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Subject: Risk Assessment Report on the new psychoactive substance 5-(2-aminopropyl)indole

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Following the Council's request to conduct a Risk Assessment on 5-(2-aminopropyl)indole, the EMCDDA hereby presents the above-mentioned Risk Assessment Report drawn up by its Scientific Committee pursuant to Article 6 of Council Decision 2005/387/JHA on information exchange, risk assessment and control of new psychoactive substances.



European Monitoring Centre  
for Drugs and Drug Addiction

**Risk assessment report  
of a new psychoactive substance: 5-(2-aminopropyl)indole (5-IT)**

In accordance with Article 6 of Council Decision 2005/387/JHA on information exchange, risk assessment and control of new psychoactive substances

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## 1. Introduction

This Risk assessment report presents the summary findings and the conclusions of the risk assessment carried out by the extended Scientific Committee of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) on the new psychoactive substance 5-(2-aminopropyl)indole. The report has been prepared and drafted in accordance with the conceptual framework and the procedure set out in the *Risk assessment of new psychoactive substances: Operating guidelines* <sup>(1)</sup>. It is written as a stand-alone document which presents a summary of the information considered during the detailed analysis of the scientific and law enforcement data available at this time. The conclusion section of the report summarises the main issues addressed and reflects the opinions held by the members of the Committee. A more detailed Technical report on 5-(2-aminopropyl)indole is annexed to this report (Annex 1).

The risk assessment has been undertaken in compliance with Article 6 of Council Decision 2005/387/JHA of 10 May 2005 on the information exchange, risk assessment and control of new psychoactive substances <sup>(2)</sup> (the 'Council Decision'). The Council Decision establishes a mechanism for the rapid exchange of information on new psychoactive substances that may pose public-health and social threats, including the involvement of organised crime. Thus, it allows the institutions of the European Union and the Member States to act on all new narcotic and psychotropic substances <sup>(3)</sup> that appear on the European Union drug market. The Council Decision also provides for an assessment of the risks associated with these new psychoactive substances so that, if necessary, control measures can be applied in the Member States for narcotic and psychotropic substances <sup>(4)</sup>.

There is information that suggests that the new psychoactive substance 5-(2-aminopropyl)indole first appeared on the drug market in Europe in late 2011. It was formally notified to the European Union Early warning system for the first time in June 2012. It has been associated with fatalities and non-fatal intoxications in four Member States. In response to this, and in compliance with the provisions of Article 5 of the Council Decision, on 12 December 2012, the EMCDDA and Europol submitted to the Council, the Commission and the European Medicines Agency (EMA) a Joint report on the new psychoactive substance 5-(2-aminopropyl)indole <sup>(5)</sup>.

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<sup>(1)</sup> EMCDDA, (2010), *Risk assessment of new psychoactive substances: Operating guidelines*, Publications Office of the European Union, Luxembourg. Available at: <http://www.emcdda.europa.eu/html.cfm/index100978.EN.html>

<sup>(2)</sup> OJ L 127, 20.5.2005, p. 32.

<sup>(3)</sup> According to the definition provided by the Council Decision, a 'new psychoactive substance' means a new narcotic drug or a new psychotropic drug in pure form or in a preparation; 'new narcotic drug' means a substance in pure form or in a preparation that has not been scheduled under the 1961 United Nations Single Convention on Narcotic Drugs, and that may pose a threat to public health comparable to the substances listed in Schedule I, II or IV; 'new psychotropic drug' means a substance in pure form or in a preparation that has not been scheduled under the 1971 United Nations Convention on Psychotropic Substances, and that may pose a threat to public health comparable to the substances listed in Schedule I, II, III or IV.

<sup>(4)</sup> In compliance with the provisions of the 1961 United Nations Single Convention on Narcotic Drugs and the 1971 United Nations Convention on Psychotropic Substances.

<sup>(5)</sup> EMCDDA and Europol (2012), *EMCDDA–Europol joint report on a new psychoactive substance, 5-(2-aminopropyl)indole*, EMCDDA, Lisbon. Available at: <http://www.emcdda.europa.eu/publications/joint-reports/5-IT>

Taking into account the conclusion of the Joint report and in accordance with Article 6 of the Council Decision, on 24 January 2013, the Council formally requested that 'the risk assessment should be carried out by the extended Scientific Committee of the EMCDDA and be submitted to the Commission and the Council within twelve weeks from the date of this notification'.

In accordance with Article 6.2, the meeting to assess the risks of 5-(2-aminopropyl)indole was convened under the auspices of the Scientific Committee of the EMCDDA with the participation of three additional experts designated by the Director of the EMCDDA, acting on the advice of the Chairperson of the Scientific Committee, chosen from a panel proposed by Member States and approved by the Management Board of the EMCDDA. The additional experts were from scientific fields that were either not represented, or not sufficiently represented on the Scientific Committee, and whose contribution was necessary for a balanced and adequate assessment of the possible risks of 5-(2-aminopropyl)indole, including health and social risks. Furthermore, one expert from the Commission, one expert from Europol and one expert from the European Medicines Agency (EMA) participated in the risk assessment. The meeting took place on 11 April 2013 at the EMCDDA in Lisbon. The risk assessment was carried out on the basis of information provided to the Scientific Committee by the Member States, the EMCDDA, Europol and the EMA. A list of the extended Scientific Committee, as well as the list of participants attending the risk assessment meeting, is annexed to this report (Annex 2).

For the risk assessment, the extended Scientific Committee considered the following information resources:

- (i) Technical report on 5-(2-aminopropyl)indole;
- (ii) Study examining the inhibition of human monoamine oxidase (MAO) by the new psychoactive substance 5-(2-aminopropyl)indole (5-IT) <sup>(6)</sup>;
- (iii) EMCDDA–Europol Joint report on a new psychoactive substance, 5-(2-aminopropyl)indole;
- (iv) Scientific articles, official reports, grey literature and Internet drug user discussion forums;
- (v) Risk assessment of new psychoactive substances: Operating guidelines; and,
- (vi) Council Decision 2005/387/JHA of 10 May 2005 on the information exchange, risk assessment and control of new psychoactive substances.

## **2. Physical and chemical description of 5-(2-aminopropyl)indole and its mechanisms of action, including its medical value**

5-(2-Aminopropyl)indole is a synthetic derivative of indole substituted at the phenyl side of the indole ring system (position 5). It is a positional isomer of alpha-methyltryptamine (AMT) which belongs to the tryptamine family, many of which have hallucinogenic and other psychoactive effects in humans. 5-(2-Aminopropyl)indole also contains the sub-structure of alpha-methylphenethylamine and therefore it could be considered to be a ring-substituted phenethylamine, many of which are stimulants. In addition, 5-(2-aminopropyl)indole is

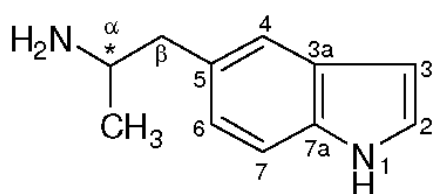
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<sup>(6)</sup> EMCDDA-commissioned study undertaken by Instituto de Ciencia y Tecnología de Alimentos y Nutrición, ICTAN-CSIC, Spain. See Appendix 1 to the Technical report.

structurally similar to the aminopropylbenzofurans, specifically 5-APB (5-(2-aminopropyl)benzofuran). Despite the structural similarities of 5-IT to AMT, 5-APB and phenethylamines such as MDA, it is difficult to predict the pharmacological profile of 5-IT based on a comparison with these other substances due to potential differences in mechanisms of action. Limited data suggests that 5-(2-aminopropyl)indole has stimulant effects and possible hallucinogenic effects.

The systematic (International Union of Pure and Applied Chemistry, IUPAC) name of 5-(2-aminopropyl)indole is 1-(1*H*-indol-5-yl)propan-2-amine. Other names are used and are listed in Annex 1. A common abbreviation used for 5-(2-aminopropyl)indole is 5-IT. Hereinafter, this abbreviation will be used interchangeably with 5-(2-aminopropyl)indole.

The molecular structure, molecular formula and molecular weight, as well as the monoisotopic mass are shown below. Aside from AMT (at position 3-) and *N*-methyltryptamine (NMT), there are four other possible isomers where the 2-aminopropyl chain is attached at positions 2-, 4-, 6- and 7- respectively. The asterisk indicates the asymmetric carbon.



Molecular formula: C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>

Molecular weight: 174.24 g/mol (base)

Monoisotopic mass: 174.1157 Da

The free base form of 5-IT has been described to form skewed prisms. The bioxalate salt form has also been documented. The forms of 5-IT detected in seizures made from the drug market are not known<sup>(7)</sup>. Nuclear magnetic resonance (NMR) data from two samples test-purchased from Internet retailers selling the substance to consumers were found to be consistent with the succinate form. Furthermore, 5-IT contains an asymmetric carbon, and as such, two enantiomers are possible. Information on the enantiomeric forms present on the market is not available. No data are available on the purity of 5-IT from the seizures or test purchases.

5-(2-Aminopropyl)indole has predominantly been seized as powders and tablets, and, in one instance, in capsules. One seizure related to tablets sold as a 'legal high' product labelled 'Benzo Fury' which also displayed an image of the chemical structure of 5-APB (5-(2-aminopropyl)benzofuran). It should be noted that chemical analysis has found that products labelled as 'Benzo Fury' may contain different new psychoactive substances (see Annex 1). 'Benzo Fury' products were originally associated with 5-APB or 6-APB (6-(2-aminopropyl)benzofuran),. In several of the fatalities an empty bag labelled 6-APB was present

<sup>(7)</sup> 'Detections' is an all encompassing term, which may include seizures and/or collected and/or biological samples. Seizure means a substance available (seized) through law enforcement activities (police, customs, border guards, etc.). Collected samples are those that are actively collected by drug monitoring systems (such as test purchases) for monitoring and research purposes. Biological samples are those from human body fluids (urine, blood, etc.) and/or specimens (tissues, hair, etc.)

at the scene, but 6-APB was not detected in post-mortem samples. A small number of tablets resembling ecstasy <sup>(8)</sup> have also been seized that were found to contain 5-IT.

In some seizures, 5-IT has been reported to be the only psychoactive substance detected. There have been a small number of seizures where 5-IT has been found in combination with: 5,6-methylenedioxy-2-aminoindane (MDAI); methylthienylpropamine (1-(thiophen-2-yl)-2-methylaminopropane, MPA) and caffeine; diphenyl(pyrrolidin-2-yl)methanol (diphenylprolinol, D2PM); and, ethylphenidate. No quantitative data were provided.

Analysis using gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) is straightforward with suitable equipment and analytical reference material. No such material was available when 5-IT emerged. Given that the isomer 3-(2-aminopropyl)indole (AMT) produces virtually identical mass spectra under some conditions, some Member States have reported that they were unable to discriminate between these two substances. The European Union Early warning system has made efforts to highlight this issue and provide technical information to the Member States to facilitate the discrimination between the two. No information was provided regarding the possible presence of the other isomers on the drug market. The implementation of suitable chromatographic techniques would be expected to allow successful separation if the reference materials are available for comparison. A full analytical profile is provided in Annex 1. Infrared spectroscopy can also be useful for bulk analysis of pure compounds.

The synthesis of 5-IT was first published in 1963. 5-(2-Aminopropyl)indole is mentioned in patents that claim derivatives of this compound, and a broad range of other arylethylamines, as prodrugs which may have potential medicinal applications. It should be noted that this does not necessarily mean that these will be commercialised. 5-(2-Aminopropyl)indole is available as an analytical reference standard and is available for use in scientific research. There are no known uses of 5-IT as a component in industrial, cosmetic or agricultural products. There are currently no other indications that 5-IT may be used for other legitimate purposes.

5-(2-Aminopropyl)indole has no established or acknowledged medical value or use (human or veterinary) in the European Union. There is no marketing authorisation (existing, ongoing or suspended) for 5-IT in the European Union or in the Member States that responded to the information request by the EMA that was launched under Article 5 of the Council Decision. There is no information that 5-IT is used for the manufacture of a medicinal product or an active pharmaceutical ingredient (API) of a medicinal product in the European Union. However, it should be noted that there is no European Union database on the synthetic routes of all registered medicinal products. Therefore, the use of 5-IT can not be ruled out with certainty.

There are no data available from scientific studies on the pharmacokinetics of 5-IT. Limited information is available from self-reports that may provide an indication of the pharmacokinetic parameters such as time of onset of desired effects, adverse effects, or duration of action of 5-IT. One user report noted that 5-IT has long-lasting stimulant effects of about twelve hours when

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<sup>(8)</sup> The term ecstasy here is used in a broad sense to refer to tablets that contain MDMA (or related substances) or are presented as containing such substances.

20 mg is taken orally. Limited, and sometimes conflicting, user reports from Internet drug user discussion forums mention the time course of effects. In some cases, this includes an apparent delayed onset. If this is the case, some users may take additional amounts of 5-IT, thus increasing the risk of acute toxicity.

In terms of **pharmacodynamics**, a study commissioned by the EMCDDA in 2013 found that racemic 5-IT is a reversible, competitive and highly selective inhibitor of monoamine oxidase-A (MAO-A) ( $IC_{50}$  of 1.6  $\mu$ M and  $K_i$  of 0.25  $\mu$ M) but not MAO-B<sup>(9)</sup>. In addition, an *in vitro* experimental comparison found 5-IT to be less potent than the known MAO-A inhibitors clorgyline and harmaline, but more potent than toloxatone and moclobemide.

A study from 1968 that investigated the inhibition of MAO by 5-IT and its five isomers reported the  $IC_{50}$  values for 5-IT, 6-(2-aminopropyl)indole (6-IT) and 3-(2-aminopropyl)indole (AMT), as 22, 4.6 and 58  $\mu$ M, respectively. These substances were also evaluated for their ability to antagonise pentylenetetrazole/reserpine-induced tonic extensor seizures in mice. 5-(2-Aminopropyl)indole appeared to be less active than 6-IT but more active than AMT with regards to anti-reserpine activity. AMT has been shown to induce stimulant effects in mice, however, 5-IT, remains to be studied in this respect.

No animal studies were identified that investigated the median lethal dose ( $LD_{50}$ ) of 5-IT.

No animal studies were identified that investigated the potential for self-administration of 5-IT.

No human studies were identified that investigated the psychological and/or behavioural effects of 5-IT.

### 3. Chemical precursors that are used for the manufacture of 5-(2-aminopropyl)indole

There is no information that suggests that 5-IT is manufactured in the European Union. One Member State reported a seizure of a bulk quantity of 5-IT powder (20.5 kg) which had been shipped from, and, apparently manufactured in, India. The chemical precursors and the synthetic routes used to manufacture the 5-IT detected in the European Union are unknown. As such, the impurities and side-products are also unknown.

One classic approach used for the synthesis of 5-IT includes a condensation reaction using indole-5-carboxaldehyde and nitroethane as starting materials. The resulting intermediate can then be reduced to produce 5-IT. Other methods and reagents of reduction may also be used. The starting materials appear to be commercially available and are not under international control. These reactions are feasible in basic laboratory settings and do not require sophisticated equipment. Further synthetic routes are possible and more details are provided in Annex 1.

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<sup>(9)</sup>  $IC$  is the inhibitory concentration,  $K_i$  is the inhibition constant.



#### 4. Health risks associated with 5-(2-aminopropyl)indole

##### *Individual health risks*

The assessment of individual health risks includes a consideration of the acute and chronic toxicity of 5-IT, as well as its dependence potential, and its similarities to and differences from other chemically-related substances.

Despite the structural similarities of 5-IT to AMT, 5-APB and phenethylamines such as MDA, it is difficult to predict the pharmacological profile of 5-IT based on a comparison with these other substances due to potential differences in mechanisms of action..

There is limited information available on the main routes of administration for 5-IT. In two non-fatal intoxications the route of administration was intranasal. A small number of user reports from Internet drug discussion forums suggest that routes of administration include: oral ingestion (swallowing), intranasal, sublingual, intravenous injection, and rectal insertion. These routes of administration are consistent with the forms of 5-IT encountered in seizures and test-purchases . There is no information in relation to the specific adverse health effects of 5-IT when administered through these routes. However, some of these routes, for example injection, may have associated health risks.

Systematic data are not routinely collected in Europe on acute toxicity related to 5-IT and no studies were identified in the literature.

Although there have been reports of detection of 5-IT in non-fatal intoxications, there is insufficient clinical details in these reports to be able to determine the clinical pattern of acute toxicity. In one case where 5-IT was the only substance detected, the observed adverse effects were tachycardia, mydriasis, agitation and tremor. In the other cases, 5-IT was detected along with other substances. In some of these, tachycardia, mydriasis and agitation were again reported. In addition, fatigue, hallucinations, unconsciousness, hypertension and hyperthermia were also observed. The presence of other substances may account, at least in part, for some or all of the effects. In addition, details of a non-analytically confirmed case were provided where the symptoms included restlessness, agitation, disorientation, shivering, sweating, mydriasis, tachycardia and hyperthermia.

Self-reports of adverse effects (including those on Internet discussion forums) include increased heart-rate, anorexia, diuresis, slight hyperthermia, muscle rigidity and jaw clenching. The limitations of self-reports, including the fact that users may be unaware or misinformed about the substance they have consumed, should be borne in mind when interpreting these reports. As such, users may have taken other substances which may account for some or all of the described effects.

As some users may be unaware that they have taken 5-IT (for example by consuming a 'Benzo Fury' product or a tablet containing 5-IT), it is likely, that the acute toxicity related to the use of the substance is under-reported.

Four Member States have reported a total of 24 fatalities where 5-IT has been detected in post-mortem samples: fifteen in Sweden, four in Hungary, four in the United Kingdom and one in Germany. These occurred between April and August 2012. They were aged between 19 and 55 years old, with only one individual older than 40 (mean age 31 with a 95% confidence interval of 27 to 34 and an interquartile range between 24 and 33). 21 were male, one was female and in the remaining two cases the sex was not reported. In seven of the cases no other substances were detected. In the remaining cases, 5-IT was present along with one or more other substances. While it is not possible to determine with certainty the role of 5-IT in all of these fatalities, in some cases it has been specifically noted in the cause of death.

While there are insufficient clinical details in the non-fatal and fatal intoxications to be able to determine the clinical pattern of acute toxicity, evidence of hyperthermia was reported in several cases.

Given the limited available information on the pharmacology of 5-IT, it is difficult to predict any potential drug interactions or contra-indications (including those that may arise from the use of alcohol and/or tobacco). However, the MAO inhibition activity of 5-IT may result in potential interactions with drugs acting on the monoaminergic system.

No experimental studies were identified that investigated the potential for chronic 5-IT toxicity in humans, including reproductive toxicity, genotoxicity and carcinogenic potential.

There appear to be no published studies on the tolerance or dependence producing potential of 5-IT.

### ***Public health risks***

The public health risks associated with 5-IT may be categorised in terms of patterns of use (extent, frequency, route of administration, etc.); availability and quality of the drug; information, availability and levels of knowledge amongst users; and, negative health consequences.

In some cases, 5-IT is being sold and consumed as a substance in its own right. Similar to other stimulant drugs, users may combine 5-IT with other substances (stimulants and/or depressants including alcohol). However, some users have taken 5-IT unknowingly along with or instead of other substances, particularly stimulants.

There is no information on the purity of 5-IT that is present on the drug market. In some of the seizures and test purchases, 5-IT has been reported to be the only psychoactive substance detected. In addition, there have been a small number of seizures where 5-IT has been found in combination with other psychoactive substances, particularly stimulants (e.g. ethylphenidate, methylthienopropamine).

There are no prevalence data on the use of 5-IT. However, 5-IT has been detected in a 'legal high' product labelled as 'Benzo Fury'. Furthermore, in three non-fatal intoxications 'Benzo Fury' was reported to have been taken. It is therefore relevant to consider the available data on the

use of 'Benzo Fury'. In addition, a small number of tablets that resembled ecstasy have also been found to contain 5-IT. Users who take such ecstasy tablets may also be exposed to 5-IT.

It is important to note that a number of different new psychoactive substances have been detected in products sold as 'Benzo Fury'. A non-representative Internet survey conducted among readers of a dance music magazine and the Guardian newspaper found that overall 2.4 % of respondents and 3 % of 'regular clubbers' from the United Kingdom reported use of 'Benzo Fury' in the last year.

One Member State reported the detection of 5-IT in biological samples from ten individuals not related to non-fatal intoxications and fatalities. The individuals were suspected to have committed minor drug offences or were people in drug treatment programmes. Further information on these cases is not available to allow further analysis.

While the routes of administration are discussed above, it is noteworthy that in a small number of cases injecting has been reported. Injecting drugs can be associated with a range of public health risks, including bacterial infections and blood borne viruses such as HIV, hepatitis C and hepatitis B.

It is likely that 5-IT will be used in similar environments as other stimulants. This would include the home, bars, nightclubs and music festivals.

There is limited information on the route of supply of 5-IT. Since June 2012, 5-IT has been seized in seven Member States, Croatia and Norway. It has typically been seized as a powder. A small number of tablets, and, in one instance, capsules have been seized. Aside from the bulk seizure noted below, the powder seizures weighed between 0.2 and 97.3 grams. In one case, seven tablets were seized that resembled ecstasy. Customs authorities in two Member States seized small packages containing 5-IT that had been posted from other Member States.

Information from the structured Internet search conducted by the EMCDDA suggests that 5-IT is commercially marketed through Internet shops selling 'legal highs' or 'research chemicals'. In some non-fatal intoxications and in one fatality it was reported that the users had sourced 5-IT on the Internet. The analysis of two test purchases which were sold as 5-IT from Internet shops confirmed the presence of the substance.

The Internet search identified a number of chemical suppliers on a trade website that claimed to be able to supply 5-IT in bulk quantities. One report was received of a bulk seizure (20.5 kg) of 5-IT shipped from India.

## **5. Social risks associated with 5-(2-aminopropyl)indole**

There is a lack of information on the social risks associated with 5-IT.

One Member State reported the detection of 5-IT in biological samples from ten individuals suspected to have committed minor drug offences or people that are in drug treatment programmes. Additional information on these cases is not available to allow further analysis.

There have been no studies that have investigated the impact of 5-IT use on educational outcomes such as attendance, concentration and exam performance. Similarly, there is no information on the effect of 5-IT use on performance/attendance at work, career progression, effects on personal relationships or neglect of family.

It is not possible at this time to estimate whether 5-IT is associated with greater healthcare costs than other stimulant drugs.

#### **6. Information on the level of involvement of organised crime and information on seizures and/or detections by the authorities, and the manufacture of 5-(2-aminopropyl)indole**

There is no information to suggest the involvement of organised crime or criminal groups in the manufacture, distribution (trafficking) and supply of 5-IT. However, as noted above, in one Member State seven tablets were seized that resembled ecstasy. Further information about this case would be required to assess whether or not there were any links to organised crime.

In one Member State where 5-IT is controlled, a case of unlawful supply was reported. This involved distribution at street level and using the postal system.

See section 4 and Annex 1 for more details of seizures or detections related to 5-IT.

#### **7. Information on any assessment of 5-(2-aminopropyl)indole in the United Nations system**

The World Health Organization is the specialised agency of the United Nations designated for the evaluation of the medical, scientific and public health aspects of psychoactive substances under the 1961 United Nations Single Convention on Narcotic Drugs, and the 1971 United Nations Convention on Psychotropic Substances. On 10 October 2012, the World Health Organization informed the EMCDDA that 5-(2-aminopropyl)indole is currently not under assessment and has not been under assessment by the United Nations system.

#### **8. Description of the control measures that are applicable to 5-(2-aminopropyl)indole in the Member States**

5-(2-Aminopropyl)indole is not listed for control in the 1961 United Nations Single Convention on Narcotic Drugs or in the 1971 United Nations Convention on Psychotropic Substances (together 'UN drug conventions').

Cyprus and Denmark control 5-(2-aminopropyl)indole under legislation by virtue of their obligations under the UN drug conventions. Twenty-five Member States, Croatia, Turkey and Norway do not control 5-(2-aminopropyl)indole by virtue of their obligations under the UN drug conventions.

Five countries use other legislative measures to control 5-(2-aminopropyl)indole. In Austria, 5-(2-aminopropyl)indole is subject to control measures according to the law on new psychoactive

substances. In Hungary, 5-(2-aminopropyl)indole is controlled as a new psychoactive substance by Government Decree 66/2012. In Sweden, 5-(2-aminopropyl)indole is regulated under the Act on the Prohibition of Certain Goods Dangerous to Health. In Germany, 5-(2-aminopropyl)indole is regulated under the Medical Products Act. In Norway 5-(2-aminopropyl)indole is regulated under the Medicines Act.

## **9. Options for control and the possible consequences of the control measures**

Under Article 9.1 of the Council Decision, the option for control that is available at European Union level is for the Member States to submit the new psychoactive substance 5-(2-aminopropyl)indole to control measures and criminal penalties, as provided for under their legislation, by virtue of their obligations under the 1971 United Nations Convention on Psychotropic Substances. There are no studies on the possible consequences of such control measures on 5-IT. If this option of control is pursued, the Committee considers that the following consequences are possible. Some of these may apply to any new psychoactive substance.

- This control option could be expected to limit the availability of 5-IT and hence the further expansion of the current open trade in this substance.
- A health consequence that may result from this control option is the benefit brought about by the presumed reduction in availability and use.
- This control option could facilitate the detection, seizure and monitoring of 5-IT related to its unlawful manufacture, trafficking and use. In so doing, it could facilitate cooperation between the judicial authorities and law enforcement agencies across the European Union.
- This control option would imply additional costs for the criminal justice system, including forensic services, law enforcement and the courts.
- This control option could lead to replacement with other (established or new) psychoactive substances which may in themselves have public health consequences.
- It is difficult to predict the impact of this control option on current or future research by the pharmaceutical or chemical industries.
- This control option could create an illicit market in 5-IT with the increased risk of associated criminal activity, including organised crime. This could include covert sales of 5-IT on the Internet or in bricks and mortar headshops.
- This control option could impact on both the quality/purity and price of any 5-IT still available on the illicit market. The extent to which this will impact on public health, criminality or levels of use is difficult to predict.

In order to examine the consequences of control, the Committee wishes to note that it will be important to monitor for the presence of 5-IT on the market post-control, should this control option be pursued.

Aside from the option for control under those stipulated in Article 9.1 of the Council Decision, other options for control may be available to Member States. These may include medicines

legislation or restricting the importation and supply of the substance as some other Member States (and Norway) have already done.

## 10. Conclusion

5-(2-Aminopropyl)indole appears to be a stimulant substance. It is a synthetic derivative of indole substituted at the phenyl side of the indole ring system. It is found mostly as a powder but also in tablets and capsules. The information available suggests that the most commonly reported routes of administration of 5-IT may be orally and intranasally. One Member State reported that injection may also occur. It has no established or acknowledged medical use (human or veterinary) in the European Union. There are no indications that it may be used for any other purpose aside from as an analytical reference standard and in scientific research.

5-(2-Aminopropyl)indole is not listed for control in the 1961 United Nations Single Convention on Narcotic Drugs or in the 1971 United Nations Convention on Psychotropic Substances. 5-(2-Aminopropyl)indole is currently not under assessment and has not been under assessment by the United Nations system. Two Member States control 5-(2-aminopropyl)indole under drug control legislation. Five Member States control 5-(2-aminopropyl)indole under other legislation.

5-(2-Aminopropyl)indole has emerged on the 'legal highs' market where it is sold as a 'research chemical' and has been detected in a 'legal high' product labelled as 'Benzo Fury'. 5-IT is sold on the Internet and in bricks and mortar headshops. In addition, it has also been detected in tablets resembling ecstasy. In some cases, analysis of samples has found 5-IT to be the sole psychoactive substance present. In other cases, it has been found in combination with other new psychoactive substances, particularly stimulants. There is limited information that it is also sold by street-level drug dealers.

5-(2-Aminopropyl)indole has been detected in seven Member States, Croatia and Norway. There are no prevalence data on the use of 5-IT. Limited information suggests that there may be some interest in using 5-IT among certain drug user groups. However, further information on the size of the demand and the characteristics of these groups is not available. There is no specific information on the social risks that may be related to 5-IT.

There is no information to suggest the involvement of organised crime in the manufacture, distribution (trafficking) and supply. There is no information to suggest that 5-IT is manufactured in the European Union. The chemical precursors and the synthetic routes used to manufacture the 5-IT detected in the European Union are unknown. There has been one report of a seizure of a bulk quantity of 5-IT powder (20.5 kg) which had been shipped from, and, apparently manufactured in, India. The starting materials used in some of the synthetic routes described in the literature are commercially available and are not under international control.

Several Member States reported that forensic and/or toxicological laboratories do not currently have validated procedures for the confirmation of 5-IT. This is in part due to the initial lack of certified reference material and the fact that 5-IT is not subject to control measures in some Member States. This may have led to under-reporting of 5-IT detections.

The acute toxicity of 5-IT appears to include symptoms that could be regarded as consistent with monoaminergic toxicity (including tachycardia and hyperthermia). In addition, there is a possibility of interactions with other substances including medicinal products and stimulants that act on the monoaminergic system. There appears to be no published studies assessing the acute or chronic toxicity, psychological and behavioural effects, nor the dependence potential of 5-IT in humans.

5-(2-Aminopropyl)indole either alone or in combination with one or more substances has been detected in 24 fatalities in four Member States and 20 non-fatal intoxications in three Member States. While it is not possible to determine with certainty the role of 5-IT in all of the fatalities, in some cases it has been specifically noted in the cause of death.

5-(2-Aminopropyl)indole appears to have been available since at least November 2011 although the evidence does not suggest it has been widely used. The fatalities associated with 5-IT occurred over a period of five months in 2012. This raises the concern that if this substance were to become more widely available and used, the implications for public health could be significant.

Many of the questions posed by the lack of evidence on the health and social risks of 5-IT, as for any new psychoactive substance, could be answered through further research. Areas where additional information would be important include: prevalence and patterns of use (including targeted studies that examine user groups and risk behaviours); market studies; chemical profiling studies; receptor binding and functional activity studies; metabolic pathway studies; behavioural studies; clinical patterns of acute and chronic toxicity in humans; the potential interaction between 5-IT and other substances (in particular those that affect the monoaminergic system); the dependence and abuse potential; and, the social risks associated with its use.

The Committee notes that a decision to control 5-(2-aminopropyl)indole has the potential to bring with it both intended and unintended consequences. Potential intended consequences include reduced levels of manufacture, availability and ultimately use. This may reduce the health and social risks and consequences arising from the use of 5-IT. It is important to recognise that a potential unintended consequence of control may be the manufacture and availability of other substances. Finally, control measures should not inhibit the gathering and dissemination of accurate information on 5-IT to users, practitioners and decision makers.

**11. List of annexes**

**Annex 1:** Technical report on 5-(2-aminopropyl)indole.

**Annex 2:** List of participants at the risk assessment meeting.





European Monitoring Centre  
for Drugs and Drug Addiction

### Technical report on 5-(2-aminopropyl)indole

Annex 1 to the Risk Assessment Report of a new psychoactive substance:  
5-(2-aminopropyl)indole

**Prepared by:** Dr Simon Elliott<sup>a</sup> and Dr Simon Brandt<sup>b</sup>

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EMCDDA contract: CC.13.SAT.02

Parts of this technical report contain data which are unpublished or in preparation for publication.

1

## Section A. Physical, chemical, pharmaceutical and pharmacological information

### A1. Physical, chemical, and pharmaceutical information

#### A1.1. Physical and chemical description

##### *Chemical description and names*

5-(2-Aminopropyl)indole is a synthetic derivative of indole substituted at the phenyl side of the indole ring system (position 5). It is a positional isomer of alpha-methyltryptamine (AMT) which belongs to the tryptamine family, many of which show hallucinogenic and other psychoactive effects in humans. 5-(2-Aminopropyl)indole also contains the sub-structure of alpha-methylphenethylamine and therefore could be considered to be a substituted phenethylamine, many of which are stimulants. Limited data suggests that 5-(2-aminopropyl)indole has stimulant effects and possible hallucinogenic effects.

The systematic (International Union of Pure and Applied Chemistry, IUPAC) name of 5-(2-aminopropyl)indole is 1-(1*H*-indol-5-yl)propan-2-amine and other names that may be encountered include  $\alpha$ -methyl-1*H*-indole-5-ethanamine and 2-(1*H*-indol-5-yl)-1-methyl-ethylamine. A common abbreviation used for 5-(2-aminopropyl)indole is 5-IT<sup>(1)</sup>. To a lesser extent the abbreviation 5-API is also used<sup>(2)</sup>. Both these abbreviations are used by Internet retailers<sup>(3)</sup> advertising 5-(2-aminopropyl)indole as well as in Internet drug user discussion forums ('drug discussion forums'). This suggests that '5-IT' and '5-API' are used as 'street names'. The Chemical Abstract Service (CAS) Registry Numbers for 5-(2-aminopropyl)indole are given in Table 1.

**Table 1.** Chemical Abstract Service (CAS) Registry Numbers for 5-(2-aminopropyl)indole

CAS Registry Numbers	Variant
3784-30-3	Unspecified amine
96875-04-6	Ethanedioate (1:1) / hydrogen oxalate / bioxalate
1336260-35-5	( <i>R</i> )-Enantiomer amine
1336564-72-7	( <i>S</i> )-Enantiomer amine

Excluding the abstractable proton on the nitrogen atom a total number of six positional isomers exist that can carry the 2-aminopropyl side chain. The synthesis of 5-(2-aminopropyl)indole (Figure 1) and its isomers was first described by Hofmann and Troxler (1963) and Troxler et al., (1968). Another isomer is *N*-methyltryptamine (NMT) which is

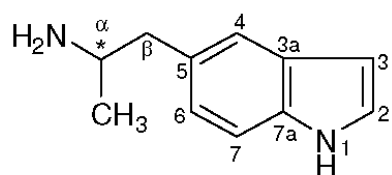
<sup>(1)</sup> The origin of the abbreviation '5-IT' is not known at this time.

<sup>(2)</sup> The origin of the abbreviation '5-API' is thought to be derived from 5-(2-aminopropyl)indole.

<sup>(3)</sup> The term 'Internet retailers' is used in this report to describe Internet shops that offer new psychoactive substances for sale often advertised as 'legal highs' and 'research chemicals'.

commonly found in nature (Ott, 1996). A more recent example of the preparation of 2-(2-aminopropyl)indole (2-IT) was presented by Alhambra et al., (2001) who employed a solid-phase approach. The synthesis of the 3-(2-aminopropyl)indole (AMT) <sup>(4)</sup> isomer was first published in 1947 (Snyder and Katz, 1947). 5-(2-Aminopropyl)indole contains an asymmetric carbon but data on the availability of its enantiomeric forms on the market (including seized, collected and biological samples referred to in this report) are currently not available.

**Figure 1.** The numbered molecular structure, formula, weight and monoisotopic mass of 5-(2-aminopropyl)indole <sup>(5)</sup>. Asterisk indicates chiral centre.



**Molecular formula:** C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>

**Molecular weights:** 174.24 g/mol (base)

**Monoisotopic mass:** 174.1157 Da

#### **Identification and analytical profile**

The free base gives a slightly violet response (Keller test) whereas the van Urk test results in the formation of a red colour (Hofmann and Troxler, 1963). Further information on the presumptive tests for 5-(2-aminopropyl)indole are not available. The reported melting points are: 81–83 °C (free base) (petroleum ether/benzene) (Hofmann and Troxler, 1963 and Troxler et al., 1968); 199–201 °C (bioxalate salt) (methanol/diethyl ether) (Hofmann and Troxler, 1963); 194 °C (dec.) (hemisuccinate) (LGC GmbH, 2012). Analysis by high performance liquid chromatography diode array detection gave  $\lambda_{\text{max}}$  values at 218.3 nm and 272.8 nm (Elliott et al., 2013).

Nuclear magnetic resonance spectroscopy (NMR) data of 5-(2-aminopropyl)indole succinate <sup>(6)</sup>: <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD):  $\delta$  7.42 (1H, br d,  $J$  = 1.1 Hz, H-4), 7.37 (1H, d,  $J$  = 8.3 Hz, H-7), 7.23 (1H, d,  $J$  = 3.2 Hz, H-3), 6.98 (1H, dd,  $J$  = 8.3 Hz,  $J$  = 1.7 Hz, H-6), 6.41 (1H, dd,  $J$  = 3.2 Hz,  $J$  = 0.8 Hz, H-2), 3.57–3.45 (1H, m (consistent with predicted dqd),  $\alpha$ -CH), 3.02 (1H, dd,  $J_{\text{gem}}$  = 13.8 Hz,  $^3J$  = 6.5 Hz, CH<sub>A</sub>H<sub>B</sub>), 2.86 (1H, dd,  $J_{\text{gem}}$  = 13.8 Hz,  $^3J$  = 8.0 Hz, CH<sub>A</sub>H<sub>B</sub>), 2.51 (4H, s, succinate), 1.26 (3H, d,  $^3J$  = 6.6 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD):  $\delta$  179.4 (succinate), 137.0 (C-7a), 129.9 (C-3a), 127.5 (C-5), 126.3 (C-3), 123.5 (C-6), 121.8 (C-4), 112.6 (C-7), 102.2 (C-2), 50.8 ( $\alpha$ -CH), 42.2 (CH<sub>2</sub>), 32.9 (CH<sub>2</sub>, succinate), 18.5 (CH<sub>3</sub>) (Elliott et al., 2013).

<sup>(4)</sup> Abbreviations and code names for AMT found in the literature include:  $\alpha$ -methyltryptamine, AMT,  $\alpha$ -MT, 3-IT, IT-290, IT-403, U-14, 162-E, Ro 3-0926, NSC 97069, and Indopan.

<sup>(5)</sup> For additional predicted data, see <http://www.chemspider.com/Chemical-Structure.25991467.html>

<sup>(6)</sup> NMR data is provided for 5-(2-aminopropyl)indole succinate as this is the form that was encountered in a collected sample that was analysed.

Mass spectrometry data: 5-(2-aminopropyl)indole (5-IT) and 3-(2-aminopropyl)indole (AMT) have been found to produce virtually identical mass spectra, especially when applying conventional electron ionization mass spectrometry (EI-MS) procedures. Thus, all six potential 2-aminopropyl isomers may be expected to yield very similar mass spectra. However, the implementation of suitable chromatographic techniques would be expected to allow successful separation if the reference materials are available for comparison.

Consistent with mass spectral behaviour reported for isomeric psychoactive tryptamines (Martins et al., 2010), key fragments observed under EI-MS conditions ( $m/z$ ) were: 44 (base peak), 131, 130, 77, 103, 117. The  $M^{*+}$  ( $m/z$  174) may be detectable at a minor relative abundance but may also be absent. CI-MS (MeOH as liquid CI reagent) gave the  $[M+H]^+$  at  $m/z$  175 as the base peak and a prominent fragment at  $m/z$  158 following the loss of  $NH_3$ . Positive electrospray tandem mass spectra (ESI-MS/MS) yielded  $m/z$  values of 77, 103, 117, 130, 143, 158 (relative abundance values dependent on collision energy) with some in-source fragmentation of the protonated molecule at  $m/z$  175. When evaluating the ability to differentiate between 5-IT and AMT under ESI-MS/MS conditions, distinct differences in the relative abundances were observed, allowing for the potential use of ion ratios for multiple reaction monitoring (MRM) transitions. Thus, for AMT:  $m/z$  175/158 (100 % abundance),  $m/z$  175/143 (78 %),  $m/z$  175/130 (30 %) and for 5-IT:  $m/z$  175/158 (100 % abundance),  $m/z$  175/143 (22 %),  $m/z$  175/130 (84 %) (Elliott et al., 2013).

The REACH (Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals) registered substances database hosted by the European Chemicals Agency (ECHA) was searched using the CAS Registry Numbers listed above and no information was found.

The lack of a rapid qualitative screening method is a limiting factor for the detection of 5-(2-aminopropyl)indole in biological samples. Furthermore, many European forensic/toxicological laboratories may not have procedures in place for analysing 5-(2-aminopropyl)indole in biological samples. In some cases this may be due to the lack of reference standards for the drug or difficulties in distinguishing between 5-(2-aminopropyl)indole and the related compound AMT.

#### *Physical description*

The free base form of 5-(2-aminopropyl)indole has been described to form skewed prisms (Troxler et al., 1968) and the bioxalate salt form has also been documented (Hofmann and Troxler, 1963). It has been reported that some Internet retailers have advertised 5-(2-aminopropyl)indole as the succinate salt. NMR data produced as part of the analysis of two collected samples of 5-(2-aminopropyl)indole (reported by the United Kingdom) were found to be consistent with the succinate form (see data above). The structured Internet search conducted by the EMCDDA for the Joint report also noted that 5-(2-aminopropyl)indole hydrochloride was being offered for sale (EMCDDA & Europol, 2012). Analytical reference standards are commercially available <sup>(7)</sup>. See section A1.2 for a description of the physical

<sup>(7)</sup> For example <http://www.logical-standards.com/index.php?mact=Products,cntnt01,details,0&cntnt01productid=1811&cntnt01returnid=57>; and,

forms that have been reported.

#### *Methods and chemical precursors used for the manufacture of 5-(2-aminopropyl)indole*

There is currently no information regarding manufacturing sites, the chemical precursors or the synthetic routes used for the 5-(2-aminopropyl)indole that has been detected on the drug market.

One classic approach used for the synthesis of 5-(2-aminopropyl)indole includes a condensation reaction using indole-5-carboxaldehyde and nitroethane. These substances are commercially available and are not under international control. The resulting 5-(2-methyl-2-nitrovinyl)indole can then be reduced with lithium aluminium hydride (LiAlH<sub>4</sub>) (Hofmann and Troxler, 1963; Troxler et al., 1968). Other methods and reagents of reduction, for example those also employed during phenylalkylamine synthesis, may equally be useful (Guy et al., 2008). The reactions are feasible in an amateur laboratory setting and do not require sophisticated equipment. In analogy to the reductive amination procedure used to obtain a range of phenylalkylamines, the use of 1-(1*H*-indol-5-yl)propan-2-one as a potential starting material might also be conceivable. However, an example of this manufacturing procedure is not available in the literature.

#### *Typical impurities encountered in seized samples*

There is currently no information available with regards to route-specific by-products produced during the synthesis of 5-(2-aminopropyl)indole. In addition there is no data currently available on the impurities detected in seized and collected samples.

In some samples, 5-(2-aminopropyl)indole has been reported to be the only psychoactive substance detected. Additionally, although not impurities, there have been a small number of reports where 5-(2-aminopropyl)indole has been found in combination with other psychoactive substances. These include: 5,6-methylenedioxy-2-aminoindane (MDAI) in Germany; methylthienylpropamine (1-(thiophen-2-yl)-2-methylaminopropane, MPA) and caffeine in tablets bearing markings resembling the Lexus logo <sup>(8)</sup> in Hungary; diphenyl(pyrrolidin-2-yl)methanol (diphenylprolinol, D2PM) in capsules in Guernsey <sup>(9)</sup>; and, ethylphenidate within a beige powder in Sweden. No quantitative data were provided for any seized or collected sample reported to the EMCDDA nor Europol.

#### **A1.2. Physical/pharmaceutical form**

Reports of seizures and collected samples have noted that 5-(2-aminopropyl)indole has been detected in: brown, pale/light brown or beige powders; beige tablets bearing markings resembling the Lexus logo; brown glittery tablets; blue/green unmarked tablets; blue unmarked tablets commercially packaged as 'BENZO FURY'; capsules; and, in residues on

<https://www.caymanchem.com/app/template/Product.vm/catalog/12042.jsessionid=4E0344937486009FBED6743CFB86E902>

<sup>(8)</sup> It is common to find markings on tablets sold as 'ecstasy' including those of popular cultural and iconic brands often having an association with quality. Lexus is a luxury Japanese car manufacturer.

<sup>(9)</sup> British Crown Dependency of the Bailiwick of Guernsey, report received from the United Kingdom national focal point.

a spoon and in the liquid recovered from a syringe. See section C for further details of the seized and collected samples of 5-(2-aminopropyl)indole.

### A1.3. Route of administration and dosage

As noted, 5-(2-aminopropyl)indole has been encountered as powders as well as tablets and capsules. These physical forms suggest that common routes of administration may be oral and by insufflation. Limited information from reports of non-fatal intoxications, deaths and drug discussion forums appear to support this (see below). The succinate salt of 5-(2-aminopropyl)indole, confirmed in the two collected samples reported by the United Kingdom, may be suitable for injection. Significantly, in this respect, Hungary has reported that 5-(2-aminopropyl)indole has been found in residues on a spoon and in the liquid recovered from a syringe. The assessment of the national focal point is that 5-(2-aminopropyl)indole is being injected in some cases.

In two non-fatal intoxications the route of administration was reported as by nasal insufflation. A user report from Shulgin and Shulgin (1997) noted an example of oral administration of 20 mg. Drug discussion forums suggest that routes of administration include: oral ingestion (swallowing, such as 'bombing' <sup>(10)</sup>), nasal insufflation (snorting), sublingual, intravenous injection, and rectal insertion <sup>(11)</sup>. Reported doses used include: '20mg' [route of administration not specified], '80 mg orally', 'bombed 100 mg', '150Mg swallowed', 'insufflated 65 mg'<sup>(12)</sup>.

## A2. Pharmacology, including pharmacodynamics and pharmacokinetics

### *Pharmacodynamics*

While detailed pharmacological investigations on 5-(2-aminopropyl)indole do not appear to have been published <sup>(13)</sup>, one study was identified that investigated the ability of 5-(2-aminopropyl)indole and its five isomers to inhibit monoamine oxidase (MAO). The assay method was based on the ability of guinea pig liver homogenate to absorb oxygen generated from serotonin as the substrate. The activity was expressed as percentage inhibition. The IC<sub>50</sub> values for 5-(2-aminopropyl)indole, 6-(2-aminopropyl)indole (6-IT) and 3-(2-aminopropyl)indole (AMT), for example, were 22, 4.6 and 58 µM, respectively. These data indicate that 6-IT was the most potent inhibitor amongst those three substances. These substances were also evaluated for their ability to antagonise pentylentetrazole/reserpine-induced tonic extensor seizures in mice. 5-(2-Aminopropyl)indole appeared to be less active than 6-IT but more active than AMT with regards to anti-reserpine activity (Cerletti et al., 1968). While the AMT isomer has been shown to induce stimulant effects in mice (including body tremor, heightened locomotor activity, mydriasis and hyperthermia) (Lessin et al.,

<sup>(10)</sup> 'Bombing' is where a drug is wrapped in cigarette paper (or similar) prior to swallowing.

<sup>(11)</sup> <http://www.drugs-forum.com/forum/showpost.php?p=1126167&postcount=9>

<sup>(12)</sup> <http://www.drugs-forum.com/forum/showthread.php?t=140331>  
<http://www.drugs-forum.com/forum/showthread.php?t=172223>  
<http://www.bluelight.ru/vb/threads/616728-The-Big-amp-Dandy-5-IT-5-API-Thread>

<sup>(13)</sup> A literature search on 5-(2-aminopropyl)indole revealed a translated article (USSR, Academy of Sciences) on serotonergic properties of several tryptamines. However, inspection of the English translation did not appear to provide any data on 5-(2-aminopropyl)indole (Buznikov et al., 1965).

1965), the extent to which this extends to the remaining isomers, including 5-(2-aminopropyl)indole, remains to be studied. A short report on the 6-(2-aminopropyl)indole isomer provided some indication that intravenous administration (0.5 mg/kg) resulted in hypertension and related sympathomimetic features in dogs (Maxwell, 1964).

Given the lack of information on the pharmacological and toxicological properties of 5-(2-aminopropyl)indole, and drawing on the study by Cerletti et al., (1968) summarised above, the EMCDDA commissioned a study designed to provide further data on the possible effects of 5-(2-aminopropyl)indole on monoamine oxidase inhibition (Appendix 1). This study used an *in vitro* assay with recombinant human MAO-A and B isoenzymes (using kynuramine as substrate) based on the procedure published by Herraiz and Caparro (2006). The study found that racemic 5-(2-aminopropyl)indole (in the form of the hemisuccinate salt) is a reversible, competitive and highly selective inhibitor of MAO-A ( $IC_{50}$  of 1.6  $\mu$ M and  $K_i$  of 0.25  $\mu$ M) but not MAO-B. In addition, an *in vitro* experimental comparison found 5-(2-aminopropyl)indole to be less potent than the known MAO-A inhibitors clorgyline and harmaline, but more potent than toloxatone and moclobemide (Table 2).

**Table 2.** Inhibition values determined from recombinant human monoamine oxidase-A assay. Racemic 5-(2-aminopropyl)indole was used in the form of the hemisuccinate salt.

Compound	$IC_{50}$ ( $\mu$ M)	$K_i$ ( $\mu$ M)	$K_i$ ( $\mu$ M) from $IC_{50}$
Clorgyline	0.016	-	0.016
Harmaline	0.020	-	0.004
5-(2-Aminopropyl)indole	1.6	0.25	0.32
Toloxatone	6.7	-	1.3
Moclobemide	>500	-	-

Shulgin and Shulgin (1997) reported long-lasting stimulant properties of around 12 hours duration following oral administration of 20 mg. Currently, no data are available on the presence and/or properties of single enantiomers. Increased potency was found to reside with the (S)-(+)-enantiomer of AMT in both animals and humans (Nichols, 1986). Whether a similar relationship exists with 5-(2-aminopropyl)indole remains to be investigated.

There are appears to be no published data on the biotransformation (metabolism) of 5-(2-aminopropyl)indole in animals or humans. Since this particular isomer carries the side chain at the 5-position it is currently unknown whether similar transformations occur that have been observed with AMT. Early work carried out *in vitro* (in rat liver microsomes) and *in vivo* samples (male albino rat urine following intraperitoneal injection of 5 mg/kg AMT and incubation with bacterial  $\beta$ -glucuronidase) indicated the presence of 6-hydroxy-AMT, 1-(1H-indol-3-yl)propan-2-one and 1-(6-hydroxy-1H-indol-3-yl)propan-2-one, respectively (Szara, 1961). A more recent example of metabolic studies in rats was provided by Kanamori et al. (2008) who observed the formation of 3-(2-aminopropyl)indolin-2-one, 2-amino-1-(1H-indol-3-yl)propan-1-ol, 6-hydroxy-AMT and 7-hydroxy-AMT in urine following enzymatic

hydrolysis. In this study, four male Wistar rats received an oral dose of 10 mg/kg of AMT with urine collected and pooled over a 24 hour period. Overall, AMT was found to be poorly metabolised, as indicated by the relative contribution of signal intensities under GC-MS conditions.

#### *Interactions with other drugs and medicines*

Given the limited information that is available on the pharmacology of 5-(2-aminopropyl)indole it is difficult to predict with accuracy any particular potential drug interactions or contraindications. However, as stated above, the ability of 5-(2-aminopropyl)indole to inhibit MAO-A in vitro may result in potential interactions with drugs acting on the monoaminergic system. In particular this may be the case for serotonergic drugs that may present a risk of inducing serotonin syndrome, the symptoms of which can include tachycardia, hyperthermia, muscle rigidity and convulsions (Boyer & Shannon, 2005). In the context of 5-(2-aminopropyl)indole use, there may be a potential risk from the (concomitant) use of medicinal products (e.g. selective serotonin reuptake inhibitors (SSRIs)) as well as stimulant drugs that act on the monoaminergic system. These include amphetamine, MDMA (and other phenethylamines) and cathinone derivatives (e.g. mephedrone, 4-methylethcathinone). In this respect, some of these drugs have been detected in the biological samples from the non-fatal intoxications and deaths detailed in section D. It may be the case that a possible synergistic interaction may have played a role in these cases.

#### *Pharmacokinetics*

No animal studies were identified that investigated the pharmacokinetics of 5-(2-aminopropyl)indole. There is limited information available from Internet reports or from drug discussion forums that could be used to determine pharmacokinetic parameters such as time of onset of desired effects, adverse effects, or duration of action of 5-(2-aminopropyl)indole. As noted, Shulgin and Shulgin (1997) provided some limited information noting that 5-(2-aminopropyl)indole has long-lasting stimulant effects in humans of about twelve hours when 20 mg is given orally.

### **A3. Psychological and behavioural effects**

No studies were identified that investigated the psychological and/or behavioural effects of 5-(2-aminopropyl)indole. As mentioned above, Shulgin and Shulgin (1997) provided some limited information noting that 5-(2-aminopropyl)indole may show long-lasting stimulant properties in humans for about twelve hours when 20 mg is given orally. The physical effects reported were increased heart-rate, anorexia, diuresis, and slight hyperthermia. No further relevant details were reported. Section D1.2.1. discusses some of the effects that have been self-reported by users on drug discussion forums.

### **A4. Legitimate uses of the product**

5-(2-Aminopropyl)indole is available as an analytical reference standard and is used in scientific research. 5-(2-Aminopropyl)indole is mentioned in patents that claim derivatives of



this compound and a broad range of other aryethylamines as pro-drugs which may have potential medicinal applications (Jenkins and Sturmer, 2012; Van Wijngaarden et al., 1988). There are currently no other indications that 5-(2-aminopropyl)indole may be used for other legitimate purposes. There are no known uses of 5-(2-aminopropyl)indole as a component in industrial, cosmetic or agricultural products.

There is no information that 5-(2-aminopropyl)indole is currently used in the manufacture of a medicinal product in the European Union. However, in the absence of a European Union database on the synthetic routes of all medicinal products this information cannot be verified. There is no marketing authorisation (existing, ongoing or suspended) for 5-(2-aminopropyl)indole neither in the European Union nor in the Member States that responded to the request for information from the European Medicines Agency (EMCDDA & Europol, 2012).

## Section B. Dependence and abuse potential

### B1. Animal *in vivo* and *in vitro* data

No published experimental animal studies were identified that examined the dependence and abuse potential of 5-(2-aminopropyl)indole.

As detailed in section A.2, 5-(2-aminopropyl)indole has been shown to act as a relatively potent, selective and reversible inhibitor of monoamine oxidase inhibitor-A (MAO-A) *in vitro*. This suggests that it might either by itself or in combination with other substances potentiate serotonergic effects. Although 5-(2-aminopropyl)indole did not inhibit human recombinant MAO-B *in vitro* up to 500 µM (Annex 1), the possibility of increased levels of other monoamines such as dopamine and noradrenaline may not be fully excluded. However, further studies are needed to investigate the dependence or abuse potential of this substance.

### B2. Human data

There are no published cases in the scientific or grey literature nor user reports describing the potential for dependence or abuse potential for 5-(2-aminopropyl)indole. Additionally, there have been no studies investigating the dependence and/or abuse potential of 5-(2-aminopropyl)indole in humans. Information from local, regional or national drug treatment agencies about the dependence and abuse potential of 5-(2-aminopropyl)indole is not available. As noted, Shulgin and Shulgin (1997) provided some limited information that 5-(2-aminopropyl)indole may show long-lasting stimulant properties in humans for about twelve hours when 20 mg is given orally.

## Section C. Prevalence of use

### *Information from seizures, collected and biological samples*

The first official notification of 5-(2-aminopropyl)indole to the European Union Early warning system was 1 June 2012 by the Norwegian national focal point. The reporting form details a seizure of one zip-lock bag containing one gram of light brown powder intercepted at Oslo Airport, Gardermoen, on 17 April 2012 by customs authorities. The identification was based on the analytical technique of GC-MS alone.

Seven Member States (Denmark, Germany, Finland, Hungary, the Netherlands, Sweden and the United Kingdom) and Norway have reported seizures of 5-(2-aminopropyl)indole.

At the time of writing the Joint report, several Member States reported that many forensic and/or toxicological laboratories did not have validated procedures for the confirmation of 5-(2-aminopropyl)indole in seized, collected and biological samples (EMCDDA and Europol, 2012). The lack of certified reference material has meant that some laboratories could not distinguish 5-(2-aminopropyl)indole from the related compound AMT which was also present in samples seized on the drug market at the time. Furthermore, in the case of biological

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samples there is no rapid qualitative screening method for the detection of 5-(2-aminopropyl)indole. Overall, this may have led to the under-reporting of 5-(2-aminopropyl)indole.

5-(2-Aminopropyl)indole has typically been encountered in seizures and collected samples in the form of powders, as well as in tablets and capsules. Where information has been provided, the quantities of powder ranged from 0.2 grams (Hungary) to 20.5 kilograms (the Netherlands). Hungary reported a seizure of seven beige tablets bearing markings resembling the Lexus logo<sup>(14)</sup>. This may suggest that 5-(2-aminopropyl)indole is being sold as 'ecstasy', as Europol have reported that tablets containing MDMA and bearing this logo, as well as a tablet punch (for imprinting logos on tablets as part of the manufacturing process) have been seized in the past. In Sweden, blue/green unmarked tablets and brown glittery tablets were also seized. In the United Kingdom, blue unmarked tablets were seized from a head shop and were found in commercial packages marked 'BENZO FURY' that also displayed an image of the chemical structure of 5-APB (5-(2-aminopropyl)benzofuran)<sup>(15)</sup>. There has been one report of residues found on a spoon and one report where 5-(2-aminopropyl)indole was recovered from the liquid in a syringe (Hungary). This may suggest that 5-(2-aminopropyl)indole is being injected by some users. See Annex 1 for details of seizures and collected samples reported to EMCDDA and Europol.

Two collected samples from the United Kingdom that were purchased from Internet retailers were found to be consistent with the succinate form of 5-(2-aminopropyl)indole.

Sweden reported the detection of 5-(2-aminopropyl)indole in ten biological samples (one blood; nine urine) from individuals suspected to have committed minor drug offences or people that are in drug treatment programmes. Further information on these cases are not available.

#### **Availability from Internet retailers**

A structured Internet search was conducted in March 2013 using the EMCDDA snapshot methodology to identify Internet retailers offering 5-(2-aminopropyl)indole for sale<sup>(16)</sup>. Five Internet retailers were identified that currently offered the substance for sale to consumers in

<sup>(14)</sup> It is common to find markings on tablets sold as 'ecstasy' including those of popular cultural and iconic brands often having an association with quality. Lexus is a Japanese car manufacturer.

<sup>(15)</sup> Although Internet retailers typically advertise 'Benzo Fury' products (or using synonyms such as 'BENZO FURY', 'BenzoFury', 'Benzo-fury', 'Benzo', and 'Fury') as containing 6-APB or 5-APB, a structured search of the European database on new drugs (EDND) found that seized and collected samples of 'Benzo Fury' products have contained: 6-APB; 5-APB; D2PM; pentylone with caffeine, lidocaine and procaine; AM-2201 (tentative identification); and, 5-(2-aminopropyl)indole. Additionally, published studies involving the analysis of collected and biological samples suggest that 'Benzo Fury' products contain: 6-APB; 5-APB; D2PM; and, 1-benzylpiperazine (BZP) with 3-trifluoromethylphenylpiperazine (3-TFMPP) and caffeine (Ayres and Bond, 2012; Baron et al., 2011; Wood et al., 2011; Wood et al., 2012).

<sup>(16)</sup> The Internet search engine 'google.co.uk' was searched (March 2013) for Internet retailers offering 5-(2-aminopropyl)indole for sale. On the advanced search page, Google was configured so that results were not narrowed by language and region. The search string used was: buy '5-IT' OR '5-API' OR '5-(2-aminopropyl)indole'. The first 100 sites were reviewed in full then sampling continued until 20 successive sites unrelated to the sale of 5-(2-aminopropyl)indole were identified. Websites that offered 5-(2-aminopropyl)indole for sale were reviewed and relevant information, such as the amount offered (mass of powder, number of capsules/pellets) and cost of purchase was recorded on a structured reporting form.

the European Union. In addition two further sites were identified that stated that 5-(2-aminopropyl)indole would be available for sale soon. On two of the sites offering the drug for sale all of the 5-(2-aminopropyl)indole products were out of stock and on one of these sites the prices given were promotional prices. Between them, the five retailers offered a total of 21 products for sale that claimed to contain 5-(2-aminopropyl)indole (11 products in powder form, two in capsules and eight for which the physical form was not stated). Three sites quoted prices in GBP, one in EUR and one did not state the price. Three sites offered 5-(2-aminopropyl)indole in powder form. The price per gram ranged from 32 to 40 GBP. The largest quantity offered for sale was 5 grams at 22.95 GBP per gram. One site offered 5-(2-aminopropyl)indole in capsule form although no prices were stated. The quantities of 5-(2-aminopropyl)indole per capsule were 80 mg and 100 mg. The site claimed that the effects of the 80 mg capsules last 3–4 hours and those of the 100 mg capsules last 5–6 hours. Some of the websites suggested that there is a similarity between 5-(2-aminopropyl)indole and 5-APB, 6-APB and MPA. No site offered products that contained both 5-(2-aminopropyl)indole and other new psychoactive substances. In addition a number of suppliers were identified on the trade website traddevv.com that claimed to supply 5-(2-aminopropyl)indole in bulk quantities. However, details of the amounts offered and prices were only available on direct application to these suppliers.

#### ***Prevalence of use***

No studies were identified that investigated the prevalence of 5-(2-aminopropyl)indole use.

One Member State reported the detection of 5-(2-aminopropyl)indole in biological samples from ten individuals not related to non-fatal intoxications and deaths. The individuals were suspected to have committed minor drug offences or were people in drug treatment programmes. Information on these cases is not available to allow further analysis.

In addition, 5-(2-aminopropyl)indole has been detected in a 'legal high' product labelled as 'Benzo fury' (see footnote 15). Furthermore, in three non-fatal intoxications 'Benzo Fury' was the product reported to have been taken. In several of the fatalities an empty bag labelled 6-APB (6-(2-aminopropyl)benzofuran) was found, but not detected in post-mortem samples. A small number of tablets resembling ecstasy have also been found to contain 5-(2-aminopropyl)indole. It is therefore relevant to discuss the available prevalence data on the use of 'Benzo Fury' products, 6-APB and ecstasy.

#### ***Information on the use of 'Benzo fury' products and 6-APB***

Two Internet surveys were identified that examined the use of 'Benzo Fury'. One of these also examined the use of 6-APB. While these surveys provide some indication of the use of 'Benzofury' products, the results are not generalisable to other groups and populations as they are non-probabilistic convenience sample surveys. In addition it is important to note both that the surveys predate the detection of 5-(2-aminopropyl)indole on the European drug market and a number of different new psychoactive substances have been detected in products sold as 'Benzo Fury' (see footnote 15).

The first survey was conducted among readers of a dance music magazine and the Guardian newspaper. It found that, overall, 2.4 % of respondents (n=7,700) and 3 % of 'regular clubbers' from the United Kingdom reported use of 'Benzo Fury' in the last year. In comparison, the self-reported last year prevalence for 'regular clubbers' of mephedrone was 30 %, MDAI was 3 % and methylone was 2 % (Mixmag, 2012). The second study conducted among self-reported users of new psychoactive substance mainly from Ireland (n=329), found that of the 159 respondents who reported using 'party pills' and 'liquid highs', 1.3 % (2/159) had used a product named 'Benzo Fury'; while none of the respondents reported use of '6-APB' (Kelleher et al., 2011).

Data from National Poisons Information Service (NPIS) in the United Kingdom indicate that there has been a small number of telephone calls and Toxbase access requests in relation to 6-APB. No information was provided on 5-(2-aminopropyl)indole itself (Health Protection Agency, 2012).

#### *5-(2-Aminopropyl)indole in tablets resembling 'ecstasy'*

Hungary reported the seizure of seven tablets that contained both 5-(2-aminopropyl)indole and methylthienylpropamine bearing markings resembling the Lexus logo (Annex 2). As noted, Europol have reported MDMA tablets and a tablet punch (for stamping logos on tablets) bearing the Lexus logo have been seized in the past. It may be the case that some ecstasy users are at risk of exposure to 5-(2-aminopropyl)indole. In this respect, drug prevalence estimates suggest that about 2 million Europeans (aged 15–64) have used ecstasy during the last year <sup>(17)</sup> (EMCDDA and Europol, 2013). However, as noted, the total number of such types of tablets containing 5-(2-aminopropyl)indole that have been reported so far is small and limited to one country.

## **Section D. Health risks**

### **D1. Acute health effects**

#### **D1.1. Animal data**

No studies were identified that investigated the acute toxicity of 5-(2-aminopropyl)indole in experimental animal models.

While detailed pharmacological investigations on 5-(2-aminopropyl)indole do not appear to have been published <sup>(18)</sup>, as noted in section A2., one study was identified that investigated the ability of 5-(2-aminopropyl)indole and its five isomers to inhibit monoamine oxidase (MAO). The assay method was based on the ability of guinea pig liver homogenate to absorb oxygen generated from serotonin as the substrate. The activity was expressed as

<sup>(17)</sup> European estimates are computed from national estimates weighted by the population of the relevant age group in each country. They are based on surveys conducted between 2004 and 2010/11 (mainly 2007–2010) and therefore do not refer to a single year. The term ecstasy is used in a broad sense to refer to substances that contain MDMA or other substances that are presented as ecstasy.

<sup>(18)</sup> As noted in footnote 13, a literature search on 5-(2-aminopropyl)indole revealed a translated article (USSR, Academy of Sciences) on serotonergic properties of several tryptamines. However, inspection of the English translation did not appear to provide any data on 5-(2-aminopropyl)indole (Buznikov et al., 1965).

percentage inhibition. The IC<sub>50</sub> values for 5-(2-aminopropyl)indole, 6-(2-aminopropyl)indole (6-IT) and 3-(2-aminopropyl)indole (AMT), for example, were 22, 4.6 and 58 µM, respectively. These data indicate that the 6-IT isomer was the most potent inhibitor amongst those three substances. These substances were also evaluated for their ability to antagonise pentylenetetrazole/reserpine-induced tonic extensor seizures in mice. 5-(2-Aminopropyl)indole appeared to be less active than 6-IT but more active than the AMT isomer with regards to anti-reserpine activity (Cerletti et al., 1968). AMT has been shown to induce stimulant effects in mice (incl. body tremor, heightened locomotor activity, mydriasis and hyperthermia) (Lessin et al., 1965), the extent to which this extends to the remaining isomers, including 5-(2-aminopropyl)indole, remains to be studied. A short report on the 6-(2-aminopropyl)indole isomer provided some indication that intravenous administration (0.5 mg/kg) resulted in hypertension and related sympathomimetic features in dogs (Maxwell, 1964).

## D1.2. Human data

### D1.2.1. User reports

As noted, Shulgin and Shulgin (1997) reported that 5-(2-aminopropyl)indole may show long-lasting stimulant properties in humans of about twelve hours following oral administration of 20 mg. Effects reported were increased heart-rate, anorexia, diuresis, and slight hyperthermia. No further information was provided.

There are some user reports on drug discussion forums that discuss the use of 5-(2-aminopropyl)indole (e.g. <sup>19</sup>). These need to be interpreted with caution as there was no analytical confirmation of the substances used. In addition, some of the users describe taking other drugs prior to or with 5-(2-aminopropyl)indole.

Some of the websites suggest that there is a structural similarity between 5-(2-aminopropyl)indole and 5-ABP, MPA and AMT. In addition one websites alludes to 5-(2-aminopropyl)indole having similar effects to 5-APB while another lists the product as '5-IT (similar to 6-APB)'.

#### *Drug Forum:*

The first discussion thread on the Drug Forum website for 5-(2-aminopropyl)indole appears to have been started in August 2010; while the first user report relating to this substance appears to be in November 2011. The thread also includes reference to 5-(2-aminopropyl)indole as an isomer of AMT, with citation of various on-line reference sources (e.g. Wikipedia) as well as Shulgin and Shulgin's 'TIKHAL' (1997).

#### *User reports*

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(<sup>19</sup>) <http://www.drugs-forum.com/forum/showthread.php?t=140331>  
<http://www.drugs-forum.com/forum/showthread.php?t=172223>  
<http://www.drugs-forum.com/forum/showpost.php?p=1126167&postcount=9>  
<http://www.bluelight.ru/vb/threads/616726-The-Big-amp-Dandy-5-IT-5-API-Thread>

One user after an apparent intravenous injection of 5-(2-aminopropyl)indole reported 'incredible rush, not so strong stimulating properties like speed, feeling is more like.. aMT + weak speed'. Another reported 'very small psychedelic properties, reminds [me] of aMT'. Following 80 mg 'bombed' a user concluded that 'I would say this somewhere in effects between 6-APB and MDAI with an amphetamine like quality but also quite reminiscent of aMT just nowhere near as psychedelic and with no nausea or body load'. Another user who 'bombed' 100 mg of 5-(2-aminopropyl)indole summarised 'no come down, very stable euphoria and clear thoughts with a little bit of trippiness'. The use of 6-APB was mentioned by a number of users and one indicated a comparison stating that 5-(2-aminopropyl)indole was 'quite comparable to 6-APB, but not quite as debilitating in its intenseness, not as euphoric, with a slightly shorter duration'.

*Bluelight:*

Amongst initial user discussions regarding the relative risk of taking 'new research chemicals' such as 5-(2-aminopropyl)indole and making predictions of effects, one user who took 20 mg (unknown route) stated that 'the duration on 5-IT is rather long, with the entire experience lasting about 10 hours, probably slightly more'. Similar duration of effect was also noted by another user who had taken 100 mg: 'I still felt it after seven hours on a 100mg dose'. However, another user in response to an extended come down experience of 22 hours postulated whether the user had actually taken 5-(2-aminopropyl)indole. Dose discussions also featured and one user surmised that 'at high doses I have seen reports of dysphoria, delirium, pain, unconsciousness, confusion, hyperthermia, tremors. I experienced only positive effects but I would not recommend a higher starting dose for these reasons'.

***D1.2.2. 5-(2-Aminopropyl)indole associated acute toxicity***

Two Member States (Sweden and the United Kingdom) reported a total of 20 non-fatal intoxications associated with 5-(2-aminopropyl)indole.

German police reported to Europol a case where a powder was seized from an unconscious person. It is not known if this is a non-fatal intoxication associated with 5-(2-aminopropyl)indole as further details are not available and therefore has not been included in this report.

*Non-fatal cases reported by Sweden*

Sweden reported 18 non-fatal intoxications where 5-(2-aminopropyl)indole was detected in biological samples. They occurred between January and August 2012.

Of the 18 cases, 16 were male and two were female. Their ages ranged between 17 and 53, however the most common age was 20 to 30 years with 11 of the 18 falling into this bracket. In six cases, the individual stated they had taken '5-IT' (a commonly used abbreviation for 5-(2-aminopropyl)indole), in three cases the stated intake was 'benzofury'. Other cases mentioned taking ethylphenidate, etizolam, MDPV and/or 6-APB. One person said they had been 'drinking only coca cola from an unknown source'; another person stated they had taken 'an unknown substance'. 5-(2-Aminopropyl)indole was analytically confirmed in each

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case although the concentration was not determined. Other drugs detected in these cases were: ethylphenidate, 4-,5- or 6-APB, 4-methylethcathinone, buprenorphine, methylphenidate (and metabolites), 4-fluoroamphetamine, oxazepam, temazepam, diazepam metabolites, methylthienylpropamine, methoxetamine, 4-hydroxymidazolam (midazolam metabolite), ketamine, GHB (gamma-hydroxybutyrate), PMMA (*para*-methoxymethamphetamine), amphetamine, *N*-methamphetamine, 4-methylamphetamine,  $\alpha$ -PVP, cannabis, thiopental, pentobarbital, benzoylecgonine (cocaine metabolite), ethanol and metabolites. It is not known whether any of these substances (e.g. benzodiazepines and barbiturates) had been administered as part of medical treatment.

The route of administration of 5-(2-aminopropyl)indole was indicated in two cases where the individuals reported having taken it by nasal insufflation. In three cases the individuals reported that they had sourced 5-(2-aminopropyl)indole from the Internet. The sources of 5-(2-aminopropyl)indole for the remaining fifteen cases are not available.

The reported symptoms included dilated pupils, sweating, restlessness, fatigue, disorientation, agitation, mydriasis, anxiety, tachycardia, hypertension and hyperpyrexia. Hallucinations were mentioned in one individual where 5-(2-aminopropyl)indole and benzodiazepines were detected only <sup>(20)</sup>.

#### *Non-fatal cases reported by the United Kingdom*

The United Kingdom reported two non-fatal intoxications associated with the second death case that is detailed in section D1.2.3. The two individuals had also reportedly ingested 'Benzo Fury' from the same source as the deceased. They were also examined at the hospital but neither appeared to have suffered any significant toxic effects. No further information on drug history or the amounts of 'Benzo Fury' taken is available.

#### **D1.2.3. 5-(2-Aminopropyl)indole associated deaths**

Four Member States (Sweden, the United Kingdom, Hungary and Germany) reported a total of 24 deaths associated with 5-(2-aminopropyl)indole (Table 3).

#### *Deaths reported by Sweden*

Sweden reported 15 deaths associated with 5-(2-aminopropyl)indole. The deaths occurred between April 2012 and July 2012. In 14 of the cases the cause of death was considered to be related to 5-(2-aminopropyl)indole. In the remaining case the cause was 'disease'. In the large majority of cases the cause of death was considered to be drug related although the ICD10 coding does not include naming 5-(2-aminopropyl)indole specifically. In the remaining cases, the cause was not ICD10 coded as being drug related and were recorded as being due to epilepsy and in another case, sudden cardiac arrest. In 14 cases the 5-(2-

<sup>(20)</sup> The example provided in the Joint report of a non-fatal intoxication involving an eighteen year old female who had taken one capsule of 5-(2-aminopropyl)indole of unknown strength actually relates to a self report that was not analytically confirmed. Although in the text of the Joint report it appears that this case was one of the 13 non-fatal intoxications reported by Sweden, further information from the national focal point has confirmed that it was not part of this case series and instead was documented prior to the introduction of biological screening for 5-(2-aminopropyl)indole. See EMCDDA & Europol (2012) for details further details of this case.



aminopropyl)indole concentration in post-mortem femoral blood ranged from between 0.7 and 5.1 µg/g blood. In one case the concentration of 5-(2-aminopropyl)indole was 18.6 µg/g femoral blood. All of the decedents were male. 13 were aged between 20 and 30 years, the remaining two were over 30 years old. In two cases 5-(2-aminopropyl)indole was the only substance reported as detected. In the remaining cases 5-(2-aminopropyl)indole was found in combination with 'pharmaceuticals' or 'other drugs of abuse'. Notably some of these drugs have monoaminergic activity, such as sertraline, venlafaxine and MDMA.

#### *Deaths reported by Hungary*

Hungary reported four deaths associated with 5-(2-aminopropyl)indole. Two of these deaths occurred in April 2012 and were originally believed to be related to AMT which was reported as detected in post-mortem biological samples. The decedents, a 40-year-old male and a 35-year-old female were found together in a flat.

The post-mortem concentrations determined as AMT were 34 mg/L and 84 mg/L respectively. These figures are provided only to show them relative to each other. The biological samples were no longer available for re-analysis. However, the reanalysis of powders found at the scene identified the presence of 5-(2-aminopropyl)indole and not AMT. The Hungarian national focal point noted that 'based on the active agent identified in the substance found next to the bodies it is assumed that the cause of the deaths was 5-(2-aminopropyl)indole intoxication rather than AMT intoxication'. As already noted, analytical reference standards were not available at the time and it was difficult to distinguish between 5-(2-aminopropyl)indole and AMT. No other substances were reported as detected.

The pathological cause of death in each case was 'circulatory failure and respiratory failure, where the direct causes of death... were the results of 5-IT intoxication' and in the case of the female 'the respiration of vomited content of stomach might have had a limited impact too'. There were signs of 'prolonged sexual intercourse, extreme hyperthermia and the use of new psychoactive substances'.

The third death occurred in May 2012, an involved a 38 year old male known 'drug abuser' who was found dead in his apartment along with injection paraphernalia. A sachet found next to the body contained 5-(2-aminopropyl)indole. 'The toxicological analysis identified 5-IT in the blood' but no quantitative information was available. No other substances were reported as detected. The cause of death was attributed to drug intoxication and respiratory failure.

The fourth death occurred in June/July 2012, a 24 year old male died having purchased a product called 'Pink' from the Internet. The substance had been dissolved in water and consumed. 'Toxicological analysis identified 5-IT in the blood and stomach'. No other substances were reported as detected. The cause of death was attributed to circulatory and respiratory failure as a result of drug use and overdose.

#### *Deaths reported by the United Kingdom*

The United Kingdom reported four deaths associated with 5-(2-aminopropyl)indole. Details are only provided for two of these cases, both of which occurred in June 2012. The decedents were both male; one was 33 years old, the other was 19 years old.

The cause of death in the first case involving the 33 year old was 'fatality following the ingestion of 'Benzo Fury' and certified as '5-(2-aminopropyl)indole (5-API; 5-IT) and Benzofuran toxicity'. The male was treated in hospital prior to death. Analysis of the blood revealed an approximate 5-(2-aminopropyl)indole concentration of 0.379 mg/L in unpreserved post-mortem blood. Other drugs detected in the blood included 5-APB (0.016 mg/L), 6-APB (0.057 mg/L), diazepam (0.037 mg/L), nordiazepam (0.009 mg/L), temazepam (0.001 mg/L) and AMT (less than 0.01 mg/L). Urine analysis detected amphetamine, 5-(2-aminopropyl)indole, 5-APB, 6-APB, AMT and benzodiazepines. In addition, 5-(2-aminopropyl)indole, 5-APB, 6-APB, AMT and diazepam were detected in the stomach contents.

In the second case involving the 19 year old, the toxicological investigation revealed 5-(2-aminopropyl)indole at a concentration of approximately 0.513 mg/L in ante-mortem blood (the deceased was admitted to hospital prior to death) and approximately 0.30 mg/L in unpreserved post-mortem blood. Other drugs detected included MDMA (0.468 mg/L ante-mortem blood, 0.502 mg/L post-mortem blood), MDA (0.036 mg/L ante-mortem blood, 0.046 mg/L post-mortem blood), 6-APB (0.005 mg/L post-mortem blood only), atropine and lignocaine. These drugs were also detected in the urine and stomach contents. It was noted that there was a high concentration of MDMA, which on its own was considered to be at a fatal level. However, a cumulative/synergistic effect of 5-(2-aminopropyl)indole was not excluded and the cause of death was recorded as 'multidrug toxicity'. This case is linked to the seizure of 116 blue tablets in branded packets labelled as 'BENZO FURY' that were found to contain 5-(2-aminopropyl)indole.

The remaining two deaths were reported in a letter to the British Medical Journal. The letter reports that 5-(2-aminopropyl)indole was detected in the post-mortem blood samples of two young adults. The authors note that 5-(2-aminopropyl)indole was 'found in combination with other drugs in one case'; while in the second case '5-APB/6-APB' was detected (Seetohul et al., 2012). It was ascertained from the national focal point that these cases were distinct from the other two cases reported by the United Kingdom. No further details are available at this time.

#### *Death reported by Germany*

Germany reported one death associated with 5-(2-aminopropyl)indole.

The report stated that on 23 May 2012, a 29 year old man who was not known as drug user was found dead in his apartment. A powder was found under his bed which was analysed and found to contain 5-(2-aminopropyl)indole. The initial urine screen indicated a positive result for amphetamine/methamphetamine. The preliminary autopsy report provided 'neither a hint on external assault and battery nor on a pathological-anatomic cause of death'. Further toxicological investigations revealed a high concentration of 5-(2-aminopropyl)indole

in the blood and urine samples (the blood sample contained >1200 ng/ml) (Schäper et al., 2013). No other substances were reported as detected. The final cause of death has not yet been recorded however, the German national focal point reported that intoxication by 5-(2-aminopropyl)indole is plausible.

This case highlights that there may be cross-reactivity issues with some screening tests. Further research is required to investigate this.

**Table 3.** Summary of deaths associated with 5-(2-aminopropyl)indole (5-IT). For reference against other reported cases µg/g is largely comparable to mg/L.

Date of death	Deceased (age/sex)	5-(2-Aminopropyl)indole toxicological findings (blood)	Other drugs detected (blood unless otherwise indicated)	CD10 coding or descriptive cause of death
<b>Sweden</b>				
April 2012	23 yr old male	18.6 µg/g	AM2201 4-APB (urine)	Toxic effects of non-medicinal substance
May 2012	31 yr old male	2.3 µg/g	0.05 µg/g hydroxyzine 0.04 µg/g etizolam	Poisoning by hallucinogen
May 2012	24 yr old male	2.4 µg/g	0.03 µg/g zopiclone 0.003 µg/g ethylphenidate 0.03 µg/g ritalinic acid	Un-attended death. No other cause found.
May 2012	28 yr old male	3.8 µg/g	26 µg levitiracetam	Epilepsy
May 2012	20 yr old male	1.1 µg/g	0.02 µg/g benzoylecgonine 0.002 µg/g THC pentadone [no quantitative data provided]	Sudden cardiac arrest
May 2012	31 yr old male	5.1 µg/g	0.04 µg/g 7-amino-clonazepam 0.01 µg/g perphenazine 0.12 µg/g ethylphenidate 2.6 µg/g ritalinic acid 0.0002 µg/g methylphenidate	Poisoning by drugs
May 2012	33 yr old male	4.2 µg/g		Poisoning by hallucinogen
May 2012	28 yr old male	2.5 µg/g	9.2 µg/g pregabalin	Poisoning by drugs
May 2012	40 yr old male	1.0 µg/g		Poisoning by hallucinogen
June 2012	31 yr old male	1.6 µg/g	0.2 µg/g alimemazine 0.1 µg/g desmethylalimemazine	Un-attended death. No other cause found
June 2012	29 yr old male	0.7 µg/g	0.18 µg/g ethylphenidate 1.9 µg/g ritalinic acid	Accidental poisoning by drugs
June 2012	55 yr old male	2.1 µg/g	0.9 µg/g carisoprodol meprobamate (not quantitated) 0.32 µg/g 7-amino-clonazepam	Poisoning by drugs

June 2012	30 yr old male	2.1 µg/g	0.7 µg/g sertraline 2.5 µg/g desmethylsertraline 1.0 µg/g venlafaxine 0.5 µg/g o-desmethylvenlafaxine	Poisoning by drugs
June 2012	24 yr old male	1.1 µg/g	0.02 µg/g benzoylecgonine 0.53 µg/g MDMA 0.03 µg/g MDA	Poisoning by hallucinogen
July 2012	28 yr old male	1.7 µg/g	0.008 µg/g ethylphenidate	Un-attended death. No other cause found
<b>Hungary</b>				
April 2012	40 yr old male	Not available	None	Circulatory failure and respiratory failure were the direct causes of death that were the results of 5-IT intoxication.
April 2012	35 year old female	Not available	None	Circulatory failure and respiratory failure were the direct causes of death that were the results of 5-IT intoxication, the respiration of vomited content of stomach might had a limited impact too.
May 2012	38 year old male	Not available	None	Brain oedema, frothy respiratory tract secretion, pulmonal oedema and minor degeneration of cardiac muscle were observed in the body. The report concludes that based on the case history and the diagnostic report drug intoxication and respiratory failure as a consequence of intoxication are the assumed causes of death.
June/July 2012	24 year old male	Not available	None	The cause of death was circulatory and respiratory failure that developed due to metabolic failure, severe brain oedema, pulmonal oedema and cardiac failure. The report concludes that, in all probability, the cause of that was drugs use and overdose.
<b>United Kingdom</b>				
June 2012	33 year old male	0.379 mg/L	0.016 mg/L 5-APB 0.057 mg/L 6-APB 0.037 mg/L diazepam 0.009 mg/L nordiazepam 0.001 mg/L temazepam <0.001 mg/L AMT	The level of 5-IT is an approximate determination in unpreserved post mortem blood. All other analytes were detected in post mortem blood. Urine analysis detected amphetamine, 5-IT, 5-APB, 6-APB, AMT and benzodiazepines. In addition, 5-IT, 5-APB, 6-APB, AMT and diazepam were detected in the stomach contents
June 2012	19 year old male	0.30 mg/L	0.502 mg/L MDMA 0.046 mg/L MDA 0.005 mg/L 6-APB Atropine Lignocaine	The level of 5-IT is an approximate determination in unpreserved post mortem blood. 0.513 mg/L 5-IT was determined in ante mortem blood. Cause of death noted to be 'multi-drug toxicity'. All other analytes were detected in post mortem blood.
Prior to 24 August 2012	'young adult'	Not available	'Other drugs'	Reported in letter to the British Medical Journal, no further details available.

Prior to 24 August 2012	young adult'	Not available	'5/6-APB'	Reported in letter to the British Medical Journal, no further details available.
<b>Germany</b>				
May 2012	29 year old male	>1200 ng/ml	None	The final cause of death has not yet been recorded however, the national focal point reported that intoxication by 5-(2- aminopropyl)indole is plausible.

## **D2. Chronic health effects**

### **D2.1. Animal data**

No studies were identified that investigated the chronic health effects of 5-(2-aminopropyl)indole in animals.

### **D2.2. Human data**

No studies were identified that investigated the chronic health effects of 5-(2-aminopropyl)indole in humans.

## **D3. Factors affecting public health risks**

### **D3.1. Availability and quality of the new psychoactive substance on the market**

5-(2-Aminopropyl)indole is offered for sale by Internet retailers as a substance in its own right. 5-(2-Aminopropyl)indole has also been detected in a 'legal high' type product branded as 'Benzo Fury'. There has also been one report from Hungary where 5-(2-aminopropyl)indole was seized as tablets resembling 'ecstasy'. Some individuals may be exposed to 5-(2-aminopropyl)indole intentionally. Others may be exposed unintentionally and unknowingly after consuming a product with no indication that it contains 5-(2-aminopropyl)indole or following its ingestion as a component of a mixture of other active substances (e.g. MDAI, 5- or 6-APB).

### **D3.2. Availability of the information, degree of knowledge and perceptions amongst users concerning the psychoactive substance and its effects**

There is relatively limited information on drug discussion forums regarding the effects and potential health / adverse effects related to the use of 5-(2-aminopropyl)indole. On some drug discussion forums the use of 5-(2-aminopropyl)indole as a drug in its own right has been discussed. This is supported by the finding that two collected samples from Internet retailers contained 5-(2-aminopropyl)indole (in powders) as well as the fact that Internet retailers offer various dosage forms claiming to contain the substance (section C). Nevertheless, it is likely that the information, degree of knowledge, and perceptions amongst users concerning 5-(2-aminopropyl)indole and its effects are likely to be limited. In addition some users may be exposed to 5-(2-aminopropyl)indole unknowingly given that it has been detected in a 'legal high' type product labelled as 'Benzo Fury' as well as tablets resembling ecstasy.

### **D3.3. Characteristics and behaviour of users**

There are self-reports from users on drug discussion forums who believe that they have specifically taken 5-(2-aminopropyl)indole. In some cases this appears to be in order to determine its relative effects compared to related compounds such as AMT in particular as well as 5- or 6-APB. This suggests a degree of risk-taking behaviour although some of the discussions have included harm reduction measures in relation to use of 'new research chemicals'.

#### **D3.4. Nature and extent of health consequence**

The limited documented information on the acute health effects of 5-(2-aminopropyl)indole have been discussed in section D1.2. There is insufficient information in the reported non-fatal intoxications and deaths where 5-(2-aminopropyl)indole has been detected to discuss in detail the circumstances of these cases. However, from the information available, it does not appear that any of these were related to road traffic accidents. The information available indicates that the presence of 5-(2-aminopropyl)indole has been analytically confirmed in a number of acute emergencies associated with the substance.

#### **D3.5. Long-term consequences of use**

As noted in sections D2.1. and D2.2. no animal or human data on the chronic health effects of 5-(2-aminopropyl)indole were identified. In particular, there have been no long-term follow up studies to determine whether 5-(2-aminopropyl)indole users are at greater risk of health deterioration later in life, or of developing chronic or life-threatening medical conditions.

#### **D3.6. Conditions under which the new psychoactive substance is obtained and used, including context-related effects and risks**

As noted, it appears that the sourcing and use of 5-(2-aminopropyl)indole is generally related to individuals attempting to source the drug itself or when it has been inadvertently taken along with or instead of other stimulants. As noted in section C, the structured Internet search conducted by the EMCDDA identified five English-language Internet retailers that offered 5-(2-aminopropyl)indole for sale to consumers in the European Union. In addition, in one case 5-(2-aminopropyl)indole was detected in a 'legal high' type product labelled as 'Benzo Fury' that was sold in a bricks and mortar headshop. It is likely that 5-(2-aminopropyl)indole is used in the same environments as other stimulants. This would be typically, but not restricted to, home environments, bars/pubs, discotheques/nightclubs and outdoor music festivals. Limited information from drug discussion forums suggest that 5-(2-aminopropyl)indole is used at home and in nightclubs.

### **Section E. Social Risks**

#### **E1. Individual social risks**

There is currently no data to be able to determine the impact of 5-(2-aminopropyl)indole in this area.

#### **E2. Possible effects on direct social environment**

There is currently no data to be able to determine the impact of 5-(2-aminopropyl)indole in this area.

#### **E3. Possible effects on society as a whole**

Sweden reported the detection of 5-(2-aminopropyl)indole in ten biological samples (one



blood; nine urine) from individuals suspected to have committed minor drug offences or people that are in drug treatment programmes. Further information on these cases are not available to allow further comment.

#### **E4. Economic costs**

Given the lack of data available on acute health emergencies and healthcare utilisation related to the use of 5-(2-aminopropyl)indole, it is not possible at this time to estimate whether the substance is associated with greater healthcare costs than other stimulant drugs.

#### **E5. Possible effects related to the cultural context, for example marginalisation**

There is currently no data to be able to determine the impact of 5-(2-aminopropyl)indole in this area.

#### **E6. Possible appeal of the new psychoactive substance to specific population groups within the general population**

There is currently no data to be able to determine the possible appeal of 5-(2-aminopropyl)indole to specific population groups within the general population.

### **Section F. Involvement of organised crime**

#### **F1. Evidence that criminal groups are systematically involved in production, trafficking and distribution for financial gain**

No information has been received by Europol of evidence that criminal groups are systematically involved in production, trafficking and distribution of 5-(2-aminopropyl)indole for financial gain.

#### **F2. Impact on the production, trafficking and distribution of other substances, including existing psychoactive substances as well as new psychoactive substances**

Based on the information available to ECMDDA and Europol it does not appear that the production, trafficking and distribution of 5-(2-aminopropyl)indole impacts on other existing psychoactive substances or new psychoactive substances.

#### **F3. Evidence of the same groups of people being involved in different types of crime**

No information has been received by Europol of evidence of the same groups of people being involved in different types of crime in connection with 5-(2-aminopropyl)indole.

**F4. Impact of violence from criminal groups on society as a whole or on social groups or local communities (public order and safety)**

No information has been received by Europol on incidents of violence in connection with 5-(2-aminopropyl)indole.

**F5. Evidence of money laundering practices, or impact of organised crime on other socioeconomic factors in society**

No information has been received by Europol on incidents of money-laundering or impact of organised crime on other socioeconomic factors in society in connection with 5-(2-aminopropyl)indole.

**F6. Economic costs and consequences (evasion of taxes or duties, costs to the judicial system)**

There is currently no data to be able to determine the impact of 5-(2-aminopropyl)indole in this area.

**F7. Use of violence between or within criminal groups**

No information has been received by Europol on incidents of violence in connection with 5-(2-aminopropyl)indole.

**F8. Evidence of strategies to prevent prosecution, for example through corruption or intimidation**

No information has been received by Europol on strategies to prevent prosecution in connection with 5-(2-aminopropyl)indole.

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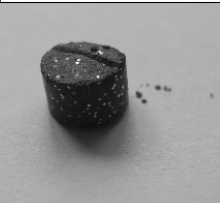
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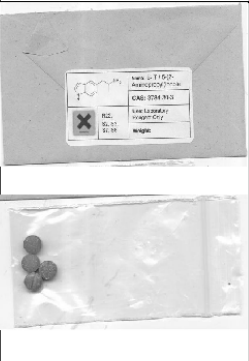
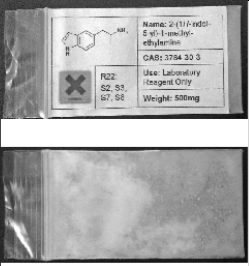

## Annex 1

### Details of seizures and collected samples of 5-(2-aminopropyl)indole (5-IT) reported to the EMCDDA and Europol


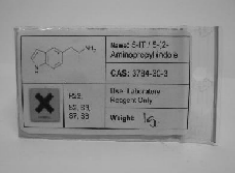
Date of seizure or collection	Amount and physical form	Seizing or collecting authority	Place of seizure or collection	Notes	Images
<b>Denmark</b>					
19/07/2012	One seizure of 5.1 g light brown powder	Customs	Haderslev	Powder was found in a small transparent bag and with a sticker: '5g 5-IT, Research Chemical, Not for human consumption'. The bag was inside a 'normal' brown envelope, and without any sender. The post came from United Kingdom. Identification based on GC-MS, UPLC-TOF, H-NMR.	
<b>Finland</b>					
01/04/2012	One seizure of 1.1 g of a light brown powder	Customs	Helsinki	Seized in incoming mail. Identification based on NMR.	
<b>Germany</b>					
02/05/2012	1.35 g and a further 0.22 g together with traces of MDAI (together with other new psychoactive substances)	Police	Hannover	The accused stated that he has bought the substances via the internet from an online shop in the United Kingdom.	
12/11/2012	Brown glittery tablets (amount unknown at present)	Police	Bavaria	Further fragmentary information on 5 additional cases of detection of 5-IT in seizures [sic] of 1-10 tablets per case was reported by the Bavarian Police and one case of 1 gram 5-IT.	

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Hungary*					
04/2012	2.4 g of a beige powder	Police	Tapolca	Confirmed as 5-(2-aminopropyl)indole	
04/2012	Residues on paper, liquid in syringe (0.75 ml)	Police	Debrecen	Confirmed as 5-(2-aminopropyl)indole	
04/2012	2.2 g of a brown powder	Police	Szombathely	Confirmed as 5-(2-aminopropyl)indole	
05/2012	Residues on spoon	Police	Szentes	Confirmed as 5-(2-aminopropyl)indole	
05/2012	10.2 g of a brown powder	Police	Tata	Confirmed as 5-(2-aminopropyl)indole	
06/2012	0.2 g of a light-brown powder	Police	Szigetvár	Confirmed as 5-(2-aminopropyl)indole	
06/2012	7 beige tablets with 'Lexus' logo, also containing, methylthienylpropamine and caffeine	Police	Kiskőrös	Confirmed as 5-(2-aminopropyl)indole. Weight of tablets: 0.285 g, diameter: 8.10 mm, thickness: 5.8 mm. The identification was carried out by TLC and GC/MS based on the laboratory's 'own' reference materials (their structure was confirmed by NMR).	
07/2012	0.2 g of brown powder	Police	Esztergom	Confirmed as 5-(2-aminopropyl)indole	
08/2012	97.3 g of a brown powder, residues on digital scale	Police	Szigetvár	In this case the investigation confirmed the fact of dealing both new psychoactive substances (according to schedule "C" Gov. Decree 66/2012) and illicit drugs (covered by the illicit drugs definition of the Penal Code). Mail delivery and selling from the flat was also confirmed. The business covered the whole country did not concentrate on the area of Szigetvár.	
09/2012	4.1 g of light brown powder	Police	Eger	Confirmed as 5-(2-aminopropyl)indole	
10/2012	0.3 g of light brown powder	Police	Debrecen	Confirmed as 5-(2-aminopropyl)indole	
12/2012	0.1 g of brown powder	Police	Szekszárd	Confirmed as 5-(2-aminopropyl)indole	
Netherlands					
Not available	20.5 kg	Customs		Not available	
Sweden					

	33 seizures incorporating 36.33 g powder and 54 tablets.	Police		The first seizure comprising 13 g beige powder was seized by the police 16/05/2012 in Ömsköldsvik city with identification based on GC-MS, GC-IRD and NMR.  Examples of seized tablets: One type of tablet in 6 materials: These are blue, green melange; round and curved with border; diameter 9.0 mm, width 4.0 mm, weight 0.25 g. Another type of tablet that occurred only in one material: brown, glittery tablet; round and flat and scored; diameter 6.0 mm, width 2.9 mm, weight 0.10 g.	
	Four seizures in total, comprising: three seizures of a brown powder weight a total of 11.07 g. One seizure of 5 tablets	Customs	Arlanda Airport, Sweden	The three packages containing powder were from Spain. The package containing tablets were sent from United Kingdom.	
<b>United Kingdom</b>					
April 2012	500 mg brown powder	State's Analyst Guernsey	Purchased from the internet	Confirmed as 5-(2-aminopropyl)indole succinate by NMR.	
May 2012	Pale brown powder	TicTac Ltd.	Purchased from internet £22.50 for 500 mg	Product label stated '5-IT' '500mg' 'NOT FOR HUMAN CONSUMPTION'.  Analysis by GCMS. Molecular formula confirmed by High Res MS. Confirmed as 5-(2-aminopropyl)indole succinate by proton NMR.	



09/06/2012	One seizure of 116 packets. Blue unmarked tablet in packet	Police	Edinburgh, Scotland	During the police investigation of one of the fatal cases from the United Kingdom where the presence of 5-(2-aminopropyl)indole was confirmed, the police were informed that the product consumed by the deceased had been purchased at a 'headshop' in Edinburgh. Police executed a search warrant at the Edinburgh premises and recovered a large quantity of items (160 productions) including bulk quantities of powders, herbal material and packaged products. One of the items submitted to the Forensic Science Laboratory contained 116 yellow packages labelled 'BENZO FURY' with a graphic displaying the structure of 5-APB. Four of these packages, selected at random, were examined and each found to contain a single blue unmarked biconvex tablet which were each analysed and found to contain 5-(2-aminopropyl)indole. Other items of interest recovered from the 'headshop' were: Yellow capsules labelled 'benzofury' found to contain brown powder containing 5/6-APB. 31 g of brown powder found to contain 5/6-APB. 174 packages (98 of one type and 76 of a second type) each containing 1 g of crystalline substance identified as methylthienylpropamine (MPA).	
08/09/2012	One seizure of seven red and white gelatine capsules with no markings on them. Also contained diphenyl prolinol (D2PM).	Customs	Guernsey	The Guernsey Border Agency seized the capsules along with a number of Class B substances from a person arriving on the Island. Analysis was carried out by the Guernsey States Analyst.	
<b>Norway</b>					
17/04/2012	One seizure of 1 g in a small bag with zip-lock	Customs	Gardermoen, Oslo Airport	Identified with MS only.	

\*The Forensic Institute of the National Tax and Customs Administration of Hungary reported no seizures of 5-(2-aminopropyl)indole.

## Appendix 1

Study examining the inhibition of human monoamine oxidase (MAO) by the new psychoactive substance 5-(2-aminopropyl)indole, CC.13.SAT.003, February 2013, Herriaz, T., in collaboration with Simon D. Brandt.

## Study examining the inhibition of human monoamine oxidase (MAO) by the new psychoactive substance 5-(2-aminopropyl)indole ('5-IT')

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In collaboration with Simon D. Brandt, Liverpool John Moores University, United Kingdom.

**EMCDDA contract:** CC.13.SAT.003

### 1. Introduction

Following an examination of the available information on 5-(2-aminopropyl)indole (5-IT) <sup>(1)</sup>, on 3 October 2012 the EMCDDA and Europol launched a Joint Report on the substance. It was presented to the Council of the European Union, the European Commission and the European Medicines Agency on 12 December 2012 <sup>(2,3)</sup>.

Consequently, a Technical report will be prepared as a matter of priority within a tight deadline in order to be available for the risk assessment as requested by the Council of the European Union. As identified in the Joint report, there is a lack of information on the pharmacology and toxicology of 5-(2-aminopropyl)indole. However, a single study published in the 1960s indicated that the substance may act as an inhibitor of monoamine oxidase (Cerletti et al., 1968). In some of the non-fatal intoxications and deaths associated with 5-(2-aminopropyl)indole that have been reported, symptoms typical of monoaminergic toxicity have been noted. These include hyperthermia along with dilated pupils, sweating, increased heart rate, high blood pressure, agitation, restlessness, disorientation and anxiety. The purpose of the contract therefore is to conduct *in vitro* studies on 5-(2-aminopropyl)indole to investigate its effects on monoamine oxidase in order to inform the risk assessment.

### 2. Objectives of the study

Experimental work was conducted to determine and evaluate the effect of 5-(2-aminopropyl)indole (5-IT) on the human monoamine oxidase (MAO) enzyme. *In vitro* assays included the use of recombinant MAO enzymes and kynuramine as substrate. Following incubation, the assays were analysed using previously validated methods based on the analysis of the reaction products by HPLC coupled to Diode Array Detector (DAD) and fluorescence detection. The two isoenzymes MAO-A and -B were considered. The inhibition parameters,  $IC_{50}$  (concentration of inhibitor that produce 50 % inhibition) and  $K_i$  (dissociation constant of enzyme and inhibitor), were determined using different concentrations of substrate and inhibitor and appropriate equations. Inhibition of MAO-A by known inhibitors was also evaluated and the inhibition

<sup>(1)</sup> For the purpose of this report the abbreviation '5-IT' is used interchangeably with '5-(2-aminopropyl)indole'.

<sup>(2)</sup> OJ L 127, 20.5.2005, p. 32.

<sup>(3)</sup> EMCDDA and Europol. (2012), *EMCDDA–Europol Joint Report on a new psychoactive substance: 5-(2-aminopropyl)indole*, EMCDDA, Lisbon.

parameters ( $IC_{50}$ ) determined. In addition, experimental work was undertaken on the mode of binding of 5-IT, its mechanism of inhibition and selectivity.

### 3. Experimental

Recombinant human monoamine oxidase-A and B were obtained from Gentest BD biosciences (Woburn, MA, USA). Kynuramine dihydrobromide, 4-hydroxyquinoline, harmaline, clorgyline, *R*-deprenyl, toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-2-oxazolidinone), and moclobemide (4-chloro-*N*-[2-(4-morpholinyl)ethyl]benzamide) were purchased from Sigma-Aldrich. 5-IT hemisuccinate (5-API hemisuccinate; 5-(2-aminopropyl)indole hemisuccinate) was purchased from LGC standards. All test compounds were dissolved in milli-q ultrapure water and diluted appropriately in 100 mM phosphate buffer pH 7.4.

#### 3.1. Monoamine oxidase assays (MAO-A and B)

The enzymatic activity of human MAO isozymes was studied using protein fractions containing this enzyme by using kynuramine as a non-selective substrate (Herraiz and Chaparro, 2006). The reaction velocity  $v$  ( $\mu\text{M}/\text{min}$ ) was obtained from oxidative deamination of kynuramine to form under the conditions of the assay 4-hydroxyquinoline that was analysed by HPLC-DAD and its concentration calculated from a calibration curve of peak area ( $\lambda$  at 320 nm) against concentration (Herraiz and Caparro, 2006). To carry out the assay, protein fractions containing MAO-A or B were diluted to the desired concentrations in 100 mM potassium phosphate buffer (pH 7.4). A 0.2 ml reaction mixture containing 0.01–0.02 mg/ml protein and 0.25 mM kynuramine in 75 mM potassium phosphate (pH 7.4) was incubated at 37 °C for 40 min. After incubation the reaction was stopped by the addition of 2N NaOH (75  $\mu\text{l}$ ), followed by the addition of 70 % perchloric acid (25  $\mu\text{l}$ ), and the sample centrifuged (10000  $\times$  g) for 6 min. An aliquot of the supernatant (20  $\mu\text{l}$ ) was injected into the HPLC and the deamination products of kynuramine formed during the enzymatic reaction determined by RP-HPLC-DAD and fluorescence detection.

#### 3.2. MAO-A and -B inhibition studies with 5-IT and other inhibitors

MAO-A or -B enzymes were incubated as above with kynuramine as a substrate and added with increasing concentrations of 5-IT or other inhibitors of MAO-A and B at the desired concentration in phosphate buffer (pH 7.4), and incubated at 37 °C for 40 min, as above.  $IC_{50}$  values (concentration of inhibitor producing 50 per cent inhibition of enzymatic activity) were calculated by fitting (% inhibition vs. concentration of inhibitor) to non-linear regression curves or by linear regression of inhibition (%) against the log of substrate concentration. Assays were carried out at least in duplicate.

Kinetic constant of Michaelis-Menten ( $K_m$ ) and the maximum velocity ( $V_{max}$ ) were obtained from nonlinear regression analysis (velocity vs. concentration) using different concentrations of substrate. The mechanism of MAO inhibition by 5-IT was assessed experimentally by obtaining the corresponding double reciprocal Lineweaver-Burk plots of the enzyme activity at different concentrations of substrate and inhibitor. The secondary plot of the slope from the double reciprocal curves versus the concentration

of inhibitor was used to calculate  $K_i$  (inhibition constant) values. Kinetic assays were carried out at least in duplicate.

To determine the type of binding of 5-IT to MAO-A (i.e. reversible or irreversible inhibition), MAO-A (0.025 mg/ml protein) in 100 mM phosphate buffer (pH 7.4) was incubated at 37 °C for 30 min with or without (control) 5-IT (100  $\mu$ M), and then centrifuged (15000 x g, 15 min) to pellet the protein, that was washed with 100 mM phosphate buffer and the procedure repeated three times. Finally, the enzyme was resuspended in phosphate buffer and used to measure MAO activity, as above. Assays were carried out at least in duplicate.

### 3.3. Chromatographic analysis by RP-HPLC

The analysis of the kynuramine deamination product 4-hydroxyquinoline was accomplished by RP-HPLC-DAD and fluorescence detection using an HPLC 1050 (Hewlett Packard) provided with a 1100 DAD (Agilent) and a 1046A-fluorescence detector (Hewlett Packard). A 150 mm x 3.9 mm, 4  $\mu$ m, Nova-pak C18 column (Waters, Milford, MA, USA) was used for separation. Chromatographic conditions were: 50 mM ammonium phosphate buffer (pH 3) (buffer A) and 20% of A in acetonitrile (buffer B). Gradient programmed from 0 % (100 % A) to 32 % B in 8 min and then 90 % B at 10 min. The flow rate was 1 ml/min, the column temperature was 40 °C and the injection volume was 20  $\mu$ l.

## 4. Results

### A) *In vitro* inhibition of human MAO-A by 5-IT

Enzymatic reactions were carried out at different concentrations of substrate and the results of the enzyme activity against the concentration of substrate fitted to nonlinear regression analysis to plot Michaelis-Menten curves (Figure 1). The reaction velocity  $v$  ( $\mu$ M/min) was obtained from the deamination of kynuramine by MAO-A to form 4-hydroxyquinoline. In that way, calculated values of  $V_{max}$  of  $0.8 \pm 0.02$   $\mu$ M/min and  $K_m$  of  $61.88 \pm 4.6$   $\mu$ M were obtained for MAO-A and kynuramine. Subsequently, inhibition of human MAO-A by the substance 5-(2-aminopropyl)indole (5-IT) was studied in presence of kynuramine as the substrate (250  $\mu$ M). The profile is shown in Figure 2, and it clearly suggests that 5-IT is an *in vitro* inhibitor of the human MAO-A with an  $IC_{50}$  value of  $1.6 \pm 0.1$   $\mu$ M.

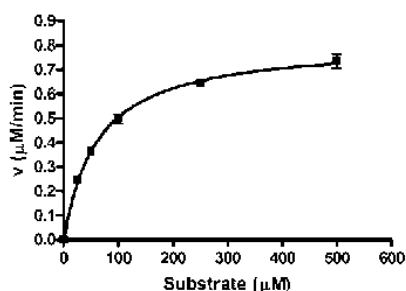


Figure 1. Michaelis-Menten curve of kynuramine deamination to form 4-hydroxyquinole by MAO-A (0.01 mg/ml MAO-A protein and kynuramine in 0.2 ml phosphate buffer. 37 °C, 40 min).

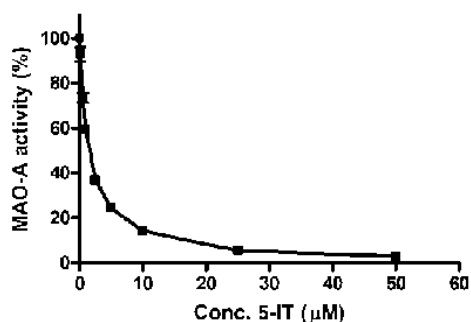


Figure 2. Inhibition of human MAO-A in presence of 5-IT

### B) *In vitro* inhibition of human MAO-B by 5-IT

Inhibition of human MAO-B by the substance 5-IT was studied in the presence of kynuramine as substrate (250 µM). The profile obtained is shown in Figure 3 and clearly shows that 5-IT was devoid of activity as an inhibitor of recombinant human MAO-B. Indeed, in the range used (0–500 µM, 5-IT) no inhibition of MAO-B was detected. Instead, under the same conditions, *R*-deprenyl, a selective inhibitor of MAO-B, highly inhibited this enzyme at sub-micromolar concentrations (Figure 4). As a result of these data, no further studies were carried out with MAO-B isoenzyme and 5-IT.

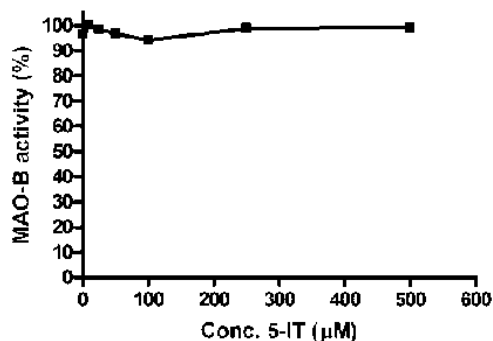


Figure 3. Activity of human MAO-B in presence of 5-IT.

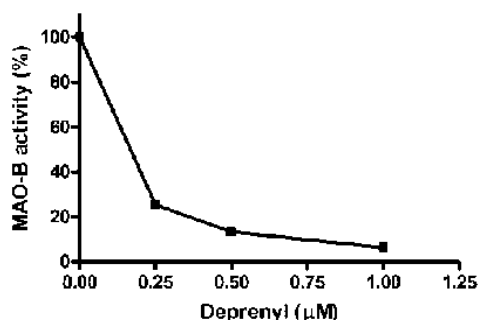


Figure 4. Inhibition of human MAO-B by (*R*)-deprenyl.

*C) Kinetic study of human MAO-A inhibition and determination of  $K_i$  (inhibition constant) values*

As 5-IT was an inhibitor of human MAO-A, kinetic assays corresponding to the activity of this enzyme in the presence of increasing concentrations of 5-IT (0–2  $\mu\text{M}$ ) were accomplished by using various concentration of substrate. The corresponding double reciprocal curves (i.e. Lineweaver-Burk plots) were obtained experimentally and are given in Figure 5, showing that 5-IT is a competitive inhibitor of MAO-A. Thus, in the presence of increasing concentrations of 5-IT, the enzyme had similar  $V_{\text{max}}$  (velocity in high concentrations of substrate) whereas  $K_m$  increased with the concentration of inhibitor. The inhibition constant  $K_i$  (dissociation constant of the enzyme-inhibitor complex) was calculated from a secondary plot of the slopes of curves in Figure 5 against the concentration of inhibitor giving a value of  $K_i$  of 0.25  $\mu\text{M}$  (Figure 6) (intercept on the x-axis).

On the other hand, as the inhibition of 5-IT over MAO-A is competitive and the inhibitor is reversible (see below), the equation of Cheng-Prusoff (i.e.  $\text{IC}_{50} = K_i (1 + S/K_m)$ ) could be used to determine  $K_i$  from the  $\text{IC}_{50}$  and  $K_m$  values (Cheng and Prusoff, 1973). The  $K_i$  obtained was 0.32  $\mu\text{M}$  which is in good agreement with the one calculated experimentally from the double reciprocal curves and secondary plot (Figures 5 and 6).

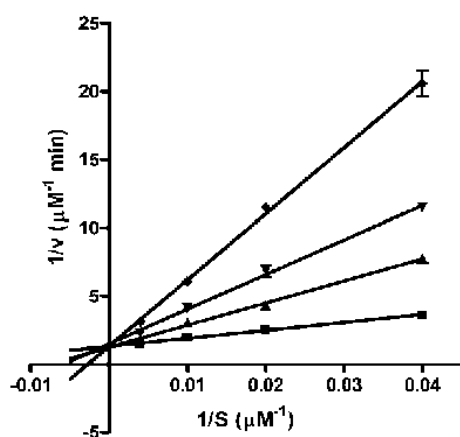


Figure 5. Lineweaver-Burk plot of the MAO-A enzymatic reaction in presence of increased concentrations of 5-IT and kynuramine used as substrate. Control without 5-IT (■); 0.5  $\mu$ M 5-IT (▲); 1  $\mu$ M 5-IT (▼); 2  $\mu$ M 5-IT (◆).

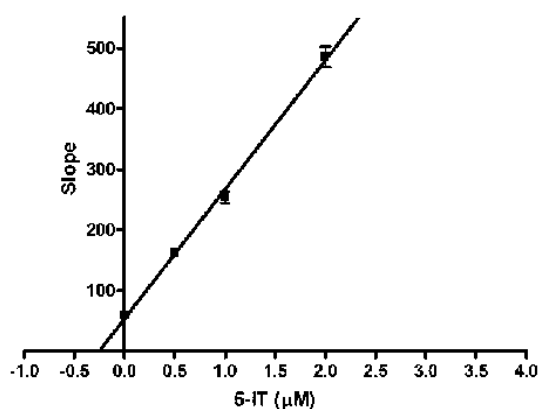


Figure 6. Secondary plot of slope of the curves of Lineweaver-Burk plot against the concentration of 5-IT as an inhibitor that was used to calculate  $K_i$ .

#### D) Type of binding (reversibility) of 5-IT with MAO-A

Following incubation of the enzyme MAO-A with 5-IT, and after washing to completely remove the 5-IT, the activity of the enzyme was fully recovered when compared with a control incubated in absence of 5-IT. Therefore, 5-IT binds to MAO-A under a reversible type of binding (Figure 7).

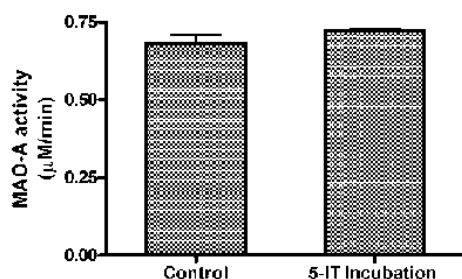


Figure 7. Human MAO-A (0.025 mg/ml, protein conc.) was incubated in duplicate with 0 or 100  $\mu$ M 5-IT at 37  $^{\circ}$ C, 30 min. The 5-IT removed through successive washing and centrifugation and then enzymatic assay was conducted.

#### E) Inhibition of MAO-A by substances used as reference and established inhibitors

Experimental data of the substance 5-IT as an inhibitor of MAO-A were compared with data obtained from other known inhibitors. For that, several substances including established inhibitors of MAO-A were studied under the experimental conditions used here. Clorgyline, a well-known irreversible inhibitor of MAO-A, demonstrated strong



inhibition of human MAO-A with an  $IC_{50}$  under the experimental conditions used of  $16 \pm 2.6$  nM (Figure 8). Similarly, the  $\beta$ -carboline harmaline, that is a reversible and potent inhibitor of MAO-A provided an  $IC_{50}$  of 20 nM (Figure 9).

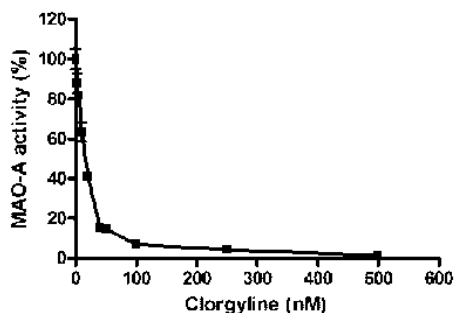


Figure 8. Inhibition of MAO-A by clorgyline.

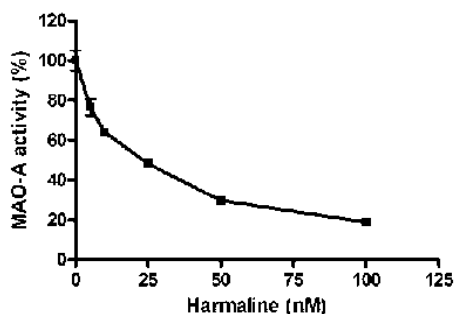


Figure 9. Inhibition of MAO-A by harmaline.

Toloxatone (Humoryl), an antidepressant launched in 1984 for the treatment of depression and which acts as a selective reversible inhibitor of MAO-A (Berlin et al., 1990) gave an  $IC_{50}$  of  $6.71 \pm 0.42$   $\mu$ M (Figure 10). Finally, moclobemide, another antidepressant that is authorised as a medicinal product in some countries and also a selective reversible MAO-A inhibitor, gave a poor inhibition of MAO-A *in vitro* with a  $IC_{50}$  value higher than 500  $\mu$ M in our assay system (Figure 11). Results obtained with moclobemide suggest that it may be a poor inhibitor of MAO-A *in vitro* although a good inhibitor of MAO-A *in vivo*, probably resulting from a metabolite of the substance (Kettler et al., 1990; Fritze et al., 1989).

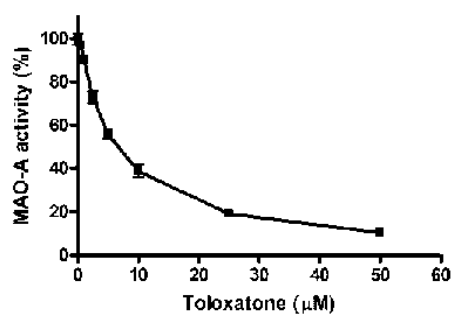


Figure 10. Inhibition of MAO-A by toloxatone.

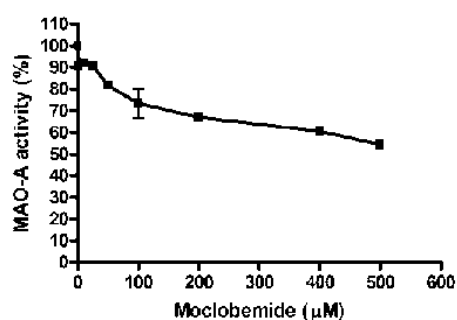


Figure 11. Inhibition of MAO-A by moclobemide.

According to the Cheng-Prusoff equation, the  $K_i$  obtained for the mentioned compounds were 0.016  $\mu\text{M}$  (clorgyline), 0.004  $\mu\text{M}$  (harmaline), and 1.34  $\mu\text{M}$  (toloxatone), respectively. Results obtained for harmaline and toloxatone were in good agreement with previous reports (Herraiz et al., 2010; Strolin-Benedetti et al., 1983).

**Table 1.** MAO-A inhibition values of compounds.

Compound	IC <sub>50</sub> ( $\mu\text{M}$ )	$K_i$ ( $\mu\text{M}$ )	$K_i$ ( $\mu\text{M}$ ) from IC <sub>50</sub>
5-(2-Aminopropyl)indole (5-IT)	1.6	0.25	0.32
Clorgyline	0.016	-	0.016
Harmaline	0.020	-	0.004
Toloxatone	6.7	-	1.3
Moclobemide	>500	-	-

## 5. Discussion

The results indicate that 5-IT is an inhibitor of human MAO-A ( $K_i$  of 0.25  $\mu\text{M}$ ) (Table 1). Nevertheless, this value suggests that its potency as a MAO-A inhibitor is at least ten times lower than that of clorgyline (i.e.  $K_i$  0.016  $\mu\text{M}$ ) that is an irreversible inhibitor of this enzyme. It is also lower than that of the  $\beta$ -carboline harmaline that is also a potent reversible inhibitor of MAO-A ( $\text{IC}_{50}$  of 1.6  $\mu\text{M}$  for 5-IT vs. 0.020  $\mu\text{M}$  for harmaline). However, 5-IT was apparently a more potent inhibitor *in vitro* than toloxatone and moclobemide. It is known that MAO-A inhibitors and antidepressants working as MAO inhibitors result in an increase of serotonin levels *in vivo*. It cannot be ruled out that recreational use of 5-IT could elevate serotonin levels by itself or in combination with other substances.

These results indicate that 5-IT is a highly selective inhibitor of MAO-A, which is a property also shared by  $\alpha$ -methyltryptamine (Tipton et al., 1982). Although the 5-IT structure contains a phenethylamine moiety it did not inhibit MAO-B. The ability of 5-IT to potentiate the hypertensive effects ('cheese effect') related to consumption of tyramine containing-foods cannot be fully excluded (Finberg and Gillman, 2011).

In a previous report, Cerletti et al., (1968) studied the inhibition of MAO by 5-IT and its positional isomers. These authors reported a value of  $\text{IC}_{50}$  of 22  $\mu\text{M}$  for 5-IT which was higher (i.e. less potent as an inhibitor) than the value reported here. Those differences might be attributed to the different assays as well as enzyme sources and fractions used. The assay of Cerletti et al., was based on the ability of guinea pig liver homogenate to uptake oxygen in an assay that used serotonin as the substrate of MAO, whereas recombinant human MAO enzymes and kynuramine deamination were used in the current study. In the same study, Cerletti et al., (1968) reported an antagonist effect of pentylenetetrazole/reserpine (an antihypertensive drug) that might also be related to MAO inhibition. Previous results of Cerletti et al., (1968) and those reported here point to 5-IT as a selective inhibitor of MAO-A in the low micromolar range. Those results suggest that 5-IT by itself or in combination with other substances could potentiate serotonergic effects. However, further pharmacological and *in vivo* studies are needed to clarify this action and its relevance to the toxicological effects of 5-IT

## 6. Conclusions

The results from this study lead to the following conclusions concerning 5-(2-aminopropyl)indole (5-IT):

- 1) 5-IT is an inhibitor of MAO-A with an  $\text{IC}_{50}$  of 1.6  $\mu\text{M}$  and  $K_i$  of 0.25  $\mu\text{M}$ .
- 2) 5-IT is a reversible inhibitor of MAO-A.
- 3) 5-IT is a competitive inhibitor of MAO-A.
- 4) 5-IT is a highly selective inhibitor of MAO-A, and does not inhibit MAO-B.
- 5) Under the experimental conditions used here, other established inhibitors of MAO-A and antidepressants provided the following  $\text{IC}_{50}$  values: clorgyline 0.016  $\mu\text{M}$ , harmaline 0.020  $\mu\text{M}$ , toloxatone 6.7  $\mu\text{M}$  and moclobemide > 500  $\mu\text{M}$ . In other words,

5-IT was less potent than clorgyline and harmaline and more potent than toloxatone and moclobemide.

In summary, 5-IT is a relatively potent, reversible and selective inhibitor of MAO-A *in vitro*. In this regard it might increase monoamine levels, particularly serotonin. However, in order to clarify the significance of these results, further pharmacological and *in vivo* studies are needed to demonstrate MAO-A inhibition *in vivo* as well as an increase of serotonin and potential monoaminergic toxicity due to the use of 5-IT-containing drugs.

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