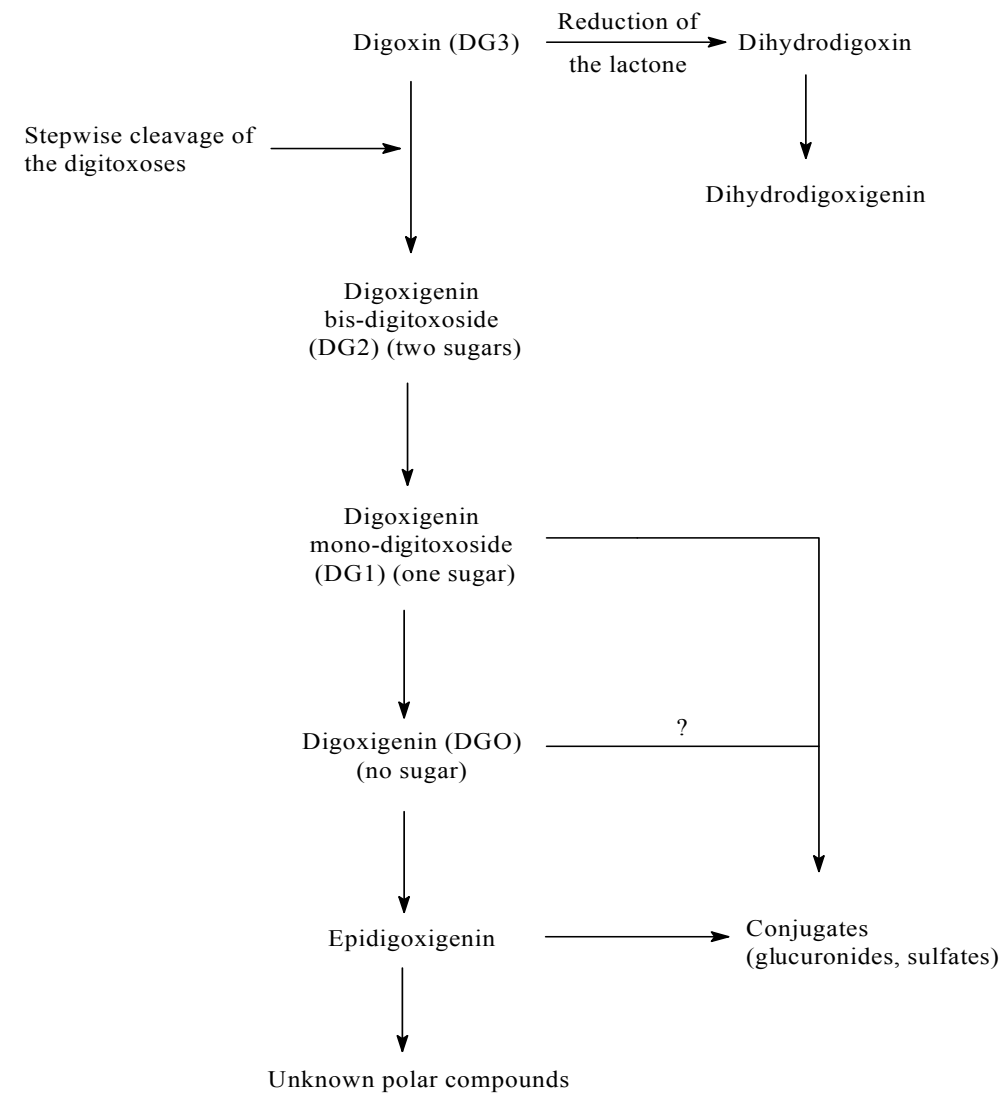
The role of OATPs in the disposition of digoxin has not been clearly defined. Data from various in-vitro systems have indicated that digoxin is not a substrate for human OATP1A2, OATP1B1, OATP1B3, or OATP2B1, although OATP4C1 may facilitate active uptake of digoxin into human kidney and liver. Digoxin is a substrate for a sodium-dependent transporter, shown to be endogenously expressed in a human kidney cell line (HEK29), and may, by its location in proximal tubular cells, partially facilitate renal clearance of digoxin (Taub *et al.*, 2011).

(d) Interactions

The bioavailability of digoxin is affected by concurrent administration of many drugs which compete for binding to P-glycoprotein. Thus, digoxin auto-regulates its absorption. Many lipophilic P-glycoprotein-inducing drugs also promote CYP3A activity, and so a complex, and poorly understood, network of interactions between drugs or endogenous metabolites may affect transport and metabolic inactivation of digoxin (Riganti *et al.*, 2011).

Fig. 4.1 Structure of digoxin and proposed metabolic pathways





From Lacarelle *et al.* (1991), Copyright © 1991, John Wiley and Sons

4.1.2 Experimental systems

(a) Absorption

The pharmacokinetics of digoxin was studied in male Sprague-Dawley rats given an intravenous bolus dose at 1 mg/kg bw. Plasma and urine samples were analysed by thin-layer chromatography to separate digoxin and its metabolites. Digoxin concentrations were described as a two-compartment model. Parent drug was rapidly eliminated from the plasma, with half-life of 2.5 hours, a volume of distribution of 3.6 L/kg, and a total body clearance of 5.77 mL/minute. Bile-duct ligation produced comparable pharmacokinetic parameters (with the exception of the total body clearance, 5.18 mL/minute). In rats with bilateral ureter ligation, the plasma half-life of digoxin was increased to 4 hours (Harrison & Gibaldi, 1976).

The function of P-glycoprotein in vivo has been investigated pharmacokinetically, using *mdr1a* (–/–) mice [*Abcb1a* (–/–)] (Schinkel *et al.*, 1995; Mayer *et al.*, 1996; Kawahara *et al.*, 1999). These mice show no major pathology, but their intestinal epithelium and brain endothelial cells have no detectable P-glycoprotein (Schinkel *et al.* 1995). Schinkel *et al.* (1995) demonstrated that concentrations of [^{3}H]digoxin in plasma and most tissues were twofold, and in brain were 35-fold, in *mdr1a* (–/–) mice given [^{3}H]digoxin intravenously compared with *mdr1a* (+/+) mice. Similarly, Kawahara *et al.* (1999) reported that digoxin accumulation in the brain was 68-fold higher. Mayer *et al.* (1996) further demonstrated that the brain concentrations of [^{3}H]digoxin continued to increase over 3 days after injection in *mdr1a* (–/–) mice, resulting in a 200-fold higher concentration than in *mdr1a* (+/+) mice. However, Kawahara *et al.* (1999) reported that disruption of the *mdr1a* gene did not to change plasma-protein binding or the blood-to-plasma partition coefficient.

Inhibition studies in vitro have shown that anionic transporters, in addition to P-glycoproteins, are involved in the absorption of digoxin (Yao & Chiou, 2006).

An additional non-*MDR1* component may contribute to active secretion of digoxin back into the lumen, to limit its intestinal absorption. In support of this, *MDR1*-transfected Madin-Darby canine kidney (MDCKII) cell monolayers showed reduced secretion of digoxin by the *MDR1* inhibitor cyclosporin A, but not by the *MDR1* inhibitor MK-571 (Lowes *et al.*, 2003).

(b) Metabolism

A proposed metabolic pathway for digoxin is shown in Fig. 4.1 (Lacarelle *et al.*, 1991).

In humans, more than 73% of an intravenous dose is excreted unchanged via the kidneys. In contrast, the rat metabolizes approximately 60% of an intraperitoneal dose, and approximately 30% is excreted via biliary and urinary routes (Harrison & Gibaldi, 1976).

Metabolism of digoxin follows a similar metabolic pathway in humans and rats, i.e. stepwise cleavage of the sugar residues to form the digoxigenin bis- and mono-digitoxoside and the aglycone digoxigenin before conjugation and elimination, but the rate is faster in rats (Harrison & Gibaldi, 1976).

The three sequential steps of oxidative metabolism of digoxin (to digoxigenin bis-digitoxoside, digoxigenin mono-digitoxoside, and digoxigenin) were studied in rat liver microsomes (Salphati & Benet, 1999). Inhibition of the CYP3A subfamily with ketoconazole or triacetyloleandomycin, or with antibodies specific to rat CYP3A2, affected oxidative metabolism; the formation of digoxigenin bis-digitoxoside and digoxigenin mono-digitoxoside decreased by up to 90%, and the rate of oxidation of digoxin and digoxigenin bis-digitoxoside was decreased by up to 85%, respectively. These oxidation reactions were unaffected by chemical or immunological inhibition of CYP2E1, CYP2C or CYP1A2. The subsequent metabolic step, i.e. oxidation of digoxigenin mono-digitoxoside, was not inhibited

by triacetyloleandomycin or by antibodies to CYP3A2, CYP2C11, CYP2E1, CYP2B1/2B2 or CYP1A2, but was however reduced (by > 80%) by inhibitors of human CYP3A. In summary, these results indicated that CYP3A, most likely CYP3A2, is the primary enzyme responsible for metabolism of digoxin and digoxigenin bis-digitoxoside in rat liver microsomes, but the enzyme that metabolizes digoxigenin mono-digitoxoside remains to be identified (Salphati & Benet, 1999).

(c) Elimination

Digoxin is eliminated primarily via the kidney through glomerular filtration and tubular secretion. P-glycoprotein has a role in the elimination of digoxin. Studies in vitro have demonstrated that mouse *mdr1a* and human *MDR1* P-glycoprotein actively transport digoxin across a polarized kidney epithelial cell layer (Schinkel *et al.*, 1995). Furthermore, experiments in vivo showed that *mdr1a* (–/–) mice eliminated [^{3}H]digoxin-12α more slowly (Schinkel *et al.*, 1995). The total body clearance was lower in *mdr1a* (–/–) mice than in the wildtype (+/+) mice; however, disruption of the *mdr1a* gene did not change the contributions of renal and bile clearances to total clearance (Kawahara *et al.*, 1999).

Digoxin is partly excreted via the biliary system. In male Sprague-Dawley rats, total body clearance values for digoxin were 10% lower in rats with bile-duct ligation, and were reduced by a further 30% by bilateral ureter ligation. The approximately 60% of total body clearance unaffected by ligations of bile duct or ureter were considered due to biotransformation of digoxin. A main excretory route for digoxigenin bis-digitoxoside was shown to be biliary as indicated by high levels of this metabolite in plasma and urine of rats with ligated bile ducts (Harrison & Gibaldi, 1976).

Intestinal P-glycoprotein in mice has been shown to contribute to excretion of [^{3}H]digoxin via the gastrointestinal epithelium. Mayer *et al.* (1996) demonstrated a shift in balance of excretion in *mdr1a* (–/–) mice given [^{3}H]digoxin (0.2 mg/kg bw) as a single intravenous or oral bolus, i.e. lower faecal elimination of [^{3}H]digoxin. This was due to reduced drug excretion via intestinal epithelium, since biliary excretion was not decreased in *mdr1a* (–/–) mice, and suggested that other transporters could be involved in the biliary excretion of digoxin. Indeed, the capacity for renal excretion remained substantial, and cumulative urinary excretion of digoxin in *mdr1a* (–/–) mice was greater than in wildtype (+/+) mice. Thus, intestinal P-glycoprotein acts by directly excreting digoxin into the intestinal lumen, and also limiting the rate of its re-uptake from the intestine by biliary excretion, thus directing faecal excretion (Mayer *et al.*, 1996). [P-glycoprotein seems to have important roles in elimination of digoxin from the systemic circulation, and also in decreasing intestinal re-uptake of digoxin after biliary excretion.]

4.2 Genetic and related effects

No data were available to the Working Group.

4.3 Other mechanistic data relevant to carcinogenicity

4.3.1 Effects on cell physiology

The physiological action of digoxin involves binding to and inhibition of the α-subunit of the Na^+/K^+ ATPase pump on the myocyte plasma membrane. This causes an increase in intracellular concentrations of sodium and calcium ions. Digoxin shares some structural homology with steroid hormones, suggesting functional similarities (Schussheim & Schussheim, 1998; Newman *et al.*, 2008). There is evidence that digitoxin at concentrations of $0.5–2.0 \times 10^{-6}$ M competes with estrogen for the estrogen cytosolic receptor in the rat uterus; however, no evidence for competition by digoxin was obtained (Rifka *et al.*, 1976; Rifka *et al.* 1978).

Other intriguing evidence for digoxin includes a case report of gynaecomastia (Aiman *et al.*, 2009), an increased relative risk of uraemic cancer in digoxin users (RR, 1.48; 45% CI: 1.32–1.65; n = 350) (Biggar, 2012), and lower relative risks of cancer of the prostate (RR, 0.76; 95% CI, 0.61–0.95) among regular users versus non-users (Platz *et al.*, 2011).

4.3.2 Effects on cell function

Digoxin reduces synthesis of the TP53 protein in human cancer cell lines; this appears to be triggered by activation of Src/mitogen-activated protein kinase signalling as a consequence of inhibition of the Na^+/K^+ ATPase pump (Wang *et al.*, 2009). Digoxin also inhibits the action of cellular DNA topoisomerases in MCF-7 cells (Bielawski *et al.*, 2006), and inhibits synthesis of hypoxia-inducible factor 1α (HIF-1α) in human Hep3B-c1 hepatoblastoma cells (Zhang *et al.*, 2008). Digoxin may inhibit synthesis of steroids (Kau *et al.*, 2005).

4.4 Susceptibility

4.4.1 Effects of age on elimination

Since young children require higher doses of digoxin per kilogram of body weight than adults to achieve pharmacological effects, there has been interest in whether expression of P-glycoprotein is age-dependent. Pinto *et al.* (2005) have studied *mdr1a* and *mdr1b* and the clearance rates of digoxin (dose, 7 µg/kg bw) in FVB mice of different ages (at birth, and age 7, 14, 21, 28 or 45 days). At birth and day 7, gene expression of *mdr1a* and *mdr1b* was very low, but mdr1b levels were significantly higher at day 21 than at days 14 or 28. Digoxin clearance rates correlated significantly with expression of P-glycoprotein, showing highest clearance values at day 21. It was concluded that increases in digoxin clearance rates after weaning may be attributed, at least in part, to similar increases in P-glycoprotein expression (Pinto *et al.*, 2005).

Evans *et al.*, (1990) showed that age affects the clearance of digoxin in rats. In male Fischer 344 rats (age, 4, 14, or 25 months) given [^{3}H]digoxin and unlabelled digoxin at a dose of 1 mg/kg bw as an intravenous bolus dose, total body clearance was 14.2, 12.1, and 7.5 mL/minute per kg, respectively, indicating a significant decrease in clearance ($P < 0.05$). No difference was seen in the terminal elimination half-life (2.0, 2.3, and 2.5 hours respectively) or steady-state volume of distribution (1.51, 1.49, and 1.27 L/kg, respectively) in rats aged 4, 14, and 25 months. Serum protein binding did not change with age; the average percentage of unbound digoxin for all rats was 61.3 ± 5.3% (mean ± standard deviation; n = 15) (Evans *et al.*, 1990).

4.4.2 Effects of renal failure on elimination

Tsujimoto *et al.* (2008) showed that, in contrast to normal serum, 10% uraemic serum inhibited the hepatic uptake of digoxin by human isolated hepatocytes (by 23%) and by rat hepatocytes (by 50%). It was further shown that the uraemic toxins 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), *p*-cresol, (both at 400 mM, which is within the plasma concentration range for patients with renal failure) and hippuric acid (at 3000 µM) significantly inhibited the uptake of digoxin. CMPF and *p*-cresol inhibited the uptake of digoxin into rat hepatocytes by 27% and 23%, respectively, and into human hepatocytes by 23% and 28%, respectively. These toxins were, however, not wholly responsible for inhibition of uptake. Indeed, 10% uraemic serum from patients contained these toxins at concentrations (CMPF, 37.6 mM; hippuric acid, 26.8 mM; and *p*-cresol, 19.5 mM) that may not have been sufficient to inhibit the uptake of digoxin. Additionally, the mechanism of inhibition of these toxins was competitive, while the inhibition shown by 10% uraemic serum was non-competitive. Thus, the

inhibitory effects of 10% uraemic serum cannot be fully explained by the three major uraemic toxins studied (Tsujimoto *et al.*, 2008).

4.5 Mechanistic considerations

The increase in the incidence of cancers of the breast and uterus after long-term treatment with digoxin (Biggar, 2012), and the observed estrogen-like side-effects of digoxin and digitoxin (Rifka *et al.*, 1976, 1978; Schussheim & Schussheim, 1998), suggested that digoxin and digitoxin act via estrogen-signalling pathways to increase cell proliferation in the mammary gland, potentially contributing to tumour development. However, mechanistic evidence was limited to a demonstration that digitoxin inhibited the binding of estradiol to specific, saturable binding sites in the rat uterine cytosol. Mammary epithelial cells contain several estrogen-binding proteins, including estrogen receptors (ERα and ERβ) and estrogen-related receptors (ERRα and ERRβ), and the signalling pathways linking receptor activation to cellular proliferation are complex (Gibson & Saunders, 2012). The molecular targets associated with the carcinogenic properties of digoxin and digitoxin have not yet been defined.

5. Summary of Data Reported

5.1 Exposure data

Digoxin is a glycoside isolated from *Digitalis lanata* and is used in the treatment of chronic heart failure and irregular heart rhythm. While use may have declined over the past 30 years, digoxin is still frequently prescribed. Global sales of digoxin were US$ 142 million in 2012, with 33% occurring in the USA. Other countries with appreciable use included Japan, Canada, and the United Kingdom.

Digitoxin, another glycoside isolated from *D. purpurea*, is used for the same indications as digoxin in certain countries; it is also found as an impurity in preparations of digoxin.

In most countries, use of "digitalis" would in practice almost always correspond to digoxin, unless digitoxin were specified.

Specifications for digitalis glycosides are provided in several international and national pharmacopoeia. In some countries, digoxin has been classified as a "hazard to water," an "environmental hazard," or as an "extremely hazardous substance."

5.2 Human carcinogenicity data

Studies in humans have assessed the risk of cancer in patients who may have used digoxin, digitoxin, or digitalis drugs as a group. The principal cancer of interest is cancer of the breast. Although risk of some other cancers has been found to be increased, the literature on other cancers was insufficient to establish patterns of increased risk.

5.2.1 Cancer of the breast

Information about the association of cancer of the breast with use of digoxin and digitoxin is available from four case–control studies (including two studies in men) conducted in four Nordic countries, France, and Switzerland, and a nationwide cohort study of women in Denmark, and other cohort studies in the USA and Norway.

Statistically significant increases in the occurrence of cancer of the breast in users of digoxin were seen in three case–control studies; in one study in women, the odds ratio was 1.3, while odds ratios were two- and fourfold in the two studies in men. The largest study, which included all women using digitalis in Denmark, reported an increased risk for current users (hazard ratio, 1.39). The positive associations with exposure to digoxin in this study were due to increased

risk in current users only: there was no association in former users and the number of new tumours declined after discontinuing drug use. Dose–response effects were difficult to examine because of the narrow dose range, and trends in risk with duration of exposure were generally not observed. In a case–case comparison among a subset of the same population, tumours occurring in digitalis users were reported to have more favourable prognostic features (estrogen receptor-positive) than in non-users. Data on the association of cancer of the breast with use of digitoxin were available from one cohort study in women in Denmark, which reported a positive association (relative risk, 1.39). These studies had limited ability to account for other risk factors for cancer of the breast, with obesity and alcohol drinking being of greatest concern.

5.2.2 Other cancer sites

Increases in the incidence of cancer of the uterus in current users of digoxin were found in one cohort study in Denmark. The same study found no increase in risk of cancers of the cervix and ovary. The risk of cancer of the prostate, another cancer that is influenced by hormones, was reduced in one high-quality cohort study from the USA, but increased in two others (one study with methodological weaknesses from Norway, and the other a very large database-screening programme from a health plan in northern California, USA). The increased risk of cancer of the uterus, and decreased risk of cancer of the prostate, is also consistent with a hormone-related mechanism, adding to the plausibility of the epidemiological findings.

Excess risks of cancers of the lung and colorectum were also observed in the cohort studies in Norway and northern California. The cohort study in Norway reported a positive association with leukaemia and lymphoma combined.

In a case–control study from southern California, USA, a positive association was observed with non-Hodgkin lymphoma in women, but not in men.

5.2.3 Synthesis

Statistically significant associations of cancer of the breast with use of digoxin were observed consistently in women and men, across different geographical regions, and with different study designs. Cancer of the breast is rare in men and strengthens the validity of association observed for cancer of the breast in women. The record-linkage studies that provided key evidence were not able to adjust for many of the recognized risk factors for cancer of the breast, notably obesity and alcohol drinking, although there was no reason to believe these would be associated with use of digoxin. Although clear effects with duration and dose were not observed, a decline in the detection of new tumours after cessation of exposure was seen in the largest study from Denmark, consistent with a possible promoting effect of digoxin. The association was specific to estrogen receptor-positive tumours of the breast in the same study.

5.3 Animal carcinogenicity data

No data were available to the Working Group.

5.4 Mechanistic and other relevant data

Oral bioavailability of digoxin is generally high, but varies due to interindividual genetic differences in expression of the efflux pump, P-glycoprotein.

The metabolism of digoxin in rats and humans involves stepwise hydrolytic cleavage of the digitoxoses to form digoxigenin bis- and mono-digitoxosides and the aglycone digoxigenin before conjugation and renal elimination.

No data were available on genetic effects of digoxin or its metabolites.

Digoxin has structural homology with steroid hormones, suggesting functional similarities. The structurally related glycoside digitoxin competes with estrogen for the rat uterine estrogen cytosolic receptor; however, no evidence for competition by digoxin was found.

Digoxin reduces synthesis of the TP53 protein in human cancer cells, inhibits cellular DNA topoisomerases, inhibits the synthesis of hypoxia-inducible factor 1α, and may inhibit synthesis of steroids.

The possible association between use of digoxin and an increased incidence of endocrine-related human cancers (primarily breast) suggests a mechanism that is estrogen receptor-mediated. However, evidence that digoxin and digitoxin act through estrogen-signalling pathways was limited to a demonstration that digitoxin inhibited the binding of estradiol to specific, saturable binding sites in rat uterine cytosol. The molecular targets associated with the carcinogenic properties of digoxin and digitoxin have not yet been identified.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

The Working Group recognized a possible association between digoxin and an increased incidence of endocrine-related human cancers. However, the evidence that digoxin and digitoxin act through an estrogen-receptor mediated mechanism was limited.

Favouring a *Group 2A* classification, the epidemiological data associating increased risk of cancer of the breast with use of digoxin were compelling. Consistent with an endocrine-mediated mechanism, the increase in risk was largely for estrogen receptor-positive tumours; further, risk of uterus cancer was increased and cancer of the prostate was decreased. The evidence in humans favoured a promoter effect that is seen only in current users.

Favouring a *Group 2B* classification, not all potential confounders were eliminated in the epidemiological studies, in particular, obesity. In addition, there were no available data from studies in experimental animals, and no known molecular mechanism by which digoxin might be a carcinogen. The weak evidence supporting an endocrine-mediated mechanism was noted as a problem.

References

Aarnoudse AJ, Dieleman JP, Visser LE, Arp PP, van der Heiden IP, van Schaik RH *et al.*(2008). Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. *Pharmacogenet Genomics*, 18(4):299–305. doi:10.1097/FPC.0b013e3282f70458 PMID:18334914

Ahern TP, Lash TL, Sørensen HT, Pedersen L (2008). Digoxin treatment is associated with an increased incidence of breast cancer: a population-based case-control study. *Breast Cancer Res*, 10(6):R102 doi:10.1186/bcr2205 PMID:19055760

Aiman U, Haseeen MA, Rahman SZ (2009). Gynecomastia: An ADR due to drug interaction. *Indian*

J Pharmacol, 41(6):286–7. doi:10.4103/0253-7613.59929 PMID:20407562

Albrecht HP, Geiss KH (2000). Cardiac glycosides and synthetic cardiotonic drugs. In: Ullmann's Encyclopedia of Industrial Chemistry. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; pp. 1–18. http://dx.doi.org/10.1002/14356007.a05_271 doi:10.1002/14356007.a05_271

Aromaa A, Hakama M, Hakulinen T, Saxén E, Teppo L, Idä lan-Heikkilä J (1976). Breast cancer and use of rauwolfia and other antihypertensive agents in hypertensive patients: a nationwide case-control study in Finland. *Int J Cancer*, 18(6):727–38. doi:10.1002/ijc.2910180603 PMID:992904

Banerjee D, Stafford RS (2010). Lack of improvement in outpatient management of congestive heart failure in the United States. *Arch Intern Med*, 170(15):1399–400. doi:10.1001/archinternmed.2010.270 PMID:20696970

Bernstein L, Ross RK (1992). Prior medication use and health history as risk factors for non-Hodgkin's lymphoma: preliminary results from a case-control study in Los Angeles County. *Cancer Res*, 52(19):Suppl: 5510s–5s. PMID:1394165

Bielawski K, Winnicka K, Bielawska A (2006). Inhibition of DNA topoisomerases I and II, and growth inhibition of breast cancer MCF-7 cells by ouabain, digoxin and proscillaridin A. *Biol Pharm Bull*, 29(7):1493–7. doi:10.1248/bpb.29.1493 PMID:16819197

Biggar RJ (2012). Molecular pathways: digoxin use and estrogen-sensitive cancers–risks and possible therapeutic implications. *Clin Cancer Res*, 18(8):2133–7. doi:10.1158/1078-0432.CCR-11-1389 PMID:22368159

Biggar RJ, Andersen EW, Kroman N, Wohlfahrt J, Melbye M (2013). Breast cancer in women using digoxin: tumor characteristics and relapse risk. *Breast Cancer Res*, 15(1):R13 doi:10.1186/bcr3386 PMID:23421975

Biggar RJ, Wohlfahrt J, Melbye M (2012). Digoxin use and the risk of cancers of the corpus uteri, ovary and cervix. *Int J Cancer*, 131(3):716–21. doi:10.1002/ijc.26424 PMID:21913187

Biggar RJ, Wohlfahrt J, Oudin A, Hjuler T, Melbye M (2011). Digoxin use and the risk of breast cancer in women. *J Clin Oncol*, 29(16):2165–70. doi:10.1200/JCO.2010.32.8146 PMID:21422417

Birkenfeld AL, Jordan J, Hofmann U, Busjahn A, Franke G, Krüger N *et al.*(2009). Genetic influences on the pharmacokinetics of orally and intravenously administered digoxin as exhibited by monozygotic twins. *Clin Pharmacol Ther*, 86(6):605–8. doi:10.1038/clpt.2009.170 PMID:19776737

British Pharmacopoeia (2009) British Pharmacopoeia. London, UK: Medicines and healthcare products regulatory agency.

Burchell HB (1983). Digitalis poisoning: historical and forensic aspects. *J Am Coll Cardiol*, 1(2 Pt 1):506–16. doi:10.1016/S0735-1097(83)80080-1 PMID:6338083

ChemicalBook (2013). ChemicalBook-Chemical Search Engine. Available from: http://www.chemicalbook.com/, accessed 10 February 2015.

Cheng JW, Rybak I (2010). Use of digoxin for heart failure and atrial fibrillation in elderly patients. *Am J Geriatr Pharmacother*, 8(5):419–27. doi:10.1016/j.amjopharm.2010.10.001 PMID:21335295

Currie GM, Wheat JM, Kiat H (2011). Pharmacokinetic considerations for digoxin in older people. *Open Cardiovasc Med J*, 5(1):130–5. doi:10.2174/1874192401105010130 PMID:21769303

Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA *et al.*; ESC Committee for Practice Guidelines (CPG)(2008). ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur Heart J*, 29(19):2388–442. doi:10.1093/eurheartj/ehn309 PMID:18799522

Digitalis Investigation Group(1997). The effect of digoxin on mortality and morbidity in patients with heart failure. *N Engl J Med*, 336(8):525–33. doi:10.1056/NEJM199702203360801 PMID:9036306

Drescher S, Glaeser H, Mürdter T, Hitzl M, Eichelbaum M, Fromm MF (2003). P-glycoprotein-mediated intestinal and biliary digoxin transport in humans. *Clin Pharmacol Ther*, 73(3):223–31. doi:10.1067/mcp.2003.27 PMID:12621387

DrugBank (2013). DrugBank: Open Data Drug & Drug Target Database. Available from: http://www.drugbank.ca/, accessed 10 February 2015.

Ehle M, Patel C, Giugliano RP (2011). Digoxin: clinical highlights: a review of digoxin and its use in contemporary medicine. *Crit Pathw Cardiol*, 10(2):93–8. doi:10.1097/HPC.0b013e318221e7dd PMID:21988950

EPA (2007). Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. Washington (DC): U.S. Environmental Protection Agency.

Eriksson M, Lindquist O, Edlund B (1984). Serum levels of digoxin in sudden cardiac deaths. *Z Rechtsmed*, 93(1):29–32. doi:10.1007/BF00202981 PMID:6495885

European Pharmacopoeia (2008). Digoxin. 7th ed. Strasbourg, France: European Directorate for the Quality of Medicines & HealthCare.

Evans RL, Owens SM, Ruch S, Kennedy RH, Seifen E (1990). The effect of age on digoxin pharmacokinetics in Fischer-344 rats. *Toxicol Appl Pharmacol*, 102(1):61–7. doi:10.1016/0041-008X(90)90083-7 PMID:2296772

Ewertz M, Holmberg L, Tretli S, Pedersen BV, Kristensen A (2001). Risk factors for male breast cancer–a

case-control study from Scandinavia. *Acta Oncol*, 40(4):467–71. doi:10.1080/028418601750288181 PMID:11504305

Fang MC, Stafford RS, Ruskin JN, Singer DE (2004). National trends in antiarrhythmic and antithrombotic medication use in atrial fibrillation. *Arch Intern Med*, 164(1):55–60. doi:10.1001/archinte.164.1.55 PMID:14718322

FDA (2013). Drugs@FDA. FDA Approved Drug Products. Available from: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/, accessed 8 September 2014

Ferrer I, Thurman EM (2012). Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. *J Chromatogr A*, 1259:148–57. doi:10.1016/j.chroma.2012.03.059 PMID:22487190

Finch MB, Johnston GD, Kelly JG, McDevitt DG (1984). Pharmacokinetics of digoxin alone and in the presence of indomethacin therapy. *Br J Clin Pharmacol*, 17(3):353–5. doi:10.1111/j.1365-2125.1984.tb02353.x PMID:6712868

Foss PRB, Benezra SA (1980). Digoxin. In: Florey K, editor. Analytical Profiles of Drug Substances. Academic press, vol 9, pp. 207–243.

Friedman GD (1984). Digitalis and breast cancer. *Lancet*, 324(8407):875 doi:10.1016/S0140-6736(84)90915-2 PMID:6148608

Friedman GD, Ury HK (1980). Initial screening for carcinogenicity of commonly used drugs. *J Natl Cancer Inst*, 65(4):723–33. PMID:6932525

Frommherz L, Köhler H, Brinkmann B, Lehr M, Beike J (2008). LC-MS assay for quantitative determination of cardio glycoside in human blood samples. *Int J Legal Med*, 122(2):109–14. doi:10.1007/s00414-007-0175-5 PMID:17569072

Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA *et al.*; American College of Cardiology/American Heart Association Task Force on Practice Guidelines; ; European Society of Cardiology Committee for Practice Guidelines; ; European Heart Rhythm Association; ; Heart Rhythm Society(2006). ACC/AHA/ESC 2006 Guidelines for the Management of Patients with Atrial Fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Circulation*, 114(7):e257–354. PMID:16908781

Gault MH, Longerich LL, Loo JC, Ko PT, Fine A, Vasdev SC *et al.*(1984). Digoxin biotransformation. *Clin Pharmacol Ther*, 35(1):74–82. doi:10.1038/clpt.1984.11 PMID:6690172

Gerloff T, Schaefer M, Johne A, Oselin K, Meisel C, Cascorbi I *et al.*(2002). MDR1 genotypes do not influence the absorption of a single oral dose of 1 mg digoxin in healthy white males. *Br J Clin Pharmacol*, 54(6):610–6. doi:10.1046/j.1365-2125.2002.01691.x PMID:12492608

Gibson DA, Saunders PT (2012). Estrogen dependent signalling in reproductive tissues - a role for estrogen receptors and estrogen related receptors. *Mol Cell Endocrinol*, 348(2):361–72. doi:10.1016/j.mce.2011.09.026 PMID:21964318

Goldin AG, Safa AR (1984). Digitalis and cancer. *Lancet*, 323(8386):1134 doi:10.1016/S0140-6736(84)92556-X PMID:6144872

Grabowski T, Świerczewska A, Borucka B, Sawicka R, Sasinowska-Motyl M, Gumułka SW *et al.*(2009). A rapid chromatographic/mass spectrometric method for digoxin quantification in human plasma. *Pharm Chem J*, 43(12):710–5. doi:10.1007/s11094-010-0384-y

Guan F, Ishii A, Seno H, Watanabe-Suzuki K, Kumazawa T, Suzuki O (1999). Identification and quantification of cardiac glycosides in blood and urine samples by HPLC/MS/MS. *Anal Chem*, 71(18):4034–43. doi:10.1021/ac990268c PMID:10500489

Harrison LI, Gibaldi M (1976). Pharmacokinetics of digoxin in the rat. *Drug Metab Dispos*, 4(1):88–93. PMID:3407

Hashimoto Y, Shibakawa K, Nakade S, Miyata Y (2008). Validation and application of a 96-well format solid-phase extraction and liquid chromatography-tandem mass spectrometry method for the quantitation of digoxin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*, 869(1–2):126–32. doi:10.1016/j.jchromb.2008.05.026 PMID:18515196

Haux J (1999). Digitoxin is a potential anticancer agent for several types of cancer. *Med Hypotheses*, 53(6):543–8. doi:10.1054/mehy.1999.0985 PMID:10687899

Haux J, Klepp O, Spigset O, Tretli S (2001). Digitoxin medication and cancer; case control and internal dose-response studies. *BMC Cancer*, 1(1):11 doi:10.1186/1471-2407-1-11 PMID:11532201

Haynes K, Heitjan D, Kanetsky P, Hennessy S (2008). Declining public health burden of digoxin toxicity from 1991 to 2004. *Clin Pharmacol Ther*, 84(1):90–4. doi:10.1038/sj.clpt.6100458 PMID:18091761

Hirabayashi H, Sugimoto H, Matsumoto S, Amano N, Moriwaki T (2011). Development of a quantification method for digoxin, a typical P-glycoprotein probe in clinical and non-clinical studies, using high performance liquid chromatography-tandem mass spectrometry: the usefulness of negative ionization mode to avoid competitive adduct-ion formation. *J Chromatogr B Analyt Technol Biomed Life Sci*, 879(32):3837–44. doi:10.1016/j.jchromb.2011.10.031 PMID:22098716

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A *et al.*(2000). Functional polymorphisms of the human multidrug-resistance gene:

multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA*, 97(7):3473–8. doi:10.1073/pnas.97.7.3473 PMID:10716719

Hollman A (1985). Plants and cardiac glycosides. *Br Heart J*, 54(3):258–61. doi:10.1136/hrt.54.3.258 PMID:4041297

HSDB (2013). Hazardous substance databank. National library of medicine. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB, accessed 10 February 2015.

Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG *et al.*(2009). 2009 focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. *Circulation*, 119(14):e391–479. doi:10.1161/CIRCULATIONAHA.109.192065 PMID:19324966

IARC (2012). Pharmaceuticals. Volume 100 A. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum*, 100:Pt A: 1–401. PMID:23189749

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

IMS Health (2012b). National Disease and Therapeutic Index (NDTI). Plymouth Meeting, Pennsylvania: IMS Health; 2004–12.

IMS Health (2012c). National Prescription Audit Plus (NPA). Plymouth Meeting, Pennsylvania: IMS Health; 2008–12.

Index Nominum (2013). International Drug Directory. Edited by the Swiss Pharmaceutical Society. Stuttgart, Germany: Medpharm Scientific Publishers.

Johne A, Köpke K, Gerloff T, Mai I, Rietbrock S, Meisel C *et al.*(2002). Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin Pharmacol Ther*, 72(5):584–94. doi:10.1067/mcp.2002.129196 PMID:12426522

Josephs RD, Daireaux A, Westwood S, Wielgosz RI (2010). Simultaneous determination of various cardiac glycosides by liquid chromatography-hybrid mass spectrometry for the purity assessment of the therapeutic monitored drug digoxin. *J Chromatogr A*, 1217(27):4535–43. doi:10.1016/j.chroma.2010.04.060 PMID:20537342

Kau MM, Kan SF, Wang JR, Wang PS (2005). Inhibitory effects of digoxin and ouabain on aldosterone synthesis in human adrenocortical NCI-H295 cells. *J Cell Physiol*, 205(3):393–401. doi:10.1002/jcp.20415 PMID:15887230

Kawahara M, Sakata A, Miyashita T, Tamai I, Tsuji A (1999). Physiologically based pharmacokinetics of digoxin in mdr1a knockout mice. *J Pharm Sci*, 88(12):1281–7. doi:10.1021/js9901763 PMID:10585223

Kirby BJ, Kalhorn T, Hebert M, Easterling T, Unadkat JD (2008). Sensitive and specific LC-MS assay for quantification of digoxin in human plasma and urine. *Biomed Chromatogr*, 22(7):712–8. doi:10.1002/bmc.988 PMID:18317988

Kleemann A (2012). Cardiovascular Drugs. In: Ullmann's Encyclopedia of Industrial Chemistry. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, pp. 1–18. http://dx.doi.org/10.1002/14356007.a05_289 doi:10.1002/14356007.a05_289

Kurzawski M, Bartnicka L, Florczak M, Górnik W, Droździk M (2007). Impact of ABCB1 (MDR1) gene polymorphism and P-glycoprotein inhibitors on digoxin serum concentration in congestive heart failure patients. *Pharmacol Rep*, 59(1):107–11. PMID:17377214

Lacarelle B, Rahmani R, de Sousa G, Durand A, Placidi M, Cano JP (1991). Metabolism of digoxin, digoxigenin digitoxosides and digoxigenin in human hepatocytes and liver microsomes. *Fundam Clin Pharmacol*, 5(7):567–82. doi:10.1111/j.1472-8206.1991.tb00746.x PMID:1778535

Lee LS, Chan LN (2006). Evaluation of a sex-based difference in the pharmacokinetics of digoxin. *Pharmacotherapy*, 26(1):44–50. doi:10.1592/phco.2006.26.1.44 PMID:16509025

Lenfant-Pejovic MH, Mlika-Cabanne N, Bouchardy C, Auquier A (1990). Risk factors for male breast cancer: a Franco-Swiss case-control study. *Int J Cancer*, 45(4):661–5. doi:10.1002/ijc.2910450415 PMID:2323842

Li S, Liu G, Jia J, Miao Y, Gu S, Miao P *et al.*(2010). Therapeutic monitoring of serum digoxin for patients with heart failure using a rapid LC-MS/MS method. *Clin Biochem*, 43(3):307–13. doi:10.1016/j.clinbiochem.2009.09.025 PMID:19833118

Li-Saw-Hee FL, Lip GY (1998). Digoxin revisited. *QJM*, 91(4):259–64. doi:10.1093/qjmed/91.4.259 PMID:9666948

Lowes S, Cavet ME, Simmons NL (2003). Evidence for a non-MDR1 component in digoxin secretion by human intestinal Caco-2 epithelial layers. *Eur J Pharmacol*, 458(1–2):49–56. doi:10.1016/S0014-2999(02)02764-4 PMID:12498906

Marcus FI, Kapadia GJ, Kapadia GG (1964). The metabolism of digoxin in normal subjects. *J Pharmacol Exp Ther*, 145:203–9. PMID:14214418

Mayer U, Wagenaar E, Beijnen JH, Smit JW, Meijer DK, van Asperen J *et al.*(1996). Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr 1a P-glycoprotein. *Br J Pharmacol*, 119(5):1038–44. doi:10.1111/j.1476-5381.1996.tb15775.x PMID:8922756

McNamara RL, Tamariz LJ, Segal JB, Bass EB (2003). Management of atrial fibrillation: review of the evidence for the role of pharmacologic therapy, electrical cardioversion, and echocardiography. *Ann Intern Med*, 139(12):1018–33.

doi:10.7326/0003-4819-139-12-200312160-00012 PMID:14678922

Medscape (2013). Medscape Drugs & Disease. Available from: http://reference.medscape.com/drug/lanoxin-digoxin-342432, accessed 5 June 2013.

Mikkaichi T, Suzuki T, Onogawa T, Tanemoto M, Mizutamari H, Okada M *et al.*(2004). Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney. *Proc Natl Acad Sci USA*, 101(10):3569–74. doi:10.1073/pnas.0304987101 PMID:14993604

Newman RA, Yang P, Pawlus AD, Block KI (2008). Cardiac glycosides as novel cancer therapeutic agents. *Mol Interv*, 8(1):36–49. doi:10.1124/mi.8.1.8 PMID:18332483

Oiestad EL, Johansen U, Stokke Opdal M, Bergan S, Christophersen AS (2009). Determination of digoxin and digitoxin in whole blood. *J Anal Toxicol*, 33(7):372–8. doi:10.1093/jat/33.7.372 PMID:19796507

Omidfar K, Kia S, Kashanian S, Paknejad M, Besharatie A, Kashanian S *et al.*(2010). Colloidal nanogold-based immunochromatographic strip test for the detection of digoxin toxicity. *Appl Biochem Biotechnol*, 160(3):843–55. doi:10.1007/s12010-009-8535-x PMID:19224402

Pähkla R, Irs A, Oselin K, Rootslane L (1999). Digoxin: use pattern in Estonia and bioavailability of the local market leader. *J Clin Pharm Ther*, 24(5):375–80. doi:10.1046/j.1365-2710.1999.00239.x PMID:10583701

Pellati F, Bruni R, Bellardi MG, Bertaccini A, Benvenuti S (2009). Optimization and validation of a high-performance liquid chromatography method for the analysis of cardiac glycosides in Digitalis lanata. *J Chromatogr A*, 1216(15):3260–9. doi:10.1016/j.chroma.2009.02.042 PMID:19268961

Pharmaceuticals and Medical Devices Agency (2011). The Japanese Pharmacopoeia Sixteenth Edition, English Version. Tokyo, Japan: Ministry of Health, Labour and Welfare. Available from: http://jpdb.nihs.go.jp/jp16e/jp16e.pdf, accessed 10 February 2015.

Pinto N, Halachmi N, Verjee Z, Woodland C, Klein J, Koren G (2005). Ontogeny of renal P-glycoprotein expression in mice: correlation with digoxin renal clearance. *Pediatr Res*, 58(6):1284–9. doi:10.1203/01.pdr.0000188697.99079.27 PMID:16306209

Platz EA, Yegnasubramanian S, Liu JO, Chong CR, Shim JS, Kenfield SA *et al.*(2011). A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. *Cancer Discov*, 1(1):68–77. doi:10.1158/2159-8274.CD-10-0020 PMID:22140654

PubChem (2013). Pubchem database. NCBI. Available from: https://pubchem.ncbi.nlm.nih.gov/, accessed 10 February 2015.

Rathore SS, Curtis JP, Wang Y, Bristow MR, Krumholz HM (2003). Association of serum digoxin concentration and outcomes in patients with heart failure. *JAMA*, 289(7):871–8. doi:10.1001/jama.289.7.871 PMID:12588271

Reuning RH, Sams RA, Notari RE (1973). Role of pharmacokinetics in drug dosage adjustment. I. Pharmacologic effect kinetics and apparent volume of distribution of digoxin. *J Clin Pharmacol New Drugs*, 13(4):127–41. doi:10.1002/j.1552-4604.1973.tb00074.x PMID:4487163

Rifka SM, Pita JC Jr, Loriaux DL (1976). Mechanism of interaction of digitalis with estradiol binding sites in rat uteri. *Endocrinology*, 99(4):1091–6. doi:10.1210/endo-99-4-1091 PMID:976189

Rifka SM, Pita JC, Vigersky RA, Wilson YA, Loriaux DL (1978). Interaction of digitalis and spironolactone with human sex steroid receptors. *J Clin Endocrinol Metab*, 46(2):338–44. doi:10.1210/jcem-46-2-338 PMID:86546

Riganti C, Campia I, Kopecka J, Gazzano E, Doublier S, Aldieri E *et al.*(2011). Pleiotropic effects of cardioactive glycosides. *Curr Med Chem*, 18(6):872–85. doi:10.2174/092986711794927685 PMID:21182478

Sakaeda T, Nakamura T, Horinouchi M, Kakumoto M, Ohmoto N, Sakai T *et al.*(2001). MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res*, 18(10):1400–4. doi:10.1023/A:1012244520615 PMID:11697464

Salphati L, Benet LZ (1999). Metabolism of digoxin and digoxigenin digitoxosides in rat liver microsomes: involvement of cytochrome P4503A. *Xenobiotica*, 29(2):171–85. doi:10.1080/004982599238722 PMID:10199593

Salvador A, Sagan C, Denouel J (2006). Rapid quantitation of digoxin in human plasma and urine using isotope dilution liquid chromatography-tandem mass spectrometry. *J Liquid Chromatogr Relat Technol*, 29(13):1917–32. doi:10.1080/10826070600757821

Saunders KB, Amerasinghe AK, Saunders KL (1997). Dose of digoxin prescribed in the UK compared with France and the USA. *Lancet*, 349(9055):833–6. doi:10.1016/S0140-6736(96)09057-5 PMID:9121258

Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P (1995). Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest*, 96(4):1698–705. doi:10.1172/JCI118214 PMID:7560060

Schussheim DH, Schussheim AE (1998). Is digoxin a designer oestrogen? *Lancet*, 351(9117):1734 doi:10.1016/S0140-6736(05)77771-0 PMID:9734913

SciFinder (2013). Digoxin: Regulatory Information. Columbus (OH): American Chemical Society. Available from: http://www.chemnet.com/cas/en/20830-75-5/digoxin.html, accessed 8 September 2014.

Sleeswijk ME, Van Noord T, Tulleken JE, Ligtenberg JJ, Girbes AR, Zijlstra JG (2007). Clinical review: treatment of new-onset atrial fibrillation in medical intensive care patients–a clinical framework. *Crit Care*, 11(6):233 doi:10.1186/cc6136 PMID:18036267

Stafford RS, Robson DC, Misra B, Ruskin J, Singer DE (1998). Rate control and sinus rhythm maintenance in atrial fibrillation: national trends in medication use, 1980–1996. *Arch Intern Med*, 158(19):2144–8. doi:10.1001/archinte.158.19.2144 PMID:9801182

Stenkvist B (1999). Is digitalis a therapy for breast carcinoma? *Oncol Rep*, 6(3):493–6. PMID:10203580

Stenkvist B, Bengtsson E, Dahlqvist B, Eriksson O, Jarkrans T, Nordin B (1982). Cardiac glycosides and breast cancer, revisited. *N Engl J Med*, 306(8):484 doi:10.1056/NEJM198202253060813 PMID:7057849

Stenkvist B, Bengtsson E, Eriksson O, Holmquist J, Nordin B, Westman-Naeser S (1979). Cardiac glycosides and breast cancer. *Lancet*, 1(8115):563 doi:10.1016/S0140-6736(79)90996-6 PMID:85158

Stuhlfauth T, Klug K, Fock HP (1987). The production of secondary metabolites by Digitalis lanata during CO2 enrichment and water stress. *Phytochemistry*, 26(10):2735–9. doi:10.1016/S0031-9422(00)83581-5

Sturm HB, van Gilst WH, Veeger N, Haaijer-Ruskamp FM (2007). Prescribing for chronic heart failure in Europe: does the country make the difference? A European survey. *Pharmacoepidemiol Drug Saf*, 16(1):96–103. doi:10.1002/pds.1216 PMID:16528759

Taub ME, Mease K, Sane RS, Watson CA, Chen L, Ellens H *et al.*(2011). Digoxin is not a substrate for organic anion-transporting polypeptide transporters OATP1A2, OATP1B1, OATP1B3, and OATP2B1 but is a substrate for a sodium-dependent transporter expressed in HEK293 cells. *Drug Metab Dispos*, 39(11):2093–102. doi:10.1124/dmd.111.040816 PMID:21849517

The International Pharmacopoeia (2011) 4th Edition. Geneva, Switzerland: World Health Organization.

Tracqui A, Kintz P, Ludes B, Mangin P (1997). High-performance liquid chromatography-ionspray mass spectrometry for the specific determination of digoxin and some related cardiac glycosides in human plasma. *J Chromatogr B Biomed Sci Appl*, 692(1):101–9. doi:10.1016/S0378-4347(96)00462-8 PMID:9187389

Tsujimoto M, Kinoshita Y, Hirata S, Otagiri M, Ohtani H, Sawada Y (2008). Effects of uremic serum and uremic toxins on hepatic uptake of digoxin. *Ther Drug Monit*, 30(5):576–82. doi:10.1097/FTD.0b013e3181838077 PMID:18708994

United States Pharmacopoeial Convention (2006) United States Pharmacopeial Convention, 30. Rockville (MD): The United States Pharmacopoeia Convention.

USP; US Pharmacopeia (2007) United States Pharmacopeial Convention. Rockville (MD), USA: United States Pharmacopeial Convention.

Varma MVS, Kapoor N, Sarkar M, Panchagnula R (2004). Simultaneous determination of digoxin and permeability markers in rat in situ intestinal perfusion samples by RP-HPLC. *J Chromatogr B Analyt Technol Biomed Life Sci*, 813(1–2):347–52. doi:10.1016/j.jchromb.2004.09.047 PMID:15556552

Vlase L, Popa D-S, Muntean D, Mihu D, Leucuta S (2009). A new, high-throughput high-performance liquid chromatographic/mass spectrometric assay for therapeutic level monitoring of digoxin in human plasma. *J AOAC Int*, 92(5):1390–5. PMID:19916377

Wang Z, Zheng M, Li Z, Li R, Jia L, Xiong X *et al.*(2009). Cardiac glycosides inhibit p53 synthesis by a mechanism relieved by Src or MAPK inhibition. *Cancer Res*, 69(16):6556–64. doi:10.1158/0008-5472.CAN-09-0891 PMID:19679550

Withering W (1785) An account of the foxglove and some of its medical uses: with practical remarks on dropsy, and other diseases. London, UK: G.G.J. and J. Robinson.

Yao HM, Chiou WL (2006). The complexity of intestinal absorption and exsorption of digoxin in rats. *Int J Pharm*, 322(1–2):79–86. doi:10.1016/j.ijpharm.2006.05.030 PMID:16781832

Yao M, Zhang H, Chong S, Zhu M, Morrison RA (2003). A rapid and sensitive LC/MS/MS assay for quantitative determination of digoxin in rat plasma. *J Pharm Biomed Anal*, 32(6):1189–97. doi:10.1016/S0731-7085(03)00050-5 PMID:12907263

Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR *et al.* (2008). Digoxin and other cardiac glycosides inhibit HIF-1α synthesis and block tumor growth. *Proc Natl Acad Sci USA*, 105(50):19579–86. doi:10.1073/pnas.0809763105 PMID:19020076