CLOZAPINE (API):
DETERMINATION OF ACCEPTABLE DAILY EXPOSURE (ADE)
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# 1. BASIC INFORMATION

<table>
<thead>
<tr>
<th>Toxicological Profile, Hazards Identification, Risk Assessment and Acceptable Daily Exposure (ADE) Monograph of Clozapine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sponsor</strong></td>
</tr>
<tr>
<td><strong>ADE Value</strong>                                                       0.5mg/day</td>
</tr>
<tr>
<td><strong>Expert name</strong></td>
</tr>
<tr>
<td><strong>Signature and Date</strong></td>
</tr>
<tr>
<td><strong>Reviewed by</strong></td>
</tr>
<tr>
<td><strong>Signature and Date</strong></td>
</tr>
<tr>
<td><strong>Chemical name</strong>                                                   3-chloro-6-(4-methylpiperazin-1-yl)-11H-benzo[b][1,4]benzodiazepine</td>
</tr>
<tr>
<td><strong>Drug product</strong>                                                    Clozapine</td>
</tr>
</tbody>
</table>

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**Notes:**
- The table above provides a summary of the toxicological profile, hazards identification, risk assessment, and acceptable daily exposure (ADE) for Clozapine.
- The chemical name is 3-chloro-6-(4-methylpiperazin-1-yl)-11H-benzo[b][1,4]benzodiazepine.
- The drug product is Clozapine.

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**Contact Information:**
- www.indivirtus.com
- upendra@indivirtus.com M: +91 9814133808
- GR Tower, 5th Floor, D-258, Industrial Area, Phase 8-A, Sector 75, Mohali, Punjab - 160055
### 2. HAZARDS IDENTIFIED

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotoxicant</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Carcinogen</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Reproductive and developmental toxicant</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Highly Sensitizing potential</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
### 3. SUMMARY OF ASSESSMENT PROCESS

<table>
<thead>
<tr>
<th>Acceptable daily exposure (ADE) value</th>
<th>0.5mg/day</th>
</tr>
</thead>
</table>

### HAZARD IDENTIFICATION

<table>
<thead>
<tr>
<th>Pharmacodynamics data</th>
<th>Clozapine action is likely mediated through a combination of antagonistic effects at D₂ receptors in the mesolimbic pathway and 5-HT₂A receptors in the frontal cortex. D₂ antagonism relieves positive symptoms while 5-HT₂A antagonism alleviates negative symptoms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute toxicity</td>
<td>Acute toxicity studies have been performed in various species. The LD₅₀ value for the mice was found to be 61 mg/kg (intravenous, M &amp; F); 90 mg/kg (intraperitoneal, M &amp; F); 210 mg/kg (oral, M); 190 mg/kg (oral, F), for the rat was found to be 58 mg/kg (intravenous, M &amp; F); 228 mg/kg (intramuscular, M); 198 mg/kg (intramuscular, F); 325 mg/kg (oral, M); 225 mg/kg (oral, F), for the guinea pig was found to be 510 mg/kg (oral, M); 681 mg/kg (oral, F) and for the dog was found to be 145 mg/kg (oral, M &amp; F).</td>
</tr>
<tr>
<td>Repeated dose toxicity</td>
<td>Repeated dose toxicity studies have been performed or conducted in rats (0, 20, 40 mg/kg/day), dogs (5, 10, 20 mg/kg/day) and monkeys (3, 20 mg/kg/day). Slight increase in the liver weight was reported in rats whereas in dog sedation, salivation and muscular tremors and in monkey sedation and slight changes in blood parameters without significant severe toxic effect (organ toxicity) were observed.</td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>No carcinogenic potential was demonstrated in long-term studies in mice and rats at doses up to 74 and 75 mg/kg respectively.</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td>Clozapine was not genotoxic when tested in the gene mutation and chromosomal aberration tests such as</td>
</tr>
</tbody>
</table>
bacterial Ames test, the *in-vitro* mammalian V79 in Chinese hamster cells, the *in-vitro* unscheduled DNA synthesis in rat hepatocytes or the *in-vivo* micronucleus assay in mice.

**Reproductive/Developmental toxicity**

Clozapine had no effect on any parameters of fertility, pregnancy, fetal weight or postnatal development when administered orally to male rats 70 days before mating and to female rats for 14 days before mating at doses of 20 and 40 mg/kg/day. It is pregnancy category B drug.

**IDENTIFICATION OF CRITICAL EFFECTS**

**Sensitive indicator of an adverse effect seen in non – clinical toxicity data**

No critical or organ toxicity has been reported in animal studies. Sedation, salivation (in dogs), slight increase in liver weight (in rats) and slight variation in blood parameters (in monkeys) were reported.

**Clinical therapeutic and adverse effects**

Clozapine is used to treat severe schizophrenia, or to reduce the risk of suicidal behaviour in people with schizophrenia or similar disorders. Adverse effects associated with clozapine are sedation, dizziness, headache, tremor, salivation, sweating, dry mouth, visual disturbances, constipation, nausea and fever.

**Therapeutic Dose**

Initial dose: 12.5 mg orally once or twice a day.
Maximum dose: 900 mg per day.
### APPLICATION OF ADJUSTMENT FACTORS - ADE CALCULATION

<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UF&lt;sub&gt;H&lt;/sub&gt;: Intraspecies Differences</strong></td>
<td>10</td>
<td>Conventionally used to allow for differences between individuals in the human population</td>
</tr>
<tr>
<td><strong>UF&lt;sub&gt;A&lt;/sub&gt;: Interspecies Differences</strong></td>
<td>3</td>
<td>For extrapolation from monkeys to humans</td>
</tr>
<tr>
<td><strong>UF&lt;sub&gt;S&lt;/sub&gt;: Sub-chronic-to-Chronic Extrapolation</strong></td>
<td>5</td>
<td>Long term (2-year) toxicity study in non-rodent (monkeys).</td>
</tr>
<tr>
<td><strong>UF&lt;sub&gt;L&lt;/sub&gt;: LOAEL-to-NOAEL Extrapolation</strong></td>
<td>1</td>
<td>Selection of NOAEL dose.</td>
</tr>
<tr>
<td><strong>UF&lt;sub&gt;D&lt;/sub&gt;: Database Completeness</strong></td>
<td>1</td>
<td>Reliable</td>
</tr>
<tr>
<td><strong>MF: Modifying Factor/ Severity of Effect</strong></td>
<td>2</td>
<td>CNS toxicity in dogs and monkeys</td>
</tr>
<tr>
<td><strong>α correction</strong></td>
<td></td>
<td>Not applied</td>
</tr>
</tbody>
</table>
4. IDENTIFICATION OF THE ACTIVE SUBSTANCE

Clozapine is a synthetic dibenzo-diazepine derivative, atypical antipsychotic and it blocks several neurotransmitter receptors in the brain (dopamine type 4, serotonin type 2, norepinephrine, acetylcholine and histamine receptors).

**IUPAC Name:** 3-chloro-6-(4-methylpiperazin-1-yl)-11\textsubscript{H}-benzo[b][1,4] benzodiazepine.

**Chemical Abstract Service (CAS) registry number:** 5786-21-0

**Chemical description and physical properties:** Clozapine is a yellow, crystalline powder with a melting range of 182°-186°C.

**Molecular formula:** C\textsubscript{18}H\textsubscript{19}ClN\textsubscript{4}

**Molecular weight:** 326.8 g/mol.

**Molecular structure:**

![Figure 1: Structure of Clozapine (1).](image)

www.indivirtus.com
upendra@indivirtus.com M: +91 9814133808
GR Tower, 5\textsuperscript{th} Floor, D-258, Industrial Area, Phase-8A, Mohali, Punjab - 160055
5. OBJECTIVE AND SEARCH STRATEGY

At present, pharmaceutical companies are investing significant effort to assess and control cross-contamination risk of drug products that are manufactured in the shared production facilities (2). Determination of health based exposure limits for a residual active substance through the derivation of a safe threshold value is employed to identify the risk posed. The derivation of threshold value like acceptable daily exposure (ADE) or threshold of toxicological concern is used to determine the risk of the active pharmaceutical substance. For determination of ADE all the available pharmacological and toxicological data including both non-clinical and clinical data should be evaluated. This involves hazard identification by reviewing all relevant data, identification of critical effects, determination of NOAEL/LOAEL of the findings that are considered to be critical effects.

In this document, brief summary of pharmacological, pharmacokinetics and toxicity data of clozapine have been presented based on the published data. The data were extracted from Drug bank, Pub chem, MSDS and Product monographs.

6. INTRODUCTION

Clozapine was the first atypical antipsychotic approved for treatment of schizophrenia.

Pharmacotherapeutic group: Anti-psychotic; ATC code: N05AH02

7. HAZARD IDENTIFICATION

a. Pharmacodynamics data

Clozapine mediated its action is likely mediated through a combination of antagonistic effects at D<sub>2</sub> receptors in the mesolimbic pathway and 5-HT<sub>2A</sub> receptors in the frontal cortex. D<sub>2</sub> antagonism relieves positive symptoms while 5-HT<sub>2A</sub> antagonism alleviates negative symptoms (4).

b. Acute toxicity studies

Table 1: Acute toxicity studies of clozapine in various animal species by different routes of administration (4).
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>SEX</th>
<th>ROUTE</th>
<th>LD$_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>M, F</td>
<td>Intravenous</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>M, F</td>
<td>Intraperitoneal</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Oral</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Oral</td>
<td>190</td>
</tr>
<tr>
<td>Rat</td>
<td>M, F</td>
<td>Intravenous</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Intramuscular</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Intramuscular</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Oral</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Oral</td>
<td>225</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>M</td>
<td>Oral</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Oral</td>
<td>681</td>
</tr>
<tr>
<td>Dog</td>
<td>M, F</td>
<td>Oral</td>
<td>145</td>
</tr>
</tbody>
</table>

c. Repeated dose toxicity studies

Toxicity study in rats

26-weeks oral toxicity study
Clozapine was administered orally (by gavage) to rats at the dose levels of 10, 20 and 40 mg/kg/day (5 days/week), for the duration of 26 weeks. Parameters such as clinical signs, body weights, haematology, clinical chemistry, urinalysis as well as full necropsy (with organ weights) and histological examination were examined. Slight increase in liver weights in the males was reported at the dose of 10 mg/kg and the doses of 20 & 40 mg/kg/day produced sedation during the early weeks and aggression during the later weeks after the treatment. Slight increase in the absolute and relative liver weights were reported and the fore-stomach was slightly dilated in males (4).

Toxicity study in dogs

13-weeks oral toxicity study
Clozapine was administered at the doses of 5, 10 and 20 mg/kg/day in gelatin capsules to beagle dogs (7 days/week) for the duration of 13-weeks. Parameters such as body weight, food intake, clinical signs, physical and neurological examinations, electrocardiography, hematology, clinical chemistry, urinalysis, as well as full necropsy (including organ weights) and histology were examined. Sedation, muscular relaxation, miosis, lacrimation, salivation, muscular tremors, prolapse of nictitating membranes, irritability and emesis were observed at all dose levels. All signs disappeared within 12 hours after the treatment with the exception of salivation, which persisted in some cases for 24 hours. No toxicological changes were reported with the exception of some increases in liver weights in some dogs compared with the controls, but there was no evidence of dose-dependence. One female at the mid dose level died after 25 days of treatment. Necropsy revealed that death was due to acute pneumonia, and was not related to treatment and no other deaths occurred at any dose level (4).

**Oral toxicity study in dogs using escalating doses**

Clozapine was administered orally in gelatin capsules to beagle dogs at dose levels that increased daily (7 days/week) for the period of 13 weeks. During the administration period the dose was gradually increased from 20 to 90 mg/kg/day and the high dose was maintained from week 9 to week 13. Thereafter, half of the dogs were sacrificed, while the remainder were entered into an 8-week drug-free recovery period before being sacrificed. Initially the dogs showed slight paresis, prolapse of the nictitating membrane, salivation, tremor and distinct dacryorrhea (excessive secretion of tears) at the doses of 20 to 30 mg/kg/day. With increasing doses these signs became progressively prominent. In addition, miosis, unnatural posture, tachypnoea and aggression was seen. Convulsion and ataxia were reported in two dogs. All adverse signs or toxic effects disappeared within two weeks after discontinuation of drug administration. ECG tracings revealed decreased heart rate, prolonged QT intervals and twin-peaked T-waves in some leads. The ECG changes disappeared 4 weeks after the withdrawal of clozapine. Microscopic examinations were unremarkable. The final dose reached was roughly 60% of the acute LD$_{50}$ (4).

**1-year oral toxicity study**
Clozapine was administered orally in gelatin capsules to beagle dogs at dose levels of 5, 10 and 20 mg/kg/day for the duration of 4 weeks and thereafter at doses of 7.5, 15 and 30 mg/kg/day (7 days/week). A control group received empty gelatin capsules. Parameters such as body weight, food intake, clinical signs, physical and neurological examinations, electrocardiography, hematology, clinical chemistry, urinalysis, as well as full necropsy (including organ weights) and histology were examined. Clinical effects due to the pharmacological action of the drug (e.g., salivation, apathy, slight tremor and diarrhoea) occurred at all dose levels in a dose-dependent manner. Minor coincidental lesions reported in some dogs but it was not due to drug administration (4).

**Toxicity study in monkeys**

**2-year oral toxicity study**
Clozapine was administered orally in gelatin capsules at the dose levels of 3 and 20 mg/kg/day (the high dose level was between 15 and 30 mg/kg/day during the early weeks) for the duration of 104 weeks (7 days/week). Parameters measured included clinical observation, body weights, hematology, blood chemistry, electrocardiography, ophthalmoscopy, as well as full necropsy with histological work-up of two monkeys per dose after 52 weeks and two further monkeys per dose after 104 weeks. Slight transient clinical signs (sedation and ptosis on day 1) and minor hematologic changes in the early weeks (slight falls in red and white blood cell counts without development of anaemia or leukopenia) was reported at the dose level of 3 mg/kg/day. ECG tracings showed slightly prolonged QT intervals in individual monkeys at sporadic intervals, mostly in the first year. This dose is considered a "no-toxic effect" dose level for the monkeys. Sedation, ptosis, salivation, impairment in weight gain and slight reduction of red and white cell counts (without anaemia or leukopenia) were reported at the dose level of 20 mg/kg/day. The ECG changes were similar to those seen at the low dose level, although there was a decrease in the incidence of these changes during the second year. Slightly increased lipopigment deposition in myocardial fibres was reported on post-mortem examination after one year of drug administration. After two years there was a distinct brown discoloration of the heart and urinary bladder mucosa associated with pigment deposition. Similar pigment was also observed microscopically in the neurons of the CNS and the mucosa of the gallbladder. Splenic weights were slightly increased and no specific organ toxicity was reported after the drug administration.
Thus, no-observed-adverse-effect-level (NOAEL) of 3 mg/kg/day was derived from the 2-year oral toxicity study in monkeys (4).

d. Carcinogenicity

Study in mice
Mice were administered clozapine orally (diet) at an initial dose of approximately 40 mg/kg/day for the duration of 78-weeks. From 32 weeks onwards, half of the treated mice were given a dose of approximately 75 mg/kg/day. The purpose of the study was primarily to detect any carcinogenic potential of the drug. Parameters such as body weight, food intake, hematology, blood chemistry, urinalysis, full necropsy (including organ weights) and histology of all major organs were examined. During the early weeks of treatment up to 40% of the mice (including controls) had occasional skin lesions of unknown etiology and no-carcinogenic potential was detected.

Study in rats
Rats were administered clozapine orally (mixed in their feed) at the dose levels of 15, 31 and 74 mg/kg/day for 100 weeks and 3, 10 and 35 mg/kg/day for 108 weeks. A control group received unmedicated feed. The purpose of the study was to detect any chronic toxic effects including carcinogenic potential of the drug in rats. Body weight, food intake, clinical signs, hematology, blood chemistry, full necropsy (including organ weights) and histology of 33 organs were evaluated. At the doses of 31 and 74 mg/kg/day doses, increased lipopigment was seen in the thyroid, brain, kidney, liver, heart, spleen and skeletal muscle of animals dying or sacrificed after one year. At the terminal examination (100 weeks) pigment was also seen in the thyroid, heart and brain at the 15 mg/kg/day dose. The presence of increased amounts of pigment was not associated with significant adverse changes. The liver showed microscopic changes at all three dose levels, namely centro-lobular vacuolization and hepatocyte swelling, in addition to increased liver weight and these effects were dose-dependent. At the dose of 31 mg/kg/day urine was reddened (probably due to a metabolite). BUN (blood urea nitrogen) and SGPT (alanine aminotransferase) levels were slightly increased at 26 and 100 weeks and degenerative changes were reported in the testes and skeletal muscle. Overall mortality was marginally increased in the treated rats compared with the controls but no dose-dependence was seen and no carcinogenic potential were detected in the study (4).
e. Genotoxicity and Mutagenicity
Genotoxic and mutagenic potential was evaluated in battery of tests such as Ames test (*Salmonella* typhimurium), DNA repair synthesis (UDS) in-vitro rat hepatocytes, V 79 Chinese hamster cells in-vitro and in-vivo micronucleus test and data indicated the negative genotoxic and mutagenic potential of clozapine (4).

f. Reproductive and teratogenic studies

**Fertility study in rats**
Clozapine was administered orally (gavage) to male and female rats at the doses of 20 and 40 mg/kg/day before mating for 70 and 14 days respectively. Control groups were administered plain water by gavage. On day 13th of pregnancy half of the dams were sacrificed and genital tract and the condition of the fetuses were examined. The remaining dams were allowed to litter and the young were sacrificed on day 21 postpartum and evaluated for abnormalities. At the end of the treatment period treated males at both dose levels had impaired weight gain as compared to the controls. Sedation was reported at the dose of 40 mg/kg/day and excitation at the dose of 20 mg/kg/day. Fertility, pregnancy, fetal and postnatal development of the young were normal throughout the study. In the females, the 2-week treatment period had no adverse effect on weight gain. The pharmacological effects observed were similar to those seen in the males. Pregnancy rate was remarkably high in the 40 mg/kg group but was associated with a slightly increased number of intrauterine deaths. No abnormalities were observed in fetuses or newborn animals. Birth and postnatal development were normal throughout the study (4).

**Perinatal study in rats**
Clozapine was administered orally (gavage) at doses of 20 and 40 mg/kg/day to mated female rats over the last third of pregnancy until day 21 post-partum. Control group was given water. Examination of the fetuses were made at birth and during the postnatal period. A dose-dependent impairment of weight gain was reported in the dams. Weight loss was reported at the dose of 40 mg/kg/day. Litter size and litter weights were within normal limits, although a slight dose-dependent reduction was seen. Survival rates and mean weights of the offspring were reduced by the end of lactation as compared to controls. The offspring showed evidence of increased excitability(4).
Teratology study in rats and rabbits
Clozapine was administered orally (gavage) during organogenesis to pregnant rats (days 5 to 16 of pregnancy in rats) and pregnant rabbits (days 6 to 18 of pregnancy in rabbits) at the doses of 20 and 40 mg/kg/day. The control groups received water. Maternal, litter and fetal parameters were evaluated and clozapine in the doses used had no apparent effect on the maternal, litter or fetal parameters. In rabbits, clozapine attenuated weight gain during drug administration, which was not compensated for during the remainder of pregnancy. Nonetheless, no treatment-related change in pregnancy, litter or fetal data was reported, apart from a slight reduction in mean fetal weights (within normal limits) (4).

Generation study in rats
The offspring of the three groups of the above study (controls, 20 and 40 mg/kg/day) were allowed to reach sexual maturity and mated, six possible combinations between groups being used (control males with 40 mg/kg females, control males with 20 mg/kg females, 40 mg/kg males with control females, 20 mg/kg males with control females, 20 mg/kg males with same dose females, 40 mg/kg males with same dose females). Pregnancy rates and litter data as well as the postnatal development of the F2-generation were studied. In none of the 6 groups was a deviation from normal values detected, nor was any intra-group difference noted. From these results, it may be concluded that clozapine administration had no effect on the F2-generation (4). Clozapine is pregnancy category B drug.

8. IDENTIFICATION OF CRITICAL EFFECTS
Scientific evaluation of published pharmacological and toxicological data including clinical and non-clinical reports helps to identify the adverse effect of the active substances. The critical effect of the active substance is one that meets the severity and persistence criteria at the lowest intake to define the hazard associated with the intake.

i. Most sensitive indicator of an adverse effect seen in non-clinical toxicity data
No critical or organ toxicity has been reported in animal studies. Reversible CNS toxicity was observed in dogs and monkeys.
ii. Clinical therapeutic and adverse effects

Clozapine is used to treat severe schizophrenia, or to reduce the risk of suicidal behaviour in people with schizophrenia or similar disorders (5). Adverse events observed in association with the use of clozapine in clinical trials at an incidence of greater than 5% were central nervous system complaints (sedation, vertigo, headache and tremor); autonomic nervous system (salivation, sweating, dry mouth and visual disturbances); cardiovascular findings (tachycardia, hypotension and syncope) and gastrointestinal complaints (constipation, nausea and fever). Complaints of drowsiness/sedation tend to subside with continued therapy or dose reduction. Salivation may be profuse, especially during sleep, but may be diminished with dose reduction (4).

9. RATIONALE FOR NO OBSERVED EFFECT LEVEL (NOAEL) VALUE SELECTION

The toxicity of clozapine was studied in rodents and non-rodents for up to 2 years. Dose dependent and reversible CNS toxicity was observed in dogs and monkeys. The rationale behind the selection of NOAEL dose of 3 mg/kg/day from 2-year monkey study is because monkeys are more relevant to the human species.

10. APPLICATION OF ADJUSTMENT FACTORS (RATIONALE FOR THE ADJUSTMENT FACTORS)

A series of modifying or safety factors are used when NOAEL is based on studies of differing types and duration in different species to provide a risk assessment for human exposure (6,7).

a. UF_H: Intraspecies Differences

A value of UF_H = 10 is conventionally used to allow for differences between individuals in the human population.

A factor of 10 is selected.
b. **UF$_A$: Interspecies differences**

The uncertainty factor for interspecies variability is used to adjust NOAEL derived from animals and for use and human applications. Applying the same calculation to the other species and expressing the results as multiples of the human surface area: body weight ratio gives the factor for the mouse = 12; for the rat = 5; for the monkey = 3; for the rabbit = 2.5; for the dog = 2. For other species where the data are not so well established the factor is taken as 10. A factor of 3 is selected based on the selection of monkey.

c. **UF$_S$: Sub chronic-to-Chronic Extrapolation**

A variable factor up to 10 takes into account the differing durations of exposure in the reported studies. For reproductive studies, a factor of 1 is used if the whole period of organogenesis is covered. A factor of 1 has been used for a study last at least one-half lifetime (1 year for rodents or rabbits; 7 years for cats, dogs and monkeys). A factor of 2 has been used for a 6-month study in rodents, or a 3.5-year study in non-rodents. A factor of 5 has been used for a 3-month study in rodents or a 2-year study in non-rodents and a factor of 10 for studies of a shorter duration. In all cases, the higher factor has been used for study duration between the time points e.g. a factor of 2 for a 9-month rodent study.

A factor of 5 is used based on long term toxicity study in monkeys.

d. **UF$_L$: LOAEL-to-NOAEL Extrapolation**

A variable factor up to 1 applied to result in which ADE being derived from NOAEL dose.

e. **MF: Modifying Factor/ Severity of Effect**

A variable factor is applied when the toxicity produced is irreversible in nature i.e. carcinogenicity, neurotoxicity or teratogenicity. A factor of 10 is used when oncogenic or neurotoxic responses are present. A variable factor is used for reproductive toxicity effects as follows: 1 for embryo or fetus toxicity or mortality associated with maternal toxicity; 5 for embryo or foetus toxicity or mortality without maternal toxicity; 5 for a teratogenic effect with maternal toxicity and 10 for a teratogenic effect in the absence of accompanying maternal toxicity.

A factor of 2 is selected the observed CNS toxicity in dogs and monkeys.
f. $U_{FD}$: Database Completeness

A factor of 1 is used as the toxicity data is found to be reliable.

$\alpha$: Bioavailability Correction

A factor of 1 is used as no bioavailability corrections used.

ADE CALCULATION:

The ADE calculation is generally presented in the format:

$$\text{ADE value (mg/day)} = \frac{\text{NOAEL} \times 50}{U_{F_H} \times U_{F_A} \times U_{F_s} \times U_{F_L} \times U_{F_D} \times \text{MF} \times \alpha}$$

$$= \frac{3 \times 50}{10 \times 3 \times 5 \times 1 \times 1 \times 1 \times 2}$$

$$= \frac{150}{300}$$

$$= 0.5 \text{ mg/day}$$

11. PK CORRECTION

No Pharmacokinetic correction was carried out since the same administration route was used for ADE calculation.
12. REFERENCES

   https://www.drugbank.ca/drugs/DB00363


   https://www.drugs.com/clozapine.html

6. ICH guideline Q3C (R7) on impurities: guideline for residual solvents,
   EMA/CHMP/ICH/82260/2006

7. VICH GL 18 residual solvents in new veterinary medicinal products, active substances and excipients (Revision), EMA/CVMP/VICH/502/1999-Rev.1, 25 May 2010
ANNEXURE I: PHARMACOKINETICS AND METABOLISM

Absorption: The absorption of clozapine is 90% to 95% after oral administration; neither the rate nor the extent of absorption is influenced by food. The absolute bioavailability of clozapine is 50% to 60% (3).

Distribution: Clozapine is approximately 95% bound to plasma proteins and the volume of distribution is 1.6 L/kg (3).

Metabolism: Clozapine is almost completely metabolized by hepatic enzymes (CYP1A2 and CYP3A4), and to some extent by CYP2C19 and 2CYPD6. Amongst all the metabolites only the desmethyl metabolite was found to be active (3).

Elimination: The elimination of clozapine is biphasic with a mean terminal half-life of 12 hours (range: 6 to 26 hours). Approximately 50% of the administered dose being excreted as metabolites in the urine and 30% in the faeces (3).
ANNEXURE II: GLOSSARY

**ADI/ADE:** Acceptable daily intake/ Acceptable daily exposure

**AUC:** Area under the curve

**GRAS:** Generally regarded as safe

**GLP:** Good laboratory practice

**GMP:** Good manufacturing practice

**LD:** Lethal dose

**LED:** Lowest-effective dose

**TDLo (Toxic Dose Low):** Lowest published toxic dose

**LOAEL:** Lowest-observed-adverse-effect level

**LOEL:** Lowest-observed-effect level

**MSDS:** Material safety data sheet

**MTD:** Maximum tolerable dose

**MPDD:** Maximum permissible daily dose

**MTEL:** Maximum tolerable exposure level

**NEL:** No-effect level

**NOAEL:** No-observed-adverse-effect level

**NOEL:** No-observed-effect level

**OEL:** Occupational exposure limit

**QSAR:** Quantitative structure–activity relationship

**SDS:** Safety data sheet

**ADI:** Acceptable daily intake. Estimate by JECFA; the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk.

**Area under the curve (AUC):** Area between a curve and the abscissa (horizontal axis), i.e., the area underneath the graph of a function; often, the area under the tissue (plasma) concentration curve of a substance expressed as a function of time.

**Bioaccumulation:** progressive increase in the amount of a substance in an organism or part of an organism that occurs because the rate of intake exceeds the organism’s ability to remove the substance from the body.

**Bioavailability:** biological and physiological availability. Extent of absorption of a substance by a living organism compared to a standard system.
Biological half-life: for a substance, the time required for the amount of that substance in a biological system to be reduced to one-half of its value by biological processes, when the rate of removal is approximately exponential.

Carcinogen: agent (chemical, physical, or biological) that is capable of increasing the incidence of malignant neoplasms, thus causing cancer.

Clastogen: agent causing chromosome breakage and (or) consequent gain, loss, or rearrangement of pieces of chromosomes.

Clearance: volume of blood or plasma or mass of an organ effectively cleared of a substance by elimination (metabolism and excretion) divided by time of elimination.

Cmax: used in pharmacokinetics referring to the maximum (or peak) serum concentration that a drug achieves in a specified compartment or test area after the drug has been administrated and before the administration of a second dose.

Critical dose: dose of a substance at and above which adverse functional changes, reversible or irreversible, occur in a cell or an organ.

Critical effect: for deterministic effects, the first adverse effect that appears when the threshold (critical) concentration or dose is reached in the critical organ:

Adverse effects with no defined threshold concentration are regarded as critical. Dose (of a substance): total amount of a substance administered to, taken up, or absorbed by an organism, organ, or tissue.

Draize test: evaluation of materials for their potential to cause dermal or ocular irritation and corrosion following local exposure; generally using the rabbit model (almost exclusively the New Zealand White) although other animal species have been used.

Elimination (in toxicology): disappearance of a substance from an organism or a part thereof, by processes of metabolism, secretion, or excretion.

Embryotoxicity: production by a substance of toxic effects in progeny in the first period of pregnancy between conception and the fetal stage.

Fetotoxicity: production by a substance of toxic effects in progeny in the second period of pregnancy between fetal stage and delivery.

First-pass effect: biotransformation and, in some cases, elimination of a substance in the liver after absorption from the intestine and before it reaches the systemic circulation.

Gavage: administration of materials directly into the stomach by oesophageal intubation.
Generally regarded as safe (GRAS): phrase used to describe the USFDA philosophy that justifies approval of food additives that may not meet the usual test criteria for safety but have been used extensively and have not demonstrated that they cause any harm to consumers.

Genotoxic: capable of causing a change to the structure of the genome.

Good laboratory practice (GLP) principles: fundamental rules incorporated in OECD guidelines and national regulations concerned with the process of effective organization and the conditions under which laboratory studies are properly planned, performed, monitored, recorded, and reported.

Good manufacturing practice (GMP) principles: fundamental rules incorporated in national regulations concerned with the process of effective organization of production and ensuring standards of defined quality at all stages of production, distribution, and marketing.

Hazard identification: determination of substances of concern, their adverse effects, target populations, and conditions of exposure, taking into account toxicity data and knowledge of effects on human health, other organisms, and their environment.

Hypersensitivity: state in which an individual reacts with allergic effects following exposure to a certain substance (allergen) after having been exposed previously to the same substance.

In silico: phrase applied to data generated and analysed using computer modelling and information technology.

In vitro: in glass, referring to a study in the laboratory usually involving isolated organ, tissue, cell, or biochemical systems.

In vivo: In the living body, referring to a study performed on a living organism.

Lethal dose (LD): amount of a substance or physical agent (e.g., radiation) that causes death when taken into the body.

Lowest-effective dose (LED): lowest dose of a chemical inducing a specified effect in a specified fraction of exposed individuals.

Lowest published toxic dose (Toxic Dose Low, TDLo): the lowest dosage per unit of bodyweight (typically stated in milligrams per kilogram) of a substance known to have produced signs of toxicity in a particular animal species.

Lowest-observed-adverse-effect level (LOAEL): lowest concentration or amount of a substance (dose), found by experiment or observation, that causes an adverse effect on morphology, functional capacity, growth, development, or life span of a target organism.
distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure.

**Lowest-observed-effect level (LOEL):** lowest concentration or amount of a substance (dose), found by experiment or observation, that causes any alteration in morphology, functional capacity, growth, development, or life span of target organisms distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

**Material safety data sheet (MSDS):** compilation of information required under the U.S. OSHA Hazard Communication Standard on the identity of hazardous substances, health and physical hazards, exposure limits, and precautions.

**Maximum permissible daily dose (MPDD):** maximum daily dose of substance whose penetration into a human body during a lifetime will not cause diseases or health hazards that can be detected by current investigation methods and will not adversely affect future generations.

**Maximum tolerable dose (MTD):** highest amount of a substance that, when introduced into the body, does not kill test animals (denoted by LD0).

**Maximum tolerable exposure level (MTEL):** maximum amount (dose) or concentration of a substance to which an organism can be exposed without leading to an adverse effect after prolonged exposure time. Maximum tolerated dose (MTD): high dose used in chronic toxicity testing that is expected on the basis of an adequate sub-chronic study to produce limited toxicity when administered for the duration of the test period.

**Median lethal dose (LD50):** statistically derived median dose of a chemical or physical agent (radiation) expected to kill 50% of organisms in a given population under a defined set of conditions.

**Mutagenicity:** ability of a physical, chemical, or biological agent to induce (or generate) heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof).

**No-effect level (NEL):** maximum dose (of a substance) that produces no detectable changes under defined conditions of exposure.

**No-observed-adverse-effect level (NOAEL):** greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of
morbidity, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure.

**No-observed-effect level (NOEL):** greatest concentration or amount of a substance, found by experiment or observation, that causes no alterations of morphology, functional capacity, growth, development, or life span of target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

**Quantitative structure–activity relationship (QSAR):** quantitative structure–biological activity model derived using regression analysis and containing as parameters physicochemical constants, indicator variables, or theoretically calculated values.

**Safety data sheet (SDS):** single page giving toxicological and other safety advice, usually associated with a particular preparation, substance, or process.

**Target (in biology):** any organism, organ, tissue, cell or cell constituent that is subject to the action of an agent.

**Temporary acceptable daily intake:** value for the acceptable daily intake proposed for guidance when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but are insufficient to conclude that use of the substance is safe over a lifetime. Note: A higher-than-normal safety factor is used when establishing a temporary ADI and an expiration date is established by which time appropriate data to resolve the safety issue should be available.