DOXEPIN 10 MG, 25 MG & 50 MG CAPSULES

2.4 Nonclinical Overview

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MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

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Abbreviations

3-MP	3-mercaptopropionic acid
5-HT	5-hydroxytryptamine (serotonin)
Aβ	β-amyloid
ACh	acetylcholine
API	active pharmaceutical ingredient
BIC	bicuculline
B_{max}	maximal binding capacity
BSA	bovine serum albumin
CBMN	cytokinesis-block micronucleus
CCI	chronic constriction injury
CNS	central nervous system
CRD	Centre for Reviews and Dissemination
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EC_{50}	50% efficient concentration
ED_{50}	50% efficient dose
EEG	electroencephalogram
EU	European Union
GI	gastrointestinal
Glu	glutamate
GPVI	glycoprotein VI
H_1	histamine-1 receptor
hERG	human ether-à-go-go-related gene
IC_{50}	50% inhibitory concentration
IL	interleukin
IP	intraperitoneal(ly)
IP3	inositol triphosphate
ISO	isoniazid
IV	intravenous(ly)
K_D	equilibrium dissociation constant
LC_{50}	50% lethal concentration
LD_{50}	50% lethal dose
LDH	lactate dehydrogenase
M	muscarinic receptor
MDA	malondialdehyde
MES	maximal electroshock

MTT	the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction
MN	micronucleus
NE	norepinephrine
NREM	non-rapid eye movement
PET	positron emission tomography
PI3K	Phosphoinositide 3-kinase
p-AKT	phosphorylated protein kinase B
p-mTOR	phosphorylated mammalian target of rapamycin
PO	per os (oral)
PSD-95	postsynaptic density protein 95
PTZ	pentylenetetrazole
SCE	sister chromatid exchange
SOD	superoxide dismutase
STR	strychnine
THIO	thiosemicarbazide
UK	United Kingdom
WT	wild type

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.1 Overview of the Nonclinical Testing Strategy

Doxepin 10 mg, 25 mg and 50 mg Capsules of Morningside Healthcare Ltd, United Kingdom (UK) are indicated for the treatment of symptoms of depressive illness, especially where sedation is required. Each type of capsules contains doxepin hydrochloride.

Doxepin hydrochloride is a dibenzoxepin derivative designated chemically as (E/Z)-3-(dibenzo[b,e]oxepin-11(6H)-ylidene)-N,N-dimethylpropan-1-amine hydrochloride (Fig. 1).

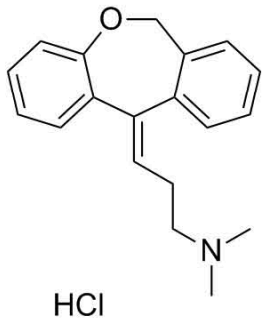

Figure 1 Structural formula of doxepin hydrochloride.

Doxepin is a psychotropic agent with tricyclic antidepressant and anxiolytic properties, and with antipruritic and sedative activities. Doxepin blocks the reuptake of norepinephrine (NE) and serotonin [5-hydroxytryptamine (5-HT)] into presynaptic terminals thereby prolonging the availability of the monoaminergic neurotransmitters within the synaptic cleft and enhancing their action leading to sedative effects ([redacted]). Doxepin has antagonistic effects on histamine-1 receptor (H_1) ([redacted]), 5-HT_2 ([redacted]), α1-adrenergic ([redacted]), and muscarinic (M) receptors ([redacted]), as well. The antipruritic effect of this agent is the result of inhibiting histamine receptors. Doxepin is a tertiary amine that can be presented as an (E) and (Z) stereoisomer from which the (Z) form corresponds to cidoxepin, also known as cis-doxepin or (Z)-doxepin, a tricyclic antidepressant. Doxepin is marketed as a mixture of geometric isomers such that the more active Z-isomer comprises only 15% of the total doxepin whereas the less active E-isomer makes up the remaining 85%. Doxepin is commonly associated with tricyclic antidepressants as it shares many properties with some of the members of this family such as amitriptyline, clomipramine, desipramine, imipramine, nortriptyline, protriptyline and trimipramine.

Depressive disorders are characterized by sadness severe or persistent enough to interfere with function and often by decreased interest or pleasure in activities ([redacted]). The exact cause is unknown but probably involves heredity, changes in neurotransmitter levels, altered neuroendocrine function, and psychosocial factors ([redacted]). Diagnosis is based on history ([redacted]). The term depression is often used to refer to any of several depressive disorders. Some are classified in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) by specific symptoms ([redacted]) such as

a) Major depressive disorder (often called major depression)
b) Persistent depressive disorder (dysthymia)
c) Other specified or unspecified depressive disorder.

Others are classified by aetiology such as

a) Premenstrual dysphoric disorder
b) Depressive disorder due to another medical condition
c) Substance/medication-induced depressive disorder.

Depressive disorders occur at any age but typically develop during the mid-teens, 20s, or 30s ([redacted]). In primary care settings, as many as 30% of patients report depressive symptoms, but less than 10% have major depression ([redacted]).

Doxepin was discovered in Germany in 1963 and was introduced in the United States (US) as an antidepressant in 1969 ([redacted]). In 2010, it was approved at very low doses for the treatment of insomnia in the US ([redacted]).

The excipients in Doxepin 10 mg, 25 mg and 50 mg Capsules of Morningside Healthcare Ltd, UK are approved and established agents in widespread use in the pharmaceutical manufacturing industry.

This Nonclinical Overview has been prepared upon a systematic search through [redacted]) to identify peer-reviewed articles evaluating the relevant preclinical pharmacodynamic, pharmacokinetic and safety/tolerability aspects of using doxepin hydrochloride as active pharmaceutical ingredient (API) in Doxepin 10 mg, 25 mg and 50 mg Capsules of Morningside Healthcare Ltd, UK. This systematic search using the keywords 'doxepin' yielded altogether [redacted] publications. The search terms were used for all fields (including title, abstract, keywords and full text. The search was narrowed by using additional keywords such as '[redacted]', '[redacted]', '[redacted]',

[redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted]. [redacted] was used for permitting [redacted] of the used terms.

This nonclinical overview has been prepared to review the relevant preclinical pharmacodynamic, pharmacokinetic and safety/tolerability aspects of using of doxepin hydrochloride as active ingredient in Doxepin 10 mg, 25 mg and 50 mg Capsules of Morningside Healthcare Ltd, UK. All pharmacodynamic, pharmacokinetic and toxicological studies described in this Nonclinical Overview were retrieved from publicly available literature.

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2.4 Nonclinical Overview

2.4.2 Pharmacology

2.4.2.1 Pharmacology and Mode of Action (Primary Pharmacodynamics)

2.4.2.1.1 Mechanism of action

Doxepin is similar in potency to amitriptyline and imipramine but is of lesser potency than desipramine in blocking the NE uptake into the rat heart ([redacted]). Unlike desipramine, it did not block guanethidine-induced pressor blood pressure responses in the cat ([redacted]). In contrast, in isolated rabbit heart, doxepin was much more potent than amitriptyline, desipramine or imipramine in inhibiting NE uptake ([redacted]). At concentrations of 10^{-4} M to 10^{-3} M, doxepin inhibited the *in vitro* uptake of NE into rat brain slices by about 30 to 40% in the hypothalamus, midbrain and pons/medulla oblongata areas, while by almost 60% in the striatum ([redacted]). In this respect, its effects seemed to be markedly weaker than those of imipramine reported in other studies. Experiments with rat ([redacted]) and rabbit ([redacted]) blood platelets have established that doxepin and desipramine compared with amitriptyline, imipramine and most of their congeners, are weak inhibitors of 5-HT uptake ([redacted]). This order of potency prevailed when 5-HT uptake into rat brain synaptosomes was studied ([redacted]). Most of the activity of doxepin in this test was exhibited by the *trans*-isomer, which comprises 85% of the commercially available drug, for the *cis*-isomer was virtually ineffective ([redacted]). These findings suggest that doxepin and desipramine would only be considered weak antidepressants based on 5-HT uptake data. Doxepin inhibits voltage-dependent K^+ channels in a concentration-dependent manner, but not in use- and/or state-dependent manners, regardless of 5-HT–NE reuptake inhibition ([redacted]). Doxepin antagonized 5-HT-induced behavioural syndrome characterised by head twitches ([redacted]). The 50% efficient dose (ED_{50}) for rats and mice was 4.43 (1.65-11.9) mg/kg and 3.22 (1.51-6.85) mg/kg, respectively. Doxepin potentiates the synaptic inhibitory effect of biogenic amines to a similar (NE) or greater [dopamine (DA)] extent than imipramine ([redacted]). Doxepin and imipramine were indistinguishable in their dose-dependent potentiation of the inhibitory effects of NE on electrically-induced postganglionic potentials in the superior cervical ganglion of the cat, but doxepin was significantly more potent than imipramine in its potentiation of the less pronounced DA-induced suppression of ganglionic transmission. Doxepin (3 mg /kg) had only a limited effect on potentiating pressor responses to NE in conscious rabbits; being similar in activity to amitriptyline

(2.5 mg/kg), but less active than protriptyline (2.5 mg/kg) or nortriptyline (2.5 mg/kg) ([redacted]).

It has been hypothesized that depressive illness are linked to a change in the form of human monoamine oxidase B (MAO_B). Doxepin, like other tricyclic antidepressant drugs, showed a greater affinity for the B- than for the A-form of rabbit lung mitochondrial MAO ([redacted]). It was of similar potency to protriptyline, but was only about half as potent as amitriptyline in inhibiting the deamination of phenethylamine (substrate for B form) or 5-HT (substrate for A form). Doxepin also inhibited human platelet MAO deamination of phenethylamine (to a greater extent than the B form of rabbit lung oxidase) at concentrations similar to those required in other studies of amitriptyline or imipramine.

In vitro studies of the anticholinergic activity of doxepin have used models of the central and peripheral muscarinic receptor binding in rat brain homogenates and the guinea pig ileum ([redacted]). Doxepin was less potent at both receptors by a factor of 4 than was amitriptyline, which had an affinity for central receptors of 10 nM and for peripheral receptors of 27nM (about $1/20^{th}$ of atropine in each case). Doxepin was a competitive inhibitor of [^{3}H]-mepyramine (a first generation antihistamine, targeting the H_1 receptor) binding to guinea-pig cerebellar homogenates. The affinity constant derived for doxepin at 30°C was $1.12 \pm 0.45 \times 10^8$ 1/M ([redacted]). Hill coefficients for curves of doxepin or mepyramine inhibition of [^{3}H]-mepyramine binding in guinea-pig cerebellum, cerebral cortex and hippocampus did not differ significantly from unity.

The specific binding of [^{11}C]doxepin, which has been used as a radioligand for mapping histamine H_1 receptors in human brain by positron emission tomography (PET), was evaluated in five animal species ([redacted]). In mice the [^{11}C]doxepin uptake was reduced by treatment with cold doxepin and two H_1 receptor antagonists, but not by H_2/H_3 antagonists. The specific binding evaluated with treatment with (+)-chlorpheniramine (H_1 antagonist) was in the range of 10–30% in mouse, rat, rabbit, and monkey, but was not detected in guinea pig. [^{3}H]Doxepin has two saturable binding sites for H_1 receptors with higher and lower affinities in brains of wild-type mice, but H_1-deficient mice showed only the weak labelling of [^{3}H]doxepin that corresponds to lower affinity binding sites ([redacted]). The binding of [^{3}H]doxepin to rat brain was examined ([redacted]). The equilibrium dissociation constant (K_D) of the high-affinity site was 0.020 nM with a maximal binding capacity (B_{max}) of 13.7 fmol/mg protein. The corresponding values for the low-affinity site were 3.6 nM and 740 fM/mg protein, respectively.

The effect of doxepin (5×10^{-8} to 2×10^{-5} M) on the resting overflow of tritium, the evoked overflow and the contractile response to electrical stimulation (2.5 Hz, 2.0 ms) was determined in mouse vas deferens previously incubated with [^{3}H]-(-)noradrenaline ([redacted]). Doxepin increased resting overflow at high concentrations (2×10^{-5} M) but produced only small changes in evoked overflow and in the contractile response at lower concentrations. At low concentration (5×10^{-7} M), doxepin produced a fall in the contractile response.

Using *in vitro* receptor binding techniques, the effects of doxepin on rat cerebral cortex receptors were investigated ([redacted]). The 50% inhibitory concentration (IC_{50}) values for all compounds obtained in the various receptor assays are summarized in Table 1.

Table 1 Effects of doxepin on rat brain receptors *in vivo*

α_1 WB4101	α_2 PAC	M QNB	5-HT_1 5-HT	5-HT_2 Spiroperidol (cortex)	D_2 Spiroperidol (striatum)	ß DHA	H_1 Mepyramine
0.030	1.83	0.177	1.81	0.093	2.51	46.7	0.0098

The IC_{50}-values (µM) were calculated using a log-logit regression analysis of values falling in the range 5% < x < 95%. Two or more experiments were used for each drug. M = muscarinic receptor; PAC = aminoclonidine; DHA = dihydroalprenololhydrochloride; D_2 = dopamine-2 receptor

2.4.2.1.2 Antidepressant activity

In rats and mice, intraperitoneal (IP) doses of doxepin 5 to 40 mg/kg produced a dose-dependent reversal of reserpine-induced catalepsy and ptosis, though open-field behaviour in reserpine-treated animals was unaffected ([redacted]). Doxepin was less potent than amitriptyline and imipramine in antagonizing reserpine-induced hypothermia in mice, with a major metabolite of doxepin, desmethyldoxepin, being as active as doxepin itself and the *cis* isomer of doxepin being more active than its *trans* isomer. In another study, both the *cis* and *trans* isomers were as active as doxepin itself in antagonizing reserpine-induced hypothermia in mice. The central depressant actions of tetrabenazine were reversed by doxepin. In animal behaviour tests of antidepressant activity, doxepin was about twice as potent as imipramine in potentiating the stimulant action of levodopa given with the monoamine oxidase inhibitor pargyline in mice.

The effect of doxepin was tested on forced swimming-induced despair behaviour in mice ([redacted]). Clonidine, B-HT 920 and guanfacine significantly prolonged the total immobility duration. Doxepin (10 mg/kg, IP) reversed clonidine-induced behavioural despair.

2.4.2.1.3 Tranquillizing and Sedative Properties

Doxepin exerts sedative or stimulant effects in animals depending upon the dose of drug, though sedative effects predominate ([redacted]). At doses of 6.25 to 12.5mg/kg, IP, doxepin stimulated spontaneous locomotor activity in mice, but higher doses of 20 to 100 mg/kg depressed the central nervous system (CNS), causing ataxia and reduced motor activity. Desmethyldoxepin was more active than doxepin in inhibiting spontaneous locomotor activity in mice. Doses of up to 50 mg/kg, PO produced similar effects in dogs, and were generally associated with parasympathetic stimulation and mydriasis. The sedative effect of doxepin was about equal to that of chlordiazepoxide in inhibiting spontaneous motility and curiosity in mice, as well as rearing and emotional defecation in the open field test in rats.

Although doxepin inhibited amphetamine-induced stereotypy in rats, especially compulsive gnawing, it did not antagonize apomorphine-induced stereotypy and did not produce catalepsy in rats ([redacted]); thus ruling out neuroleptic activity. At high (50 mg/kg) and lower (5 to 10 mg/kg) doses in mice and at lower doses in rats (5 to 25 mg/kg), doxepin inhibited amphetamine-induced hyperactivity, whereas the hyperactivity was enhanced with high doses (50 mg/kg) in rats. In another study in rats, doses of 20 mg/kg of both doxepin and in particular its *cis* isomer enhanced amphetamine-induced hyperactivity, whereas the *trans* isomer tended to antagonize the hyperactivity.

Doxepin suppressed conditioned avoidance responses in rats only in large doses (> 40 mg/kg), which caused muscle relaxation, whereas chlordiazepoxide, thioridazine and chlorpromazine inhibited conditioned responses at doses that caused sedation ([redacted]). In mice, 10 to 30 mg/kg doses of doxepin produced a progressively greater suppression of avoidance behaviour. The effect of 10 mg/kg doxepin was similar to that of amitriptyline but more marked than that of desipramine ([redacted]). The *cis* isomer of doxepin appears to be slightly more potent than doxepin or its *trans* isomer in inhibiting conditioned avoidance behaviour ([redacted]). In operant conditioning schedules in rats, doxepin produced a dose-dependent depression of food-reinforced responses, equipotent with amitriptyline and imipramine but more potent than butriptyline ([redacted]). Unlike diazepam, pentobarbital, chlorpromazine and haloperidol, the avoidance-reinforced response was only slightly depressed by doxepin and the other tricyclic antidepressants and even then at the highest doses.

H_1 receptor knockout mice were used to investigate if the sleep-promoting effects of doxepin and diphenhydramine are dependent on blockade of the H_1 ([redacted]). When doxepin was administered, non-rapid eye movement (NREM) sleep in wild type

(WT) mice increased for 4 h, with an increase in the numbers of NREM sleep bouts of 256–512 s and 512–1024 s. These effects were not observed in the H_1 knockout mice. Furthermore, diphenhydramine increased NREM sleep for 6 h in WT, and not in the H_1 knockout mice after the injection. These results indicate that both doxepin at 15 mg/kg and diphenhydramine at 10 mg/kg induce NREM sleep through blockade of H_1. The *cis* isomer of doxepin was more active than its *trans* isomer in potentiating urethane-induced sedation ([redacted]). Doxepin and amitriptyline were equipotent in prolonging hexobarbital-induced sleep in mice, with the *cis* isomer being more potent than doxepin or its *trans* isomer. Spontaneous electrical activity in monkey brains was depressed by doxepin in a similar way to that seen with amitriptyline.

The effects of doxepin were assessed in punishment and pentylenetetrazol drug discrimination paradigms used to identify antianxiety activity in rats ([redacted]). In the punishment procedure, doxepin was not found to have an effect on the mean fixed ratio (punished) response.

2.4.2.2 Secondary Pharmacodynamics

Doxepin possesses peripheral anticholinergic activity as demonstrated by the production of mydriasis in mice, and central anticholinergic activity as shown by the protection of mice against the toxic effects of the cholinesterase inhibitor paraoxon ([redacted]); with desmethyldoxepin being less active than doxepin and the *cis* isomer of doxepin being more active than the *trans* isomer. Doxepin was less potent than amitriptyline, but more potent than imipramine, in producing mydriasis in mice and in blocking methacholine-induced mortality in mice, with the *cis* isomer being more potent than doxepin or its *trans* isomer.

At concentrations of less than 1 µg/mL, doxepin inhibited spasm induced by 5-HT (1.5 µg/mL), histamine (2.5 µg/mL), acetylcholine (ACh, 0.2 µg/ml and barium chloride (100 µg/ml) in isolated guinea pig ileum ([redacted]). The antagonism of 5-HT and ACh was less pronounced in isolated guinea pig trachea than in ileum. The anticholinergic action was relatively weak in both preparations compared with the antagonism of 5-HT or histamine, and this order of potency prevailed in intact guinea pigs with bronchospasm induced by the three transmitter substances. Doxepin also caused a 25 to 100% inhibition of epinephrine-induced contractions of rabbit aortic strips at concentrations of 0.001 and 0.1 µg/mL respectively, with inhibition of angiotensinamide-induced contractions only at considerably higher (100 µg/mL) concentrations ([redacted]). Doxepin is less potent than amitriptyline in inhibiting ACh-induced spasm in isolated guinea pig ileum with the cis isomer being more potent than doxepin and its *trans* isomer. Doxepin (1 µM) was more potent as postsynaptic α-adrenoceptor antagonists than in inhibiting responses to ACh ([redacted]

[redacted]). Doxepin is a potent inhibitor of prostaglandin E_2 and $F_{2\alpha}$ biosynthesis in guinea pig lung ([redacted]).

The effects of doxepin on β-amyloid (Aβ)-induced memory impairment and neuronal toxicity were studied ([redacted]). Rats were treated with Aβ1-42 and doxepin was injected to validate its effects on cognitive function by the Morris water maze test. Aβ1-42-treated SH-SY5Y human neuroblastoma cell line was also used to detect the effects of doxepin and to explore the underlying mechanism. After treatment with 1 mg/kg of doxepin, Aβ1-42-treated rats showed markedly lower escape latency and higher platform-finding strategy score. Low doses of doxepin significantly reversed the effects of Aβ1-42 on the protein expression levels of postsynaptic density protein 95 (PSD-95), synapsin 1, phosphorylated protein kinase B (p-AKT) and phosphorylated mammalian target of rapamycin (p-mTOR) in rats. Phosphoinositide 3-kinase (PI3K) inhibitor (LY294002) treatment could markedly reversed the effects of doxepin on Aβ1-42-treated SH-SY5Y cells. The protective effect of doxepin was associated with the enhancement of PSD-95 and synapsin 1 expression via PI3K/AKT/mTOR signaling pathway.

Stimulation of the collagen receptor glycoprotein VI (GPVI) leads to phospholipase Cγ2-dependent inositol triphosphate (IP3) production with subsequent platelet activation, due to increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Platelet adhesion, activation, and aggregation are essential for primary haemostasis, but are also critically involved in the development of acute arterial thrombotic occlusion. Doxepin inhibits GPVI-dependent platelet Ca^{2+} signalling and collagen-dependent thrombus formation ([redacted]).

The protective effect of doxepin on cultured neuronal injury induced by oxidative stress was investigated ([redacted]). Exposure of cultured neurons to glutamate (Glu 0.5 mmol/L, 15 min), sodium dithionite dithionite (0.5 mM/L, 24 h) or haemoglobin (Hb100 mg/L, 24 h) developed neurotoxicity expressed in the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction (MTT) assay, the increase of lactate dehydrogenase (LDH) leakage, malondialdehyde (MDA) content and intracellular $[Ca^{2+}]_i$ accumulation, as well as the decrease of superoxide dismutase (SOD) activity. Doxepin 1-100 nM/L significantly inhibited all above changes thereby protected cultured neurons against oxidative stress-induced injury by suppressing intracellular $[Ca^{2+}]_i$ accumulation, decreasing lipid peroxide generation and stimulating antioxidant enzyme.

The effects of doxepin were investigated in a mouse model of neuropathic pain to determine the role of cytokine activation in the effects of this drug ([redacted]). In the Albino-Swiss mice subjected to chronic constriction injury (CCI), doxepin (10

mg/kg) attenuated the symptoms of neuropathic pain and diminished the CCI-induced increase in the levels of spinal interleukin (IL)-6 and -1b mRNA, but not the protein levels of these cytokines, measured on Day 12. Chronic administration of doxepin for 12 days produced allodynia and hyperalgesia in naive mice that may be associated with an increase in the levels of pronociceptive cytokines resulting from 5-HT_3-induced hypersensitivity.

2.4.2.3 Safety Pharmacology

Cardiaovascular effects

In most animal species, intravenous (IV) doxepin generally lowers blood pressure, increases heart rate and provokes cardiac arrhythmias ([redacted]). Intracardiac conduction blockade may be responsible for the arrhythmic effects due to NE potentiation ([redacted]), while anticholinergic effects appear to play a minor part. In cumulative doses up to about 9 to 15 mg/kg in mice, doxepin caused tachyarrhythmias, leading at higher doses (> 20 mg/kg) to a progressive and finally lethal bradycardia ([redacted]). ß-adrenoceptor blocking drugs, but not atropine or physostigmine, produced dose-dependent inhibition of doxepin-induced tachyarrhythmias in mice, and none of the drugs prevented or postponed death. Chronic treatment of rats with doxepin (10 mg/kg, IP) decreased the specific binding of ^{3}H-dihydroalprenolol, a ß-adrenergic blocker, by 30.6% as compared to control ([redacted]). This observation suggests that the resistance of adenylate cyclase to noradrenaline following chronic administration of antidepressants is due to subsensitivity of noradrenergic ß-receptors ([redacted]). In anaesthetized dogs, doxepin produced a dose-related decrease in blood pressure and total peripheral resistance, a slight and transient increase in cardiac output, and a slight increase in heart rate. Intraarterial doxepin was more potent than papaverine in increasing femoral arterial blood flow in dogs ([redacted]). In the isolated cat heart, doxepin 50 to 200 µg/mL produced a transient increase in coronary blood flow and a transient negative inotropic effect, which were maximal within 30 seconds of administration. The rate of contractions was decreased slightly by doxepin 200 µg/mL ([redacted]). Doxepin produced significant hypotension in conscious rabbits ([redacted]). It is unlikely that an anticholinergic component is involved in the cardiotoxic effects of doxepin, since in rodents its tachyarrhythmic effects were unaffected by large doses of atropine or physostigmine, but blocked by ß-adrenoceptor antagonists. In anaesthetized cats, doxepin 5mg/kg did potentiate pressor responses to NE, and in common with most other tricyclic antidepressants, it reduced the pressor effect of epinephrine ([redacted]).

The effect of doxepin on human ether-à-go-go-related gene (hERG) channels was investigated ([redacted]). hERG encodes channels responsible for the cardiac rapid delayed rectifier potassium current, I_{Kr}. Whole-cell patch-clamp recordings were made of recombinant HERG channel current (I_{HERG}), and of native I_{Kr} 'tails' from rabbit ventricular myocytes. Doxepin inhibited I_{HERG} with an IC_{50} value of 6.5 ± 1.4 μM and native I_{Kr} with an IC_{50} of 4.4 ± 0.6 μM. The inhibitory effect on I_{HERG} developed rapidly upon membrane depolarization, but with no significant dependence on voltage and with little alteration to the voltage-dependent kinetics of I_{HERG}. Neither the S631A nor the N588K inactivation-attenuating mutations reduced significantly the potency of inhibition. The S6 point mutation Y652A increased the IC_{50} for I_{HERG} blockade by about 4.2-fold; the F656A mutant also attenuated doxepin's action at some concentrations. HERG channel blockade is likely to underpin reported cases of QT interval prolongation with doxepin. This study also establishes doxepin as an effective inhibitor of mutant (N588K) HERG channels responsible for variant 1 of the short QT syndrome.

Nervous system effects

The anticonvulsant characteristics of doxepin were evaluated in numerous experimental seizure models, including maximal electroshock (MES)-, pentylenetetrazole (PTZ)-, isoniazid (ISO)-, 3-mercaptopropionic acid (3-MP)-, bicuculline (BIC)-, thiosemicarbazide (THIO)-, and strychnine (STR)-induced seizures ([redacted]). In addition, the acute adverse-effect profile of doxepin with respect to impairment of motor coordination was assessed with a mouse rotarod test. The evaluation of the time-course and dose-response relationships for doxepin provided evidence that the peak maximum anticonvulsant activity and acute adverse effects occurred 5 min after IP administration. The results also revealed that doxepin had excellent anticonvulsant activity against maximal electroshock-induced seizures in mice with a median ED_{50} of 6.6 mg/kg. The assessment of acute adverse effects in the rotarod test showed that doxepin-induced acute neurotoxicity, and its median toxic dose (TD_{50}) was 26.4 mg/kg. Additionally, doxepin showed anticonvulsant activity in several chemically-induced seizure models, including ISO, 3-MP, BIC, and THI.

Doxepin produced atropine-like central effects in conscious rabbits ([redacted]). It produced dose-dependent mydriasis and electroencephalogram (EEG) synchronisation, at levels of 1 to 5 mg/kg, IV, without producing overt signs of sleep. Similar levels of doxepin also inhibited in a dose-dependent manner the EEG activation produced by physostigmine or methamphetamine, but its influence on the behavioural effects of methamphetamine was, like that of atropine, enhancement.

Effects on locomotor activity depended on the dose. At doses of 6.25 to 12.5mg/kg in mice, doxepin potentiated spontaneous locomotor activity, whereas higher doses (20 to 50mg/kg) inhibited hyperactivity ([redacted]).

Doxepin prevented convulsions induced by pentylenetetrazole or maximal electroshock in mice, with ED_{50} values of 7.6 to 20 mg/kg, IP ([redacted]).

Tricyclic antidepressants can diminish pain reaction in laboratory animals. In a test involving induced rabbit dental pain, doxepin, amitriptyline, imipramine and trimipramine were more potent than nortriptyline, protriptyline and desipramine but less potent than morphine ([redacted]).

Gastrointestinal effects

Doxepin inhibited gastric acid secretion when given intracerebroventricularly or IV in pylorus-ligated rats, which is indicative of a site of action in the CNS ([redacted]). Doxepin may inhibit gastric acid secretion via a central mechanism presumably involving suppression of vagal (parasympathetic) outflow to the gastric mucosa.

Doxepin at 5, 10, 20, and 30 mg/kg significantly reduced deprivation-induced fluid consumption in a standardized test system to 80.8 ± 17.0, 70.3 ± 10.6, 48.3 ± 17.1, and 21.7 ± 14.7 %, respectively, as compared to pre-drug level taking it 100% ([redacted]).

Doxepin similarly to amitriptyline inhibited the insulin release from the perfused rat pancreas, but to a lesser extent than cyproheptadine ([redacted]). Both early and late insulin secretion were suppressed when induced by a high glucose stimulus.

2.4.2.4 Pharmacodynamic Drug Interactions

Isobolographic analysis for equivalent doses of drugs was applied to examine the nature of interaction between tramadol and doxepin in a neuropathic pain model in rats ([redacted]). Allodynia and hyperalgesia were assessed after IP administration of each drug alone or in combination. Doxepin was effective in reducing thermal hyperalgesia and mechanical allodynia. Combined administration of tramadol and doxepin demonstrated synergistic action in reducing thermal hyperalgesia and additive action in reducing mechanical allodynia.

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.3 Pharmacokinetics

2.4.3.1 Absorption

Following administration of single oral (PO) doses to rats and dogs, doxepin is well absorbed and plasma levels reach a maximum within 30 minutes to 1 hour, thereafter declining rapidly ([redacted]). Plasma levels of unchanged drug are low. In 3 dogs dosed over 5 days, plasma levels of doxepin and desmethyldoxepin usually reached a plateau by Day 2 or 4 ([redacted]). Plasma levels of doxepin and its metabolites were still detectable 3 days after the last dose in the dogs given 100 mg daily for 5 days or rats given 50 mg/kg/day for 5 days.

2.4.3.2 Distribution

Initially, drug levels are high in the liver, kidney, spleen and lung ([redacted]), but initial brain levels do not appear to be as high as reported following similar doses of amitriptyline or imipramine. In rabbits, concentrations in the heart are 40 to 200 times greater than those measured in the plasma at the same time. Appreciable amounts of the active metabolite desmethyldoxepin are also found in tissues, and other metabolites in liver and urine, but only this demethylated metabolite and doxepin itself are found in brain. Doxepin appears to have an affinity for melanin of the eye where it is still detectable for up to 70 days after a single oral dose, but i*n vitro* studies with beef eyeball show it to be less strongly bound than either amitriptyline or chlorpromazine ([redacted]). Plasma and tissue concentrations of doxepin tend to increase with repeated or continued administration of the drug to rats. Tissue levels rapidly decline after administration is discontinued. Increases in tissue concentration are most marked, compared with single-dose administration, in liver, kidney, fat, muscle and eyeball, but there is no difference between light and dark skin. A semilogarithmic plot of radioactivity in some tissues vs. time indicates multiphasic loss of material (Fig. 2).

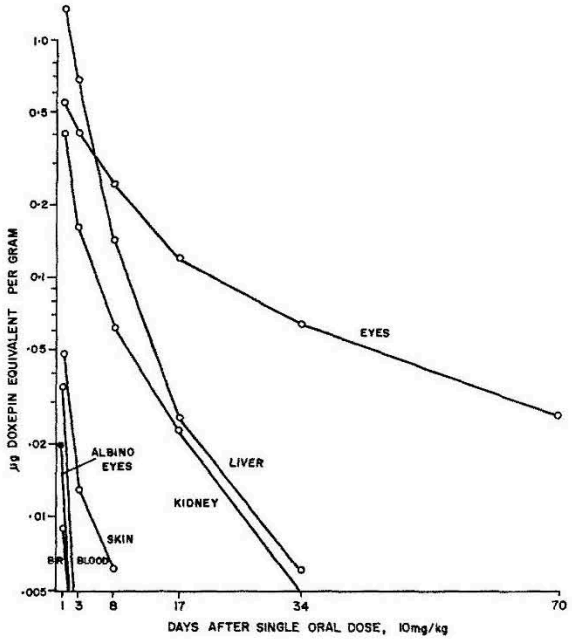

Figure 2 Tissue levels of radioactivity in the rat after a single oral dose of doxepin (10 mg/kg).

2.4.3.3 Metabolism

Identified metabolites in urine, faeces and bile in rats include desrnethyldoxepin, doxepin-N-oxide, and a hydroxydoxepin and its glucuronide derivative. In dogs, the same metabolites have been recovered in urine as well as desmethyl hydroxydoxepin, but appreciable quantities of unchanged drug are also excreted ([redacted]). The major routes of metabolism of imipramine and amitriptyline involve hydroxylation on the aromatic rings, hydroxylation on the ethylene bridge, N-demethylation, and N-oxidation. In addition, the side chain can be removed from imipramine; such a reaction has not been reported for amitriptyline where the side chain is joined to carbon (as in doxepin) rather than to nitrogen. The major route in the rat appears to be N-oxide formation and hydroxylation; a portion of the hydroxydoxepin is subsequently converted to the glucuronide. In the do, most of the hydroxydoxepin is conjugated and appreciable quantities of unchanged drug and N-oxide are excreted. In addition, there are also uncharacterized polar metabolites in the urine of both species. The metabolic transformations of doxepin are summarized in Fig. 3.

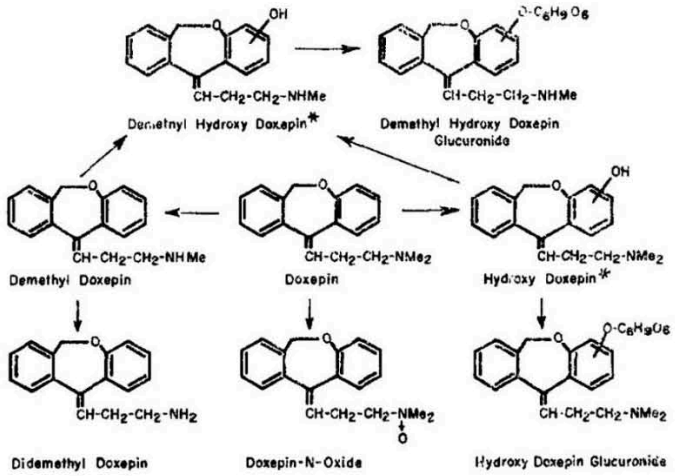

Figure 3 Doxepin metabolites. *Hydroxylation could also be on other aromatic ring.

There was no evidence of Z/E interconversion after administration of the pure doxepin isomers to rat *in vivo*, or after incubation of rat or human liver homogenates with pure isomers ([redacted]). *In vitro* data suggested that the distortion of the Z:E ratio of N-desmethyldoxepin was a consequence of faster metabolism of the E-isomer in comparison with Z-N-desmethyldoxepin rather than 'enrichment' of the Z-isomer at the expense of the E-isomer.

2.4.3.4 Elimination

The administered drug is excreted mainly in the urine. In the rat, 60% of the radioactivity administered appears in the 24-hour urine following single oral dosage, with about 25% in the faeces ([redacted]). Most of the dose in rats appears to be eliminated in the urine within 8 hours; very little is excreted in bile. Excretion of unchanged doxepin is low, less than 5% in rats ([redacted]). The excretion of radioactive material in the urine and faeces determined in rats after oral administration of doxepin is given in Table 2.

Table 2 Excretion of radioactivity by hooded rats after oral administration of labelled doxepin

Excreta	Time (h)	% of Dose
Urine	0 – 24	56.76 ± 2.27
	24 – 48	2.24 ± 0.53
	48 – 120	1.14 ± 0.39
	Total	60.13 ± 2.24
Faeces	0 – 24	24.30 ± 1.59
	24 – 48	1.47 ± 0.33
	48 – 120	0.77 ± 0.24
	Total	26.55 ± 1.97
Total	86.68 ± 1.96	88.51 ± 4.47

Four animals per group received 10 mg/kg. Results are expressed as % of dose administered (mean ± SD).

The kidney appeared to be the major excretory organ, the urine containing 60 % of the radioactivity administered. The similarity in excretory patterns after the two routes of administration indicates that doxepin was rapidly and completely absorbed from the gastrointestinal (GI) tract.

After examination of four animal species (dog, rabbit, guinea pig, rat), rat was closest to human in terms of the Z:E ratio of the geometric isomers of N-desmethyldoxepin excreted in the 0 ± 24-h urine ([redacted]). Changes in the urinary Z:E ratio of the metabolite were observed after PO but not after IV or IP administration to rat.

2.4.3.5 Pharmacokinetic Drug Interactions

The effect of short-term oral administration of doxepin on voluntary drinking of ethanol was studied in the rat as a function of sex ([redacted]). The effects of doxepin on ethanol and acetaldehyde metabolizing enzymes of hepatic and selected endocrine tissues were made in the presence and absence of ethanol. A reduction in voluntary ethanol intake was determined after the initial doxepin dose. This effect was not apparent during continued drug administration. Subsequently, a statistically insignificant reduction of ethanol drinking was noted 24 h and 72 h post-drug termination. Hepatic alcohol and aldehyde dehydrogenase were not altered from respective controls by doxepin in the presence and absence of ethanol. Epididymal and testicular aldehyde dehydrogenase were inhibited by doxepin from control in rats maintained on ethanol or water, respectively. The results suggest lack of adverse effect of doxepin on peripheral metabolism of alcohol metabolizing enzymes as compared to adverse interaction with acetaldehyde metabolizing enzyme in the endocrine tissues studied.

The binding of doxepin to bovine serum albumin (BSA) was investigated by spectroscopic (fluorescence, UV–vis absorption and circular dichroism) techniques ([redacted]). Doxepin interacted with BSA by both hydrogen bond and hydrophobic interactions. The biological significance of this is evident since albumin serves as a carrier molecule for multiple drugs and the interaction of doxepin may modify their pharmacokinetic profile.

The adsorption of doxepin onto cholestyramine was demonstrated *in vitro* with use of

1.2 M/L HCl at 37°C to simulate gastric fluid ([redacted]). Binding to cholestyramine was approximately 80% for doxepin, and this was about the same degree of binding noted with a non-pharmaceutical, non-ionic resin widely used in the diagnostic toxicology laboratory (Amberlite XAD-2). This suggests that cholestyramine should be used with caution in patients receiving doxepin. It also

suggests that cholestyramine may be a potentially useful adjunctive therapy in treatment of overdose with doxepin.

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.4 Toxicology

2.4.4.1 Single Dose Toxicity

The 50% lethal dose (LD_{50}) values of doxepin in various animal species are shown in Table 3 by different routes of administration ([redacted]).

Table 3 LD_{50} values of doxepin in various animal species by different routes of administration

Species	Route	LD_{50} (mg/kg)
Rat	PO	346 - 460
	IV	18.8
Mouse	PO	148 - I78
	IV	14.6 - 19.6
Dog	PO	200
	IV	16

Administration of a toxic dose of doxepin usually results in death within 5 minutes when given IV and within 1 hour when given PO. Toxic signs in all species generally include CNS effects such as ataxia, general and respiratory depression, tremors, convulsions, prostration then death. Peripheral vasodilatation and/or constriction, piloerection and exophthalmia, have been noted occasionally, while in dogs, urination and defecation. Vomiting and extensor rigidity have also been noted. The cause of death was severe cardiac arrhythmia. Pulmonary oedema was also noted.

2.4.4.2 Repeat Dose Toxicity

In a 5-week study, in which rats were given 25, 50, 100, 150, or 200 mg/kg, PO of doxepin daily, normal haematological and urinalysis values were measured ([redacted]). Most of the animals died at the highest dose levels, some of them at the dose of 100 mg/kg daily and one animal at the dose of 50 mg/kg daily. A decrease in body weight gain occurred in the higher dose groups, the effect being more pronounced in males than females. No adverse effects were detected on autopsy or following microscopic examination.

In a 6-month chronic toxicity study in rats, no adverse effects were detected at 5, 10 and 20 mg/kg, PO daily doses, while higher doses of 80 mg/kg in males and females, and 40 mg/kg in males, caused a decrease in weight gain but no deaths. Aspiration lipoid pneumonia was seen on microscopic examination in sacrificed animals in the 40 and 80 mg/kg daily groups

In a 12-month chronic toxicity study in rats, slight hepatic fatty metamorphosis was observed in rats given doxepin at 50 mg/kg, PO daily dose ([redacted]).

No macroscopic, microscopic, haematological or biochemical changes were observed in dogs given 25 – 50 mg/kg, PO daily for 30 days. Mild sedation and vomiting occurred at a dose of 25 mg/kg. At a dose of 50 mg/kg, increased heart rate, miosis, sedation and twitching were observed. In a 12-month chronic toxicity study in dogs, ptosis, sedation, tremors and vomiting occurred at 50 mg/kg, PO daily dose ([redacted]). There were occasional episodes of vomiting at 25 mg/kg, PO but those given 5 mg/kg, PO were practically asymptomatic. There were no abnormal laboratory test values.

Toxic signs in all species generally include CNS effects such as ataxia, general and respiratory depression, tremors, convulsions, prostration then death ([redacted]).

2.4.4.3 Genotoxicity

There is no evidence indicating mutagenic potential. Doxepin was negative in *in vitro* (bacterial reverse mutation, chromosomal aberration in human lymphocytes) and *in vivo* (rat micronucleus) assays ([redacted]).

The potential genotoxic and cytotoxic effects of doxepin were studied *in vitro* on human peripheral lymphocytes cytokinesis-block micronucleus (CBMN), sister chromatid exchange (SCE), and single cell gel electrophoresis (alkaline comet assay) ([redacted]). Four different concentrations of doxepin (1, 2.5, 5, and 10 mg/mL) were administered to human peripheral lymphocytes for 24 h. The tested concentrations were found to exhibit no cytotoxic and mitotic inhibitory effects. SCE increase caused by 5 and 10 mg/mL of escitalopram was found statistically significant, while no statistically significant increase was observed in DNA damage and micronucleus (MN) formation. Doxepin (10 mg/mL) significantly increased arbitrary unit and SCE formation. These findings suggest that the investigated concentrations of doxepin were non-cytotoxic but at higher concentrations potentially genotoxic.

2.4.4.4 Carcinogenicity

No evidence of carcinogenic potential was observed when doxepin was administered orally to hemizygous Tg.rasH2 mice for 26 weeks at doses of 25, 50, 75 and 100 mg/kg/day. Doxepin has shown no oncogenic effect when administered daily to rats for 104 weeks at doses of 10, 30 and 75 mg ([redacted]).

2.4.4.5 Reproductive and Developmental Toxicity

There were no changes observed in litter size, number of live births or lactation in animals (species not stated) given doxepin at daily doses of up to 25 mg/kg, PO for 8 or 9 months ([redacted]). A decreased conception rate resulted when male rats were given doxepin 25mg/kg, PO daily for prolonged periods (time not stated). This effect has also been observed in animals given other psychotherapeutic agents. Macroscopic and microscopic examination of the offspring revealed no evidence of drug-related dysmorphogenic effects. However, at dosages of 90 and 270 mg/kg, PO (a dose level greatly exceeding the maximum safety level) from Day 9 to Day 14 of gestation in pregnant rats, either death, miscarriage or reduction in body weight occurred in the dams and the mortality of foetuses at term was high, with a reduced body weight in live foetuses in the 270 mg/kg, PO group. The birth rate and survival rate at 3 weeks after birth was also decreased in the 270 mg/kg group. These effects were not observed in pregnant rats given 10 or 30 mg/kg, PO. No dysmorphogenic effects were noted as determined by the absence of external, visceral or skeletal malformations.

2.4.4.6 Other Toxicity Studies

In doses up to 50 mg/kg, doxepin, as with other tricyclic antidepressants, does not appear to have tolerance and physical dependence producing liability. Ten days treatment did not reveal tolerance to the inhibitory effect of doxepin on spontaneous locomotor activity in mice. Abnormal behaviour ascribable to physical dependence was not observed in mice and rats during or after abrupt withdrawal of forced drinking. Doxepin did not suppress abstinence signs elicited in barbital dependent mice ([redacted]).

2.4.4.7 Local Tolerance

Solutions of 0.3 mL of doxepin at 50, 75, and 100 mM and control (only the vehicle solution) were applied as a patch to the shaved dorsal skin of rats to test its effects on antinociception ([redacted]). No local tolerance issue are reported in the study. Doxepin cream (5%) is a topical medication used for the short-term treatment of pruritus (itching of the skin) due to atopic dermatitis (eczema) or lichen simplex chronicus (thickening of skin due to prolonged itching and scratching).

2.4.4.8 Ecological Toxicity

Microbial toxicity and fate analysis of doxepin and its transformation products were evaluated in the aquatic environment ([redacted]). Photodegradation experiments in aqueous media were carried out in a 1000 mL batch immersion-type photo-reactor using 800 mL (100 mg/L doxepin) of sample, under constantly

polychromatic UV irradiation by means of a medium-pressure mercury lamp. After 16 min of UV irradiation DOX was almost completely eliminated, but not fully mineralized. The acute and chronic toxicity of the photolytic mixture towards *V. fischeri* was significantly increased after 2 min of irradiation, showing the highest effect after 4 min (acute luminescence inhibition, growth inhibition) and 8 min (chronic luminescence inhibition), respectively.

Eccotoxicological evaluation of doxepin carried out ([redacted]). Four aquatic bioindicators (*Vibrio fischeri*, *Daphnia magna*, *Selenastrum capricornutum*, and *Danio rerio*) were used and the lethal, sublethal and/or inhibitory effects were obtained. High solubility for doxepin seems to be related with its bioavailability and thus, leads to higher toxicity. However, 50% efficient concentration (EC_{50}) ecotoxocity values for doxepin is between 100 and 1000 mg/L for all the studied environmental bioindicators, therefore, it should be considered as potentially harmful for the environment.

The zebrafish embryo toxicity test was selected to evaluate the acute toxicity of several drugs including doxepin ([redacted]). Lethal and sublethal effects were detected. For all of the drugs tested, these values were higher than the concentrations found in the natural environment. The maximum concentration found for doxepin was 0.699 µg/L, in wastewater treatment plant influents in Greece. Therefore, there was a low environmental toxicological risk.

2.4.4.9 Toxicology of Components of the Formulation

Silica, colloidal anhydrous. Colloidal silicas are most often prepared in a multi-step process where an alkali-silicate solution is partially neutralized, leading to the formation of silica nuclei. The subunits of colloidal silica particles are typically in the range of 1 to 5 nm. It is used as a free-flow agent to assist powder flow, e.g. in tabletting, by reducing the angle of repose of bulk powder. Acute oral toxicity (LD_{50}) in the rat: 3160 mg/kg. Acute dermal toxicity (LD_{50}) in the rabbit: >2000 mg/kg. Acute toxicity of the dust [50% lethal concentration (LC_{50})] in the rat: >2.2 mg/l 1 hours ([redacted]).

Magnesium stearate is used as antiadherent in the manufacture of medical tablets, capsules and powders. Acute PO toxicity (LD_{50}) in the rat is >10000 mg/kg. Chronic use may cause damage to the liver and the skin. Hazardous in case of ingestion. Slightly hazardous in case of skin contact (irritant), of inhalation ([redacted]).

Partially pregelatinized maize starch is used in the pharmaceutical industry for a wide variety of reasons, such as an excipient, a tablet and capsule diluent, a tablet and capsule disintegrant, a glidant, or as binder. Disintegrants enable tablets and capsules to break down into smaller fragments (dissolve) so that the drug can be released for absorption.

Starches also absorb water rapidly, allowing tablets to disintegrate appropriately. Starches are also used in the food manufacturing industry for processing, and as food thickeners or stabilizers. Consumption of excessive quantities of raw starch has resulted in obesity and iron-deficiency anaemia in humans. However, there is no evidence to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future. Inhalation of dusts may cause respiratory irritation. Prolonged inhalation may be harmful. It is not considered a carcinogen by IARC, American Conference of Governmental Industrial Hygienists (ACGIH), NTP, or OSHA. Due to lack of data, the classification is not possible ([redacted]).

Capsule shell

Gelatine is a translucent, colourless, brittle (when dry), flavourless food derived from collagen obtained from various animal by-products. It is commonly used as a gelling agent in food, pharmaceuticals, photography, and cosmetic manufacturing. Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation. It may cause adverse reproductive effects and birth defects (teratogenic) based on animal test data. Large doses may cause GI upset ([redacted]).

Titanium dioxide, E 171 (also known as Pigment White 6 or Colour Index number CI77891) is the principal white colorant used in many cosmetic and food products today. Mild skin irritant, moderate eye irritant. LD_{50} values: oral, rat - >25000 mg/kg; dermal, rabbit - >10000 gm/kg. LC_{50} (inhalation, rat, 4h): > 6820 mg/m^3 ([redacted]).

Iron oxide red (E172) belongs to iron oxides. There are sixteen known iron oxides and oxyhydroxides. Iron oxides and oxide-hydroxides are widespread in nature, and are widely used as inexpensive, durable pigments in paints, coatings and coloured concretes. Colours commonly available are in the yellow/orange/red/brown/black range. When used as a food colouring, it has E number E172. Black iron oxide may cause mechanical skin and eye irritation. Repeated and prolonged exposure to iron oxide dust may cause a benign pneumoconiosis called siderosis. Not considered a carcinogen by IARC, National Toxicology Program (NTP), ACGIH or Occupational Safety and Health Administration (OSHA) ([redacted])

Printing Ink - White:

Shellac (E904), refined Bleached shellac is a resin secreted by the female lac bug. It is dissolved in ethanol to make liquid shellac, which is used as a brush-on colorant, food glaze and wood finish. Shellac functions as a tough natural primer, sanding sealant, tannin-blocker, odour-blocker, stain, and high-gloss varnish. This product has not been tested on animals to obtain toxicological data. Shellac is not reported to produce

reproductive toxicity, mutagenic effects, embryotoxic effects, teratogenic effects in humans, and reproductive effects in humans ([redacted]).

Titanium dioxide, E 171 (also known as Pigment White 6 or Colour Index number CI77891) is the principal white colorant used in many cosmetic and food products today. Mild skin irritant, moderate eye irritant. LD_{50} values: oral, rat - >25000 mg/kg; dermal, rabbit - >10000 gm/kg. LC_{50} (inhalation, rat, 4h): > 6820 mg/m^3 ([redacted]).

Polyethylene glycol (E1520 or E1521) (PEG) is a polyether compound with many applications from industrial manufacturing to medicine. Acute oral toxicity (LD_{50}) in the rabbit: 26800 mg/kg. Acute dermal toxicity (LD_{50}) in the rabbit: >20000 mg/kg. Acute toxicity of the vapor (LC_{50}) in the rat: >13 8 hours. Slightly hazardous in case of skin contact (irritant, permeator), of eye contact (irritant), of ingestion, of inhalation ([redacted]).

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.5 Integrated Overview and Conclusions

This Nonclinical Overview of the medicinal product candidates Doxepin 10 mg, 25 mg and 50 mg Capsules of Morningside Healthcare Ltd, UK has been compiled for evaluating literary data about the nonclinical efficacy and safety properties of doxepin hydrochloride in preparation of Applications for Marketing Authorisation of Doxepin 25 mg and 50 mg Capsules of Morningside Healthcare Ltd, UK according to European Union (EU) Directive 2001/83/EC Article 10(1) and that of Doxepin 10 mg Capsule of Morningside Healthcare Ltd, UK according to EU Directive 2001/83/EC Article 10(3).

Doxepin blocks the reuptake of NE and 5-HT into presynaptic terminals thereby prolonging the availability of the monoaminergic neurotransmitters within the synaptic cleft and enhancing their action leading to sedative effects. Doxepin also has antagonistic effects on H_1, α1-adrenergic, and muscarinic receptors. The pharmacological profile of doxepin combines significant activity in animal models of depression with pronounced sedative and tranquillizing properties. It also possesses peripheral and central anticholinergic activity, together with antispasmodic and mild peripheral vasodilating effects. In virtually all these tests, the *cis* geometric isomer of doxepin is more active than doxepin itself, which in tum is more active than its *trans* isomer. Desmethyldoxepin, a major metabolite of doxepin, is pharmacologically active and has more marked sedative properties than doxepin does. The suppression of conditioned avoidance behaviour in rats, generalized slowing of spontaneous electrical activity with an increase in amplitude on the electroencephalogram in monkeys, and potentiation of the effects of barbiturates and alcohol in mice reflect the depressant properties of this agent. Its stimulant properties were demonstrated by its ability to antagonize the sedative effects of the synthetic reserpine analogue, tetrabenazine, in rats, to block and reverse the effect of reserpine in mice, and to potentiate the effect of amphetamine on random activity in rats. Doxepin is a less potent inhibitor of NE uptake than other tricyclic antidepressants and demonstrates weak anticholinergic activity.

Following administration of single oral doses to rats and dogs, doxepin is well absorbed and plasma levels reach a maximum within 30 minutes to 1 hour, thereafter declining rapidly. The administered drug is excreted mainly in the urine. In the rat, 60% of the radioactivity administered appears in the 24-hour urine following single oral dosage, with about 25% in the faeces. Most of the dose in rats is eliminated in the urine within 8 hours; very little is excreted in bile. Excretion of unchanged doxepin is low, less than 5% in rats. The kidney appeared to be the major excretory organ, the urine containing 60 % of the radioactivity administered.

Doxepin produces dose-dependent decreases in blood pressure, increases in heart rate, and cardiac arrhythmias in most species at corresponding dose levels greater than therapeutic doses in man. Single dose toxicity has been demonstrated in mice, rats, and dog. Toxic signs in all species generally include CNS effects such as ataxia, general and respiratory depression, tremors, convulsions, prostration then death. In a 12-month chronic oral toxicity study in dogs, ptosis, sedation, tremors and vomiting occurred at 50 mg/kg daily dose. There were occasional episodes of vomiting at 25 mg/kg but those given 5 mg/kg were practically asymptomatic. Doxepin was negative in *in vitro* (bacterial reverse mutation, chromosomal aberration in human lymphocytes) and *in vivo* (rat micronucleus) assays. No evidence of carcinogenic potential was observed when doxepin was administered orally to mice for 26 weeks. There were no changes observed in litter size, number of live births or lactation in animals (species not stated) given doxepin at daily oral doses of up to 25 mg/kg for 8 or 9 months. The birth rate and survival rate at 3 weeks after birth was decreased at high dose (270 mg/kg).

In summary, the antidepressant and anxiolytic activity and safety of doxepin hydrochloride have been consistently demonstrated in nonclinical experiments. Doxepin is a well-established drug, marketed for over 50 years. Excipients of the formulation are approved and established agents in widespread use in the pharmaceutical manufacturing industry.

In conclusion, the pharmacodynamic data and the safety profile of doxepin hydrochloride obtained in animals are in line with observations made during the over 50 years of its application in human therapy, and continue to support its human application.

MODULE 2 OVERALL SUMMARIES

2.4 Nonclinical Overview

2.4.6 Literature References

Module	#	References
4	1.	[redacted]
4	2.	[redacted]
4	3.	[redacted]
4	4.	[redacted]
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