[redacted]

REGISTRATION DOSSIER ON A BUPIVACAINE HYDROCHLORIDE AND FENTANYL ASSOCIATION AT 1.0 and 1.25 mg/ml TO 2 µg/ml RATIOS IN INFUSION BAGS FOR EPIDURAL ADMINISTRATION

Module 2.4

NON CLINICAL OVERVIEW

FINAL VERSION

[redacted]

Rdacted under section 40 of the FOI Act

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LIST OF ABBREVIATIONS AND RELATED DEFINITIONS

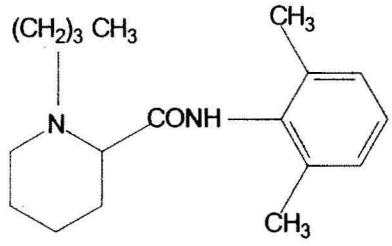
AAG	=	α_1-acid glycoprotein
AE(s)	=	adverse event(s)
CAS	=	chemical abstracts service
CNS	=	central nervous system
CSF	=	Cerebro-spinal fluid
ED_{50}	=	Dose pharmacologically effective for 50 % of the population exposed to the drug
GC	=	gas chromatography
HPLC	=	high performance liquid chromatography
i.v.	=	intravenous administration
LD	=	lethal dose
LD_{50}	=	dose that kills 50 % of the animals
MLAC	=	Minimum local analgesic concentration
MV	=	Maternal vein
NPD	=	nitrogen phosphorous detector, also called TSD
p.o.	=	oral administration
RIA	=	radio immuno assay
s.c.	=	subcutaneous administration
TSD	=	thermoionic specific detector, also called NPD
UV	=	Umbilical vein
VAPS	=	Visual Analogue Pain Scores

2.4.1 *OVERVIEW OF NON CLINICAL TESTING STRATEGY*

2.4.1.1 CHEMICAL PROFILE OF BUPIVACAINE

Bupivacaine corresponds to the chemical structure depicted in Fig. 1.

Figure 1 – Chemical structure of bupivacaine



$C_{18}H_{28}N_2O$ mol.wt.288.43, CAS 2180-92-9, melting at 107-108°C.

This compound is usually present in pharmaceutics as hydrochloride salt, melting at 255-256°C, with CAS 18010-40-7 as anhydrous, and 14252-80-3 as monohydrate.

As bupivacaine has a stereogenic carbon atom, two enantiomers exist, namely S(-) and R(+). It is used in therapy as a racemate.

2.4.1.2 PATENTS OF BUPIVACAINE

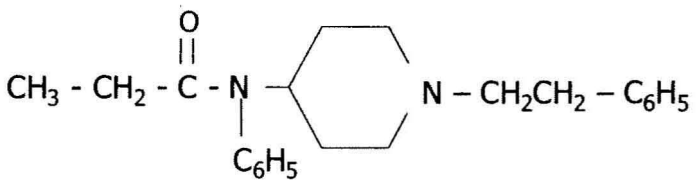
Bupivacaine was patented in 1959 and 1960 to Bofors, and then in 1969 to Sterling Drugs [redacted].

2.4.1.3 CHEMICAL PROFILE OF FENTANYL

Fentanyl corresponds to the chemical structure depicted in Fig. 2.

Fentanyl is usually present in pharmaceutical formulations as citrate salt, melting at 149-151°C, with CAS 990-73-8.

Figure 2 – Chemical structure of fentanyl



C_{22} H_{28} N_2O, CAS 437-38-7, mol. wt 336.4, melting at 83-84°C

2.4.1.4 PATENT OF FENTANYL

Fentanyl was patented in 1964 to Janssen, U.S. pat. N. 3,141,823.

2.4.1.5 PHARMACEUTICAL FORMULATION

The applicant wants to register the associations at fixed doses of bupivacaine hydrochloride of 0.1% and 0.125% + fentanyl 2 µg/ml in infusion bags for analgesia by epidural administration.

The bags have the composition reported in the following Table 1:

Table 1 – Composition of bupivacaine hydrochloride and fentanyl associated solution in infusion bags

Active ingredients:	Bupivacaine 0.1% + Fentanyl 2 µg/ml	Bupivacaine 0.125% + Fentanyl 2 µg/ml
Bupivacaine hydrochloride	[redacted]	[redacted]
Fentanyl *as citrate salt*	[redacted]	[redacted]
Excipients:		
Injectable sterile saline	[redacted]	[redacted]
pH range	[redacted]	[redacted]

No relevant impurities are present in the active ingredients and in the excipients, as extensively reported in the Quality Dossier, which is part of the registration documentation.

2.4.1.6 PHARMACEUTICAL INDICATIONS

Bupivacaine hydrochloride is a local and locoregional anaesthetic, because it is able to produce anaesthesia in wide body regions, including spinal anaesthesia, which is obtained by injection into the subarachnoid space [redacted].

Fentanyl is a potent opioid analgesic used alone or associated to other anaesthetics for induction and maintenance of anaesthesia [redacted]

The bupivacaine hydrochloride/fentanyl association for epidural infusion is indicated for post-operative and labor analgesia, except where specifically contraindicated.

2.4.1.7 ISSUES CONSIDERED IN THE PRESENT REVIEW

Throughout the present expert report, pharmacology, toxicology, pharmacokinetics and interactions of bupivacaine hydrochloride and fentanyl with other drugs are comprehensively discussed with reference to specific publications and reviews.

As both bupivacaine hydrochloride and fentanyl are two very old drugs, studied in animals for formal toxicology tens of years ago and used in therapy on millions of patients, the present non-clinical overview considers data not only on animals, but also in humans. This for a more comprehensive presentation of the whole subject, and to complete the safety data of these two drugs, due to the lacking from literature of same toxicity data in animals [redacted].

2.4.2 PHARMACOLOGY

2.4.2.1 BUPIVACAINE HYDROCHLORIDE

As local anaesthetic, bupivacaine hydrochloride is used mainly for epidural and spinal anaesthesia [redacted].

Bupivacaine, like other local anaesthetics, prevents the generation and the conduction of the nerve impulse. When increasing concentrations of bupivacaine are applied to a nerve fibre, the threshold for excitation increases, the impulse conduction slows, the rate of rise of the action potential declines, the action potential amplitude decreases, and, consequently, the ability to generate an action potential is abolished. All these effects result from the binding of the local anaesthetic to sodium channels; this binding results in a blockade of the sodium current. If the sodium current is blocked over a critical portion of nerve, propagation of an impulse over the blocked area is no longer possible [redacted], [redacted]

A number of precautions should be observed when bupivacaine hydrochloride is injected [redacted]. Resuscitation equipment and appropriate drugs should be immediately available. The safe use of these agents in pregnancy, with respect to adverse effects on foetal development, has not been fully established.

Local anaesthetics, and thus bupivacaine, possess also antiarrhythmic activity [redacted], [redacted]. Lidocaine hydrochloride is in fact widely used in cardiology, mainly in the management of arrhythmic crisis in patients with myocardial infarction [redacted]. Even if the two activities, namely local anaesthetic and antiarrhythmic, are very different, their mechanism is the same, as described herein. Bupivacaine hydrochloride however, is not used in therapy as antiarrhythmic.

2.4.2.2 FENTANYL

2.4.2.2.1 ACTIVITY, USES AND DOSES

Fentanyl, a phenylpiperidine derivative, is a potent opioid analgesic chemically related to pethidine and is primarily a μ-opioid agonist.

Fentanyl is used as an analgesic, an adjunct to general anaesthetics, and as an anaesthetic for induction and maintenance. It is also used as a respiratory depressant in the management of mechanically ventilated patients under intensive care. When used in association with an antipsychotic such as droperidol, it can induce a state of neuroleptanalgesia in which the patient is calm and indifferent to his surroundings and is able to cooperate with the surgeon.

Fentanyl is often administered by intramuscular or intravenous injection as the citrate salt or in transdermal patches as the base. It is also administered by epidural injection or by transmucosal route as the citrate salt. It is more liposoluble than morphine and, following an intravenous injection of 100 μg, the effects of fentanyl begin almost immediately, although maximum analgesia and respiratory depression may occur only several minutes later; the average duration of action is 30 to 60 minutes, although analgesia may last only 10 to 20 minutes in an unpremedicated adult. Increasing the dose to 50 μg per kg body weight, fentanyl can give pain relief for 4 to 6 hours.

As an adjunct to general anaesthesia, fentanyl is usually given by intravenous injection. Dosage recommendations show a wide range depending on the technique. Patients with spontaneous respiration may be given 50 to 200 μg of fentanyl as an initial dose with supplements of 50 μg. It is recommended that doses above 2 μg per kg body-weight should be used in conjunction with assisted ventilation. Significant respiratory depression

follows doses higher than 200 µg. Patients whose ventilation is assisted may be given 300 to 3500 µg (up to 50 µg per kg) as an initial dose, with supplements of 100 to 200 µg or higher depending on the patient's response. High doses have been reported to moderate or attenuate the response to surgical stress [redacted].

For treatment of acute pain by the epidural route, fentanyl is administered by epidural bolus doses, continuous epidural infusion or a combination of both, including by Patient-Controlled Epidural Analgesia. Doses reported in a literature review [redacted] of 28 studies, are in the following ranges: Boluses of 20 to 200 µg, Infusions of 20 µg/hr to 63 µg/kg/hr, and combined treatment of Bolus 20 to 100 µg + Infusion of 8 to 200 µg/h.

2.4.2.2.2 EPIDURAL INJECTION IN RATS

Differences in safety and activity of fentanyl administered per subcutaneous or epidural injection have been investigated in rats.

A series of side effects of this analgesic opioid was monitored (breath depression, muscle rigidity, blockade of pinna, blockade of cornea reflexes) together with the dose necessary to provoke analgesia (ED_{50}).

Results of two separate studies show that when fentanyl is administered via subcutaneous injection, analgesia and respiratory depression appear at similar doses [redacted] On the other hand, fentanyl blocked the pinna and cornea reflexes and produced skeletal muscle rigidity only at a concentration of drug at least three fold higher than the concentration required to induce analgesia [redacted].

When fentanyl was administered per epidural injection, the dose necessary to cause analgesia was not sufficient to provoke any detectable respiratory effect, neither at the

time of appearance of analgesia, nor later [redacted]. Quantitative results are reported in Table 2.

These findings are consistent with the observation that also in patients, epidural injection of fentanyl is less likely to produce respiratory depression, than after intravenous administration.

Table 2

Respiratory and analgesic effects of fentanyl after epidural and subcutaneous injection in rats [redacted].

	Fentanyl	
	Subcutaneous	**Epidural**
Analgesia ED_{50} (µg/kg)	12 (6.7-20)	3.2 (1.8-5.4)
Ventilation ED_{50} (µg/kg)	20 (6.7-60)	//

2.4.2.2.3 EPIDURAL ADMINISTRATION IN HUMANS

The use of fentanyl analgesia by different routes of administration in the management of acute pain in adults has been thoroughly reviewed [redacted]. Regarding safety, the secondary effects have been compared via different routes and with different opioids.

Respiratory depression is common after intravenous fentanyl infusion but most events are not clinically significant. Other side effects after i.v. administration include nausea and vomiting, pruritis and urinary retention.

In a review of 11 studies comparing fentanyl administered i.v. vs. epidural for analgesia in acute pain after various surgical procedures, the following can be summarized between the two routes of administration:

Analgesia – No significant difference (NSD) or greater with epidural admin.

Plasma concentration – Either NSD or lower with epidural admin.

Respiratory Effects – Either NSD or greater with i.v. admin.

Other Side Effects – Either NSD or greater with i.v. admin. (Nausea & Vomiting)

Dose requirement – NSD (7 studies), 28 – 55% lower with epidural (3 studies), 25% higher with epidural (1 study)

In more than 40 published clinical trials, most suggest that epidural fentanyl is less likely than morphine to produce clinically significant ventilatory depression. However, respiratory arrest has been reported, but clinically significant respiratory depression appears to be relatively rare. Fentanyl is reported to be associated with fewer minor adverse effects (nausea, vomiting, pruritus, sedation, urinary retention) than morphine. The most common side effect from epidural administration of fentanyl is pruritis but is less intense than from epidural morphine and more localized. Treatment is rarely necessary. The incidence of nausea and vomiting is comparable to other epidural opioids.

2.4.2.3 BUPIVACAINE / FENTANYL ASSOCIATION

Activity and safety of bupivacaine hydrochloride/fentanyl association administered by continuous epidural infusion is well documented from a wide therapeutic use in patients. Furthermore, in addition to the widespread, routine use in clinical practice of the association, the Applicant has marketed since 2001 over 50,000 units of bupivacaine-fentanyl infusions. The fact that no expected or unexpected adverse effect has ever been reported, provides confirmation regarding the safety of the association.

In order to produce a more complete view of this interesting association administered per epidural infusion, results obtained in labour analgesia are here briefly summarized.

In a clinical trial, parturient women were treated with 0.125% bupivacaine and 2 µg/ml fentanyl epidural infusion for several hours (max. 15 hours) from the beginning of labour until delivery. Maternal blood and umbilical blood were drawn for fentanyl and bupivacaine concentration determination at different times.

Results suggest that the use of continuous epidural infusion of low dose bupivacaine and fentanyl during labour (over periods up to 15 hours) does not result in significant drug accumulation either in mother or neonate [redacted]. No adverse neonatal effects were observed. A summary of bupivacaine and fentanyl concentrations in maternal vein (MV) and umbilical vein (UV) blood is reported in the following Table 3 together with bupivacaine data from previous clinical trials [redacted].

Table 3
Drug concentration data

	MV concentration	UV concentration	UV / MV ratio
Data from [redacted]			
Bupivacaine (µg/ml)	0.50 ± 0.16	0.15 ± 0.06	0.30
Fentanyl (µg/ml)	0.17 ± 0.10	0.16 ± 0.09	0.94
Data from [redacted]			
Bupivacaine (µg/ml)	0.54 ± 0.10	0.18 ± 0.04	0.33

A number of studies concerning treatment of post-operative and labor pain report that epidural fentanyl enhances analgesia achieved with the local anaesthetic bupivacaine, thereby allowing lower dosages of either drug alone. The minimum local analgesic concentration of epidural bupivacaine is thus reduced.

In a double-blinded study of 223 labouring woman, patients received either plain bupivacaine, or bupivacaine with fentanyl at 1, 2, 3 or 4 µg/ml [redacted]. The minimum local analgesic concentration (MLAC) is the effective concentration in 50% of subjects. The observed reduction in MLAC was 18%, 31%, 55% and 72% with the addition of 1, 2, 3 and 4 µg/ml fentanyl, respectively, demonstrating significant negative linear trend in the amount of bupivacaine required with increasing fentanyl doses. The incidence of pruritis increased significantly, however, with the addition 4 µg/ml fentanyl.

In a double-blinded study of 60 patients receiving patient-controlled extradural analgesia after Caesarean section, patients received either bupivacaine 0.1%, fentanyl 4 µg/ml, or a combination at bupivacaine 0.05% + fentanyl 2 µg/ml [redacted]. The results show that the addition of fentanyl to bupivacaine reduced the dose of bupivacaine by up to 68%, improved analgesia at rest and decreased motor block. The addition of bupivacaine

to fentanyl reduced fentanyl dose by up to 57%. Further, epidural opioids are considered to provide effective analgesia at lower doses than systemic opioids.

Therefore, due to the synergistic effect, it may be concluded that safety is enhanced by administration of the association by epidural as opposed to each substance administered individually in higher quantities which are more likely to provoke the known adverse effects of these drugs. Furthermore, the impact of the association on other non-clinical parameters would be lower than when each substance is administered separately in higher quantities.

2.4.3 PHARMACOKINETICS

2.4.3.1 BUPIVACAINE BIOASSAY METHODS

Bupivacaine is a highly liposoluble drug, which major metabolite is desbutylbupivacaine (DBB), formed in the liver by oxidative dealkylation of the parent drug. Another important metabolite of this drug is 4'-hydroxy bupivacaine (4'-OH-B).

Several gas chromatographic methods have been reported for the measurement of serum bupivacaine and for the simultaneous determination of bupivacaine and DBB [redacted]. Bupivacaine in human serum can be determined also by liquid chromatography (HPLC-UV) using a detection wavelength of 210 nm [redacted]. The same analytical technique (and the same detection wavelength) has also been employed to quantitatively and simultaneously determine bupivacaine and its two major above mentioned metabolites (DBB and 4'-OH-B) [redacted] both in human serum and urine.

2.4.3.2 BUPIVACAINE ABSORPTION

Administration of bupivacaine hydrochloride for regional anaesthesia of the head and neck in a mean total dose of 3.4 mg per kg body-weight has produced mean peak plasma concentrations of 3.56 and 4.95 µg/ml when administered with or without adrenaline, respectively, without producing toxicity. Similarly, intrapleural administration of bupivacaine 0.5% in a dose of 2.5 mg per kg has produced mean peak plasma concentrations of 2.57 and 3.22 µg/ml when given with or without adrenaline, respectively, without producing toxicity. A further study in which a 72-hour interpleural infusion of bupivacaine hydrochloride with adrenaline was administered to cholecystectomy patients showed appreciable interpatient variability in steady-state plasma drug concentrations (range 1.3 to 3.2 µg/ml; mean 2.1 µg/ml); no patient suffered any adverse effects.

Stellate ganglion block with bupivacaine hydrochloride 0.25% has produced a mean peak plasma concentration of 0.40 and 0.47 µg/ml after doses of 10 or 20 mg respectively. Administration of bupivacaine 0.5% in a dose of 3 mg per kg with or without adrenaline for sciatic and femoral nerve block produced mean peak plasma concentrations below 0.8 µg/ml.

Bupivacaine proved to be rapidly absorbed from the synovial membrane of the knee during arthroscopy but plasma concentrations did not exceed 350 ng/ml after controlled pressure-irrigation with isotonic solutions. Although one group of workers found that the maximum plasma concentrations of bupivacaine after intra-articular injection of 30 ml of a 0.5% solution for arthroscopy was 875 ng/ml they suggested that adrenaline should probably be added to minimise absorption [redacted].

After epidural administration of 150 mg with adrenaline, mean peak plasma concentrations of 1.1 μg/ml were reported at 0.3 h and peak cerebrospinal fluid concentrations averaged 30 μg/ml at 0.5 h [redacted]

2.4.3.3 DISTRIBUTION, PROTEIN BINDING AND METABOLISM OF BUPIVACAINE

Bupivacaine is about 95% bound to plasma proteins. Reported half-lives are from 1.5 to 5.5 hours in adults and about 8 hours in neonates. It is metabolised in the liver and is excreted in the urine principally as metabolites, only 5 to 6% as unchanged drug.

Bupivacaine is distributed in breast milk in small quantities. It crosses the placenta but the ratio of foetal to maternal concentrations is relatively low. Bupivacaine also diffuses into the cerebrospinal fluid [redacted].

2.4.3.4 INTERACTIONS OF BUPIVACAINE

Pharmacokinetic interactions could occur for protein binding, as follows. Bupivacaine is markedly bound to α_1-acid glycoprotein (AAG), a protein which increases after trauma, surgery, burns, myocardial infarction, in chronic inflammatory disorders such as Crohn's disease, and in cancer. Protein binding may therefore be increased in these conditions, whereas it may be reduced in neonates, in the nephrotic syndrome, and in liver disease when AAG concentrations are lower than normal. This may result in marked variations in the free fraction of bupivacaine.

Additional interactions are the following:

Antiarrhythmics. There is an increased risk of myocardial depression when bupivacaine hydrochloride and antiarrhythmics are concomitantly administered [redacted].

Beta blockers. Propranolol proved to reduce the clearance of bupivacaine by 35% in 6 healthy subjects. There is therefore the risk of increased bupivacaine toxicity if these two drugs are concomitantly administered [redacted]

Histamine H_2-receptor antagonists. Studies of the effect of H_2-receptor antagonists on the pharmacokinetics of bupivacaine have yielded variable results. While one research group found that pre-treatment with cimetidine decreased the clearance of bupivacaine, others have failed to find any significant pharmacokinetic effects [redacted]. Similarly pre-treatment with ranitidine has either increased plasma concentrations of bupivacaine or had no significant interaction [redacted].

Neuromuscular blockers. The metabolism of local anaesthetics derived from esters may be inhibited by anticholinesterases, thus increasing the risk of systemic toxicity [redacted]

2.4.3.5 CHIRALITY OF BUPIVACAINE

As bupivacaine has a stereogenic carbon atom, two enantiomers of this drug exist, namely S (-) and R (+). The drug is currently used as racemate in therapy or as the levobupivacaine stereoisomer.

Studies have ascertained some differences in systemic disposition of bupivacaine, which were attributed to an enantioselective plasma binding of the two enantiomers [redacted].

2.4.3.6 FENTANYL BIOASSAY METHODS

Fentanyl can be assayed in plasma by GC, HPLC, and RIA methods. Table 4 lists a series of bioassay method of fentanyl, able to produce useful pharmacokinetic data.

Three main techniques were employed in fentanyl pharmacokinetics studies performed before 1975. They were based on radiochemical assays (principally using ^{3}H labelled drug), in which selectivity to differentiate fentanyl from fentanyl metabolites was obtained by chromatography and by the use of hydrophobic solvent extraction. This technique was useful to identify fentanyl metabolites (Section 2.4.3.9).

A radioimmunoassay having sufficient sensitivity for routine use was reported in the mid and late 1970s [redacted]. This technique has been extensively used in various pharmacokinetic studies, but it is sensitive to haemolysis within blood samples. Similarly, the advent of gas chromatography with nitrogen-selective detection (GLC-NPD), and mass spectrometry detection methods, have provided sufficiently sensitive assays for fentanyl, alfentanil and sufentanil determination [redacted];

[redacted]

Table 4 – Bioassay methods of fentanyl

Author(s)	Method, apparatus and detector	Sensitivity
[redacted])	GC-mass spectrometry	LOQ 500 pg/ml
[redacted])	GC-TDS	LOQ 400 pg/ml
[redacted])	GC-TDS	LOQ 3.3 ng/ml
[redacted])	GC-TDS	LOQ 20 pg/ml
[redacted])	HPLC	LOQ 2 ng/ml
[redacted]	RIA	n.r.

n.r. = not reported in the cited publication

2.4.3.7 *FENTANYL ABSORPTION*

In contrast to the majority of other opiates, fentanyls are most frequently given by epidural or intravenous routes, and absorption studies of other routes of administration do not pertain to the Application.

The absorption of morphine and fentanyl after intravenous, intrathecal and epidural administration was studied in goats [redacted]. Bulk flow was considered to be the principal mechanism of opioid elimination from the CSF. After epidural administration, morphine and fentanyl were absorbed into CSF at the same rate, but the relative amount of drug absorbed may be higher for morphine than for fentanyl. Pertinent results are presented in Table 5.

Table 5 – Fentanyl and Morphine absorption in goats

	Fentanyl	**Morphine**
Plasma concentration-time curve (body clearance) after i.v. admin.	3.9-5.8 ml/min/kg (N=3) (2-compartement model)	84 ±23 ml/min/kg (N=5) (3-compartement model)
Elimination rates from CSF after intrathecal admin.	0.6 – 2.4 ml/h/kg (N=3)	0.3 – 2.0 ml/h/kg (N=3)
Time to Max. CSF concentration after epidural admin.	0.22 ± 0.13 h (N=8)	0.22 ±0.14 h (N=6)
CSF availability after epidural admin.		
low dose	0.8%	2.3%
high dose	3.3%	11.3%

In one study carried out on patients, the ability of fentanyl to pass into blood and through the dura mater into the CSF was investigated after epidural administration. Results suggested that there was a minimal vascular uptake following epidural administration of bolus doses of 1 μg/kg of fentanyl, and there was a rapid absorption of the drug across the dura mater as indicated by the steep increase of lumbar CSF concentration. Fentanyl in

CSF was therefore readily available to interact with the opioid receptors in the dorsal horn region of the spinal cord. Moreover, as a result of passive CSF flow, fentanyl concentrations were also found in the cervical region in all patients [redacted] at doses considerably lower (between 3.4 and 37 times lower) than those found in the lumbar region. In the same study, each patient also received the same amount of fentanyl by the i.v. route. Blood fentanyl levels decreased rapidly due to extensive distribution of fentanyl into tissues. All patients reported reductions in Visual Analogue Pain Scores (VAPS) following epidural administration with no reports of nausea or light-headedness. However, some of these side effects were reported in half of the patients receiving i.v. fentanyl.

As the application is addressed to epidural infusion, no additional absorption data are discussed in this expert report.

2.4.3.8 PROTEIN BINDING AND DISTRIBUTION OF FENTANYL

In various experiments, fentanyl proved to be bound to plasma proteins by 79 to 87%, the free fraction ranging 13-21% [redacted].

The physiological distribution of fentanyl has been reported in a series of animal studies during the late 1960s and early 1970s. Fentanyl is widely distributed to organs and tissues of the mammalian body [redacted]. After intravenous injection, high concentrations occur quickly in well-perfused tissues, notably lung, kidney, spleen, heart and brain. Maximum concentrations in the intestine wall, liver and muscle lag slightly behind the well-perfused tissues, and the maximum concentrations in fat occur at approximately 30 minutes after injection.

This reflects the high affinity and low perfusion of the adipose tissue in comparison with the other mentioned tissues.

The following Table 6 summarizes the distribution of fentanyl infused i.v. to rats at the dose of 30 mg/kg/min over 1 min.

Table 6 – Peak concentrations of fentanyl in rat tissues [redacted]

	x	SD	%CV	t_{max} (min)
Brain	157	27	17	2
Heart	513	185	36	1
Kidney	183	31	17	2
Stomach	93	12	13	6
Small Intestine	73	12	16	2
Large Intestine	28	8.8	31	4.5
Pancreas	240	50	21	6
Spleen	119	16	13	41
Testes	44	4.0	9	21
Muscle	30	5.8	19	4.5
Skin	15	4.5	30	41
Fat	48	9.4	20	41

x = mean values of fentanyl concentrations (ng/g) in different tissues.

The main routes of distribution after administration of fentanyl into the epidural space in adults include [redacted]:

- movement across the meninges into the cerebrospinal fluid (CSF)
- movement from the CSF into the opioid receptor or other nonspecific binding site in the spinal cord
- rostral migration via the CSF to supraspinal sites
- vascular absorption in the epidural or spinal vascular system
- uptake into epidural fat

2.4.3.9 *ELIMINATION*

In animals and in humans, fentanyl is cleared primarily by metabolic route. Fentanyl excreted as such, via urine, was estimated to be around 6% of the administered dose [redacted].

Fentanyl in clinical use is considered a drug with fast onset but short duration of analgesic effects [redacted].

Fentanyl is metabolised primarily by N-dealkylation to norfentanyl, and by hydroxypropionyl fentanyl and hydroxypropionyl norfentanyl, respectively [redacted] Other postulated pathways include piperidine ring hydroxylation, aromatic hydroxylation, aromatic hydroxylation and amine hydrolysis [redacted]. Interestingly, fentanyl undergoes extrahepatic metabolism in some animal species and its metabolic pathways are sensitive to acid-base balance in the mouse and rat [redacted]

2.4.4 TOXICOLOGY

2.4.4.1 *GENERAL*

Toxicity data in animals are obtained for a new chemical entity to define the doses that can be administered to humans for the first time, without affecting safety. Guidelines suggest to start from a relatively low dose and to increase it following an increasing dose scheme, until AEs are experienced, which indicates the borderline between tolerated and untolerated doses [redacted].

When, however, toxicological data are requested for old drugs like bupivacaine and fentanyl, the only way to achieve them is to search in the literature. Toxicological profiles

however were not, and still now are not, usually published or are only partially published, so that it is often difficult, and in some cases impossible, to describe a thorough toxicological profile of these old drugs. Bupivacaine was discovered in 1959 and fentanyl in 1964 and they are still now largely used all over the world, alone as well as in association by the epidural route, for their analgesic and anaesthetic activities.

2.4.4.2 CARCINOGENICITY

After 40 years of use in humans, neither bupivacaine nor fentanyl have been classified as agents that are possibly, probably or are carcinogenic to humans by the International Agency for Research on Cancer who have evaluated over 900 agents, including many substances used in pharmaceutical products (IARC – Overall Evaluations of Carcinogenicity to Humans). It is pertinent to note that the bupivacaine-fentanyl association subject of the Application is administered on a short-term basis for treatment of acute pain.

2.4.4.3 LOCAL TOLERANCE

Both bupivacaine alone and in combination with fentanyl, have been administered in humans by the epidural route for several decades. Any potential issues on a local level at the site of administration are due to catheter insertion (e.g. epidural abscess, hematoma) and not related to the substances themselves.

So millions of patients have been treated with these two important drugs since their discovery (for about 40 years), which would mean that all their possible side effects on humans are already well reported and known. This diminishes the need to focus on a complete picture of non-clinical, toxicological data.

However, data present in scientific publications on animal toxicity of bupivacaine hydrochloride and fentanyl are reported in the next Sections 2.4.4.4, 2.4.4.5 and subsections.

2.4.4.4 BUPIVACAINE HYDROCHLORIDE

2.4.4.4.1 Acute toxicity

Acute toxicity of bupivacaine hydrochloride was described in various animal species and by various administration routes by [redacted]. These authors have compared results of bupivacaine hydrochloride with those of mepivacaine hydrochloride and tetracaine hydrochloride administered as such and associated to adrenaline.

In mice weighing 20 ± 2 g, test solutions (0.20 ml/20 g body weight) were injected into a tail vein at a rate of 0.20 ml/2 sec. Mice were observed intermittently during the 3 hours following injection, and mortalities recorded after 24 hours.

Test solutions were injected subcutaneously into the caudal part of the mice back; otherwise, the procedure was as described above.

In guinea pigs, weighing 500 ± 100 g, the test solutions (2.5 ml/500 g) were injected into the peritoneal cavity within 30 sec; otherwise, the procedure was as described above.

In adult rabbits, weighing from 3500 to 5500 g, the test solutions were injected into the caudal part of the back (8 ml/kg).

A summary of the results of the above tests is reported in the following Table 7.

Table 7

Acute toxicity of bupivacaine·HCl, mepivacaine HCl and tetracaine·HCl in mice, guinea pigs and rabbits in the absence and in the presence of adrenaline. LD_{50} Mean values ± SE in mg/kg

Species	Route	Mepivacaine·HCl	Bupivacaine·HCl	Tetracaine·HCl
Mice	i.v.	40.3 ± 3.2 n=192	7.8 ± 0.4 n=36	8.0 ± 1.0 n=36
	+ adrenaline	22.7 ± 1.2 n=36	2.1 ± 0.6 n=36	2.4 ± 0.8 n=36
	s.c	270 ± 20 n=285	82 ± 6 n=36	62 ± 5 n=36
	+ adrenaline	318 ± 22 n=320	95 ± 8 n=36	101 ± 7 n=36
Guinea Pigs	i.p.	173 ± 16 n=50	50 ± 8 n=36	60 ± 11 n=36
Rabbits	s.c. + adrenaline	160 ± 20 n=11	50 ± 10 n=11	50 ± 11 n=11

As expected, with i.v. injection the presence of adrenaline augmented the toxicity of the three tested compounds. On the contrary, with s.c. injection adrenaline lowered the toxicity, as it delayed the diffusion of drugs administered into circulating blood.

2.4.4.4.2 Repeated Dose

Available data on repeated dose with bupivacaine hydrochloride are restricted to the Cumulative Toxicity Study reported by [redacted].

This test was carried out on both mice and rabbits after subcutaneous injection. Techniques were the same described in Section 2.4.4.4.1. The injections were repeated each 30 min, producing results after 4, 6 and 8 consecutive injections.

Results obtained are summarized in the following Tables 8 and 9.

As concerns both the convulsion episodes and the number of deaths, bupivacaine HCl proved to be less toxic than mepivacaine HCl.

Mepivacaine however was administered at doses 3-4 times higher than bupivacaine.

Table 8

Cumulative toxicity of mepivacaine and bupivacaine·HCl in mice on subcutaneous injection. Single dose versus multiple doses.

Single total dose	Reaction after one injection		Dose per injection	Reaction after four injections		Reaction after six injections		Reaction after eight injections	
mg/kg	Convuls.	Deaths	mg/kg	Convuls.	Deaths	Convuls.	Deaths	Convuls.	Deaths
Mepivacaine.HCl									
400	12/12	12/12	50.0	1/10	0/10	5/10	5/10	10/10	9/10
350	10/12	7/12	42.5	0/4	0/4	1/2	0/2	2/6	1/6
Bupivacaine.HCl									
120	2/2	2/2	15.0	0/2	0/2	1/2	0/2	2/6	0/6
100	19/20	17/20	12.5	0/5	0/5	0/10	0/10	1/20	1/20

Table 9

Cumulative toxicity of mepivacaine HCl, bupivacaine·HCl and tetracaine·HCl with adrenaline in rabbits on subcutaneous injection. Single dose versus multiple doses.

Single total dose mg/kg	Reaction after one injection		Dose per injection mg/kg	Reaction after eight injections	
	Convuls.	Deaths		Convuls.	Deaths
2% mepivacaine.HCl + Adrenaline 1:200,000					
192	3/3	0/3	24	5/5	0/5
160	6/8	5/8	20	2/8	0/8
0.5% bupivacaine.HCl + Adrenaline 1:200,000					
48	2/3	1/3	6	2/5	0/5
40	7/8	3/8	5	0/10	0/10
0.5% tetracaine.HCl + Adrenaline 1:200,000					
48	3/3	3/3	6	5/5	0/5
40	7/8	1/8	5	8/8	1/8

Results show that no deaths occurred after repeated dose, with the exception of one case of death with tetracaine HCl.

As for the frequency of convulsions after repeated doses, bupivacaine proved to be the least toxic among the three compounds tested.

2.4.4.4.3 Reproductive and Developmental Toxicity

The maternal-foetal distribution of bupivacaine was extensively investigated by various authors in rabbit [redacted], [redacted] They have shown that foetal : maternal plasma concentration (F:M) ratios of bupivacaine are relatively low at delivery following extradural analgesia. It has been claimed that such low ratios might result from extensive tissue uptake, rather than slow placental transfer, even though ratios do not increase consistently with dose-delivery interval, as would be expected during tissue equilibration. Rabbit placental perfusion experiments have demonstrated consistently slow transfer of bupivacaine, associated with high maternal protein binding [redacted].

Bupivacaine was infused i.v. in nine anaesthetized pregnant rabbits near term. Pups were removed at 10-15 min intervals and bupivacaine concentrations measured in foetal plasma, brain, placenta, amniotic fluid, maternal plasma sampled synchronously and maternal brain at the end of the experiment. Mean maximum foetal : maternal (F:M) ratio was 0.31 (SD 0.16) (range 0.18-0.64). Mean foetal brain : plasma ratios ranged from 2.04 to 5.09. There was no progressive increase in foetal brain bupivacaine concentration with time. Maternal brain : plasma ratio was 1.62 (0.81). However, maximum foetal brain concentration was only 0.27-0.86 of maternal. Concentrations increased with time in amniotic fluid, but did not exceed those in maternal plasma.

The above data are consistent with the evidence from the wide use of bupivacaine hydrochloride for tens of years that this drug does not evoke reproductive or developmental adverse events / effects.

2.4.4.4.4 Other Toxicity Data

As above discussed, any problem related to genotoxicity and carcinogenicity must be ruled out with bupivacaine hydrochloride, a drug used in millions of humans for decades. This wide use has produced complete profiles of any adverse effect of the drug.

2.4.4.5 FENTANYL

2.4.4.5.1 Reproductive and developmental toxicity

Fentanyl has been widely used as anaesthetic for extradural analgesia in labour and delivery, for this reason a large number of studies have been performed throughout the years in order to assess teratogenic activity of this drug. Moreover, the ability of fentanyl to pass through the placental barrier and cause problems to the foetus has been investigated in different animal models.

Placental passage and effects of fentanyl on uterine blood flow were studied in pregnant sheep, after intravenous administration of different doses of fentanyl (50, 75 and 100 µg) [redacted]. Effects of this drug on sheep mother and foetus were also studied after epidural administration of 50 and 100 µg [redacted]

Blood levels of fentanyl were measured both in mother and foetus.

With both routes of administration and all the injected doses, no dramatic change was observed in any maternal or foetal parameter, as reported in Table 10.

Table 10

Maternal and foetal effects on some parameters after different doses of fentanyl administered per intravenous [redacted] and epidural [redacted] injection in pregnant sheep.

		Intravenous administration (50, 75, 100 µg)	Epidural administration (50, 100 µg)
Maternal effects	Cardiovascular parameters	No significant alterations	Slight drop in heart rate (50 µg); slight increase in heart rate (100 µg). Slight decrease in pulmonary arterial pressure and pulmonary vascular resistance (100 µg)
	Acid-base status (blood pH, $PaCO_2$, serum bicarbonate, base excess)	75- and 100-µg doses increased PaO_2	Slight drop in PaO_2 (50 µg); no changes with 100 µg
	Uterine blood flow	No significant alterations	No significant alterations
	Intrauterine pressure	No significant alterations	No significant alterations
Foetal effects	Cardiovascular parameters	75- and 100-µg doses increased heart rate	No significant alterations
	Acid-base status (blood pH, $PaCO_2$, serum bicarbonate, base excess)	75- and 100-µg doses increased PaO_2	Slight decrease in pH and increase in $PaCO_2$ (50 µg); decrease in PaO2 (100 µg)

Toxic effects of fentanyl were studied on chicken embryos too [redacted], by inoculating 8-day fecundated eggs with 5 or 50 µg of fentanyl. No teratogenic effects were noticed with any of the employed fentanyl doses, even if the percentage of eggs that reached complete development was markedly lower for the eggs inoculated with higher fentanyl doses (86.5 % control; 65.4 % for 5 µg; 26.9 % for 50 µg).

Teratogenicity of fentanyl was also tested after repeated dose in rats. Female pregnant Sprague-Dawley rats were dosed for two weeks with different dosage regimens of fentanyl: 10, 100, 500 µg/kg/day. There were no major or minor reproductive abnormalities or teratogenic findings in any of the treated groups [redacted].

Only in combination with nitric oxide, fentanyl showed some effect: the addition of fentanyl to nitrous oxide increased the mortality rate among the rats, even though it did not significantly add to the adverse reproductive or teratogenic effects of nitrous oxide [redacted].

2.4.4.5.2 Safety data on laboratory animals

A great amount of clinical data is available for fentanyl, due to its 40-year long use; this compound has been used also as a comparison for newer derivatives. Therefore, some toxicity data on laboratory animals have been published too. Acute fentanyl toxicity in rats (LD_{50}) has been reported to be 62 mg/kg, while the efficacy dose (ED_{50}) is 0.08 mg/kg [redacted].

Safety margins of fentanyl in mice and rats have been reported to be respectively 1:454 and 1:277, after i.v. injection [redacted].

2.4.4.5.3 Tolerability of new fentanyl formulations

Fentanyl citrate has been used in several different formulations (injectable, transdermal delivery systems, oral transmucosal) for a number of years. Fentanyl citrate has also been formulated for intranasal nebulization, a formulation that could allow a faster onset of the analgesic action.

Clinical studies aimed at assessing tolerability and acceptability of such a formulation in cancer pain relief have shown that, after administration of 20 µg of fentanyl citrate, no systemic adverse events were noted and that the only inconvenience encountered was itching of the nasal mucosae [redacted].

2.4.5 INTEGRATED OVERVIEW AND CONCLUSIONS

Bupivacaine hydrochloride is a local and locoregional anaesthetic, able to produce anaesthesia in body regions, including spinal anaesthesia.

Fentanyl is a potent opioid analgesic used for induction and maintenance of anaesthesia.

The association of bupivacaine hydrochloride and fentanyl associated in the fixed dose ratios of 1.0 and 1.25 mg/ml to 2 µg/ml in infusion bags is indicated in post operative and labor analgesia. This association is the object of the application requested by the applicant.

The present non clinical overview, Module 2.4, is addressed to complete the application dossier, as requested by operating guidelines in Europe.

In this expert report pharmacodynamics, pharmacology, pharmacokinetics and toxicology of the two active ingredients, namely bupivacaine hydrochloride and fentanyl citrate, were extensively reviewed from data present in the literature.

Both the above active ingredients are well-known and have been used in therapy for several decades alone and in association. A vast amount of clinical data supports their use in association by the epidural route. Further supported by the present non clinical overview reporting data from animals, integrated with some relevant data ascertained in humans in order to produce a more exhaustive picture of this interesting association, it can be concluded that the product is safe for its intended clinical use.

2.4.6 LIST OF LITERATURE

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Text redacted under sections 41 and 43 of the FOI Act