Napropamide impurities and metabolites: a complementary approach using chemical synthesis and biosynthesis

Abstract

Napropamide is a herbicide approved in Europe for pre-emergence control of a range of grasses and weeds. As all agrochemicals regulated by the EFSA, it needs to undergo the registration process every ten years, which involves the characterization of both its impurity and metabolite profiles. The expertise of contract research organizations gains relevance when a wide range of compounds is required in a short period of time. The present white paper describes the collaborative effort of GalChimia and Hypha Discovery to provide a comprehensive family of compounds relevant for the risk assessment of napropamide. The extensive experience of GalChimia in organic synthesis is nicely complemented by that of Hypha Discovery in microbial biotransformations, enabling the preparation of a wide range of impurities and metabolites in the most efficient manner.

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GalChimia

HYPHA DISCOVERY

Highlights

- The registration of agrochemicals involves strict requirements regarding the detailed characterization of impurity and metabolite profiles. In order to provide this information, all species of interest need to be identified, synthesized, and characterized.
- GalChimia and Hypha Discovery, two CROs with complementary expertise in organic chemistry and biosynthesis, have established a collaboration to provide clients with the opportunity to source impurities and metabolites of agrochemicals using multiple techniques.
- In this example, Galchimia's expertise in organic chemistry and custom synthesis enabled the preparation of two impurities and one metabolite related to napropamide.
- In addition, Hypha Discovery delivered by microbial biotransformation two glucuronidated metabolites, which would have been too complex to prepare by traditional synthetic methods.

1. Introduction

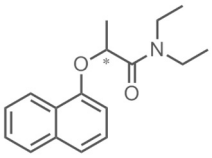
Pesticides are some of the most tested and regulated products in the world. Both industry and public agencies review them on a regular basis to ensure their safety for people, wildlife, and the environment. In most developed markets, pesticides are regulated according to risk assessments, which are repeated periodically to ensure that no new factors emerge that may alter the analysis. Such periodic risk assessments help also monitor for long-term and unforeseen effects.

In Europe, the marketing and use of pesticides is covered by Regulation EC 1107/2009, in addition to the specific data requirements collected in Regulations EU 283/2013 and 284/2013. The initial authorization of an active substance expires after 10 years, while the application for renewal needs to be submitted up to 3 years before the expiry date. The registration process involves three bodies: the Rapporteur Member State (RMS), the European Food Safety Authority (EFSA), and the European Commission (Scheme 1).

Scheme 1. The agrochemical registration process.

The safety of a registered product depends not only on the toxicological properties of the active substance, but also on the impurities formed during its synthesis and the metabolites generated upon degradation. As such, the registration of an active substance involves the detailed evaluation of the toxicity profiles of said impurities and metabolites (through the abovementioned risk assessments), which in turn requires their unambiguous identification and isolation/synthesis.

In this regulatory scenario, an agrochemical of interest is napropamide (*N*,*N*-diethyl-2-(1-naphthylenoxy)-propanamide) (Scheme 2). This herbicide was approved in the EU in 2011 and it is currently undergoing the process for re-registration (expiration extended to December 2023). Napropamide is employed for pre-emergence control of a range of annual grasses and broadleaf weeds, where it works by inhibiting the synthesis of very-long-chain fatty acids, thus blocking the progression of cell elongation and preventing seedlings from developing properly. Examples of commercial products are Devrinol (United Phosphorus Limited) and Naprop (Globachem).



Scheme 2. Napropamide structure showing its chiral center.

As shown above, the structure of this acetamide presents a chiral center, although the commercial material is a racemic mixture composed of equimolar amounts of the (*S*)- and (*R*)-isomers. Nevertheless, it has been reported that (*R*)-napropamide is eight times more toxic than the (*S*)-counterpart.[1] The more biologically active (*R*)-isomer, denoted also napropamide-M,[2] is currently undergoing the EFSA peer-review process for approval as a distinct active substance (pending status as of May 2020).

With the aim of providing solutions to the legal requirements for the re-registration of napropamide, GalChimia and Hypha Discovery decided to combine their efforts and prepare a comprehensive family of napropamide-related compounds

(impurities and metabolites). This report provides a summary of the work carried out by both organizations using two different but complementary approaches: organic synthesis and biosynthesis. The preparation of relevant impurities (Section 2) and metabolites (Section 3) is described, as well as information on their purity and the analytical techniques employed for their characterization.

2. Napropamide Impurities

The identification, quantification, and control of impurities in an active substance are important parts of agrochemical development.[3] The impurities generated during the manufacturing process of an active substance may originate from three different sources:

1) The starting materials, which may contain contaminants or isomers that are dragged through the manufacturing process.

2) The synthetic protocol, where most impurities are unreacted intermediates of synthesis or by-products formed in side-reactions.

3) Degradation during storage, that is, the transformation of the active ingredient with the time through different chemical processes (oxidations, photochemical reactions, etc.).

The identification of impurities is required when these are present at concentrations above 0.1%. This often involves the isolation and/or synthesis and full characterization of said impurities.

Considering the above, the analysis of the synthetic route for an active ingredient is crucial to identify the potential impurities that may be formed (e.g., structural isomers, disubstituted or coupled products, etc.). This is important not only to determine the origin of the impurities, but also to design improved syntheses where such undesired side-reactions are avoided.

CASE STUDY

Synthesis of napropamide impurities

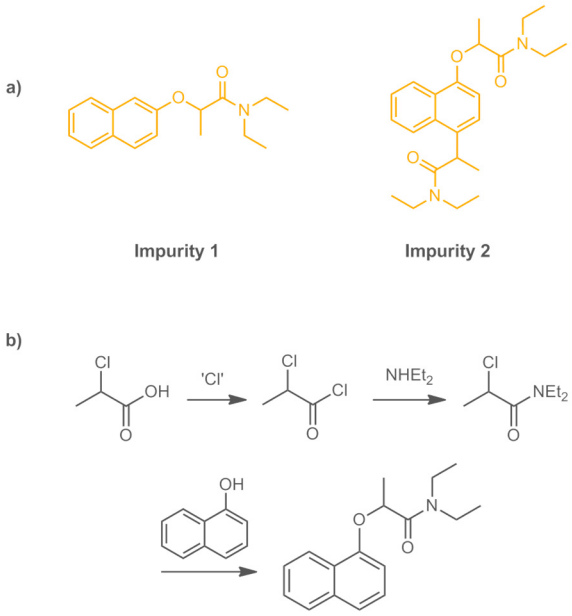
GalChimia was approached by a client for the preparation of standards of two potential impurities in their product: 2-(2-naphthoxy)-*N*,*N*-diethylpropionamide (**Impurity 1**) and 2-((4-(1-(diethylamino)-1-oxopropan-2-yl)naphthalen-1-yl)oxy)-*N*,*N*-diethyl propanamide (**Impurity 2**) (Scheme 3a).

Our chemists first analyzed the most general synthetic route for the preparation of napropamide (Scheme 3b). Starting from α-chloropropionic acid, the corresponding acid chloride is obtained and subsequently reacted with diethylamine to generate the amide. Finally, reaction with 1-naphthol affords napropamide.[4] The crucial step in this synthesis is clearly the coupling of naphthol and the propanamide, while the stoichiometry and purity of the reagents need to be carefully monitored to avoid undesired reactivities.

Considering the synthetic route shown in Scheme 3b, **Impurity 1**, which is a structural isomer of napropamide, is likely generated due to the presence of small amounts of 2-naphtol in the starting material, while **Impurity 2** would be produced through Friedel–Crafts alkylation at position 4 following activation by the alkoxy substituent in position 1.

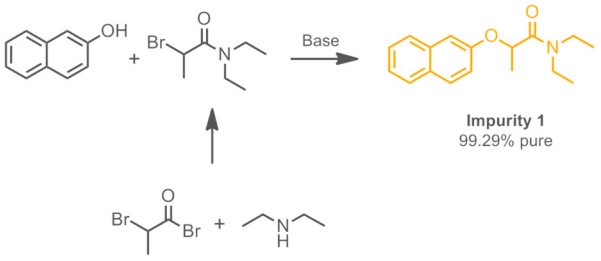
Impurity standards: The expertise of a CRO

The expertise of a contract research organization can help speed up the process for the identification of unknown impurities. Experienced chemists will be able to recognize faster the most likely candidates, for which standards can then be prepared. Alternatively, you may wish to optimize the process for the manufacture of an active substance to reduce/remove the formation of undesired impurities.
GalChimia is a reference CRO specialized in organic and custom synthesis, in addition to provide Analytical and Process Development Services. In our catalog, you can find a wide range of standards of relevant agrochemicals. If you cannot find what you are looking for, talk to us about our custom synthesis service.



Scheme 3. a) Structures of potential impurities and b) general synthesis of napropamide.

After a thorough analysis of the literature, synthetic routes for these compounds were designed. The preparation of **Impurity 1** was straightforward from 2-naphthol and the corresponding alkylating agent, which was also prepared by us (Scheme 4).



Scheme 4. GalChimia's synthetic route to Impurity 1.

In contrast, the greater complexity of the second target led to a more challenging synthetic protocol (Scheme 5). Friedel–Crafts reaction of napropamide with ethyl chlorooxalate afforded the disubstituted naphthalene quantitatively, followed by a reduction step that proceeded smoothly. A final sequence of methylation, ester hydrolysis, and condensation with Et_2NH led to the desired compound.

Both products were thus successfully prepared and fully characterized by ^{1}H-NMR spectroscopy and HPLC-MS analyses, which confirmed the desired structures with purities over 98%. Standards of these compounds can be requested through the Chemical Catalog of GalChimia (Compound IDs: GA02057 and GA02058).

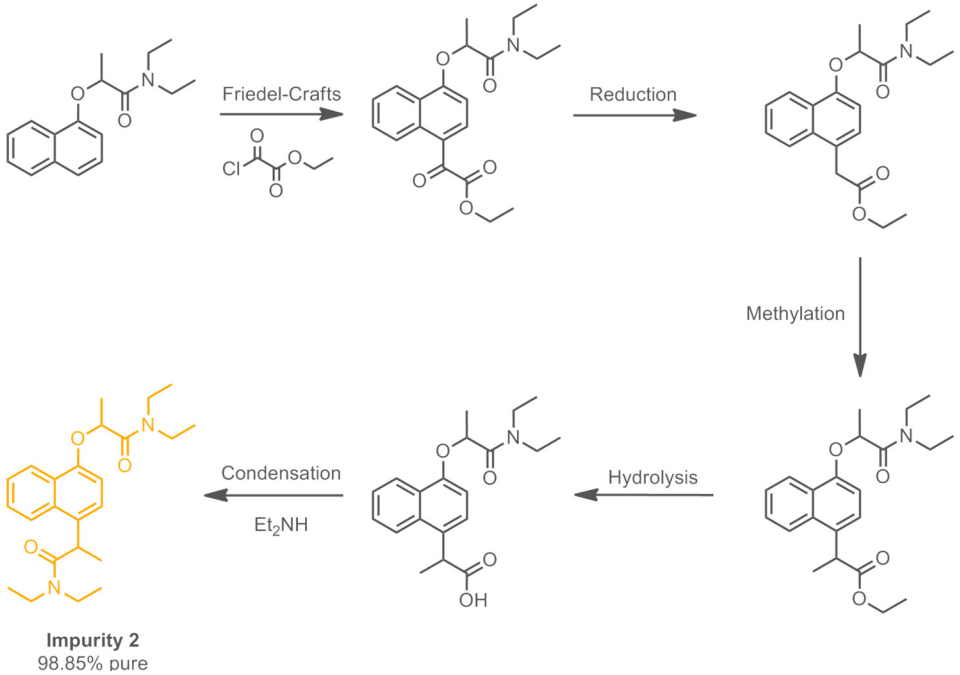
3. Napropamide Metabolites

Metabolites are degradation products generated when an active substance is metabolized by humans, animals, plants, or microorganisms. Major metabolites need to be unambiguously identified and their characteristics (toxicity, ecotoxicity, e-fate, etc.) evaluated in detail. The number of major metabolites for an active ingredient can vary from a few to over a dozen.

In plants and soil, napropamide behaves as a typical alkylamide. The loss of the alkyl groups attached to the nitrogen is followed by conversion of the amide into 2-(naphthalen-1-yloxy)propanoic acid (**NOPA**, Table 1). In general, its metabolism involves desethylation, ring hydroxylation, and/or hydrolysis, among other oxidative processes, leading to the main metabolites **NQ**, **PA**, and **1-naphthol** (Table 1). Photodegradation is also a significant pathway by which environmental loss of napropamide occurs on the soil surface (half-life of 28 days), while degradation in soil is rather slow, as mediated by microorganisms.

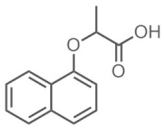
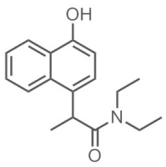
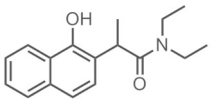
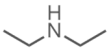
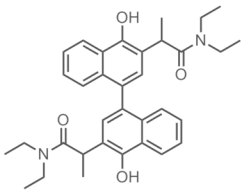
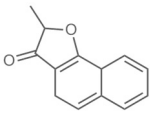
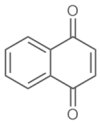
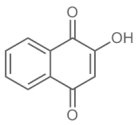
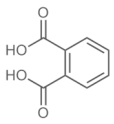
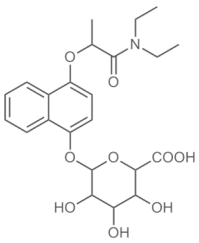
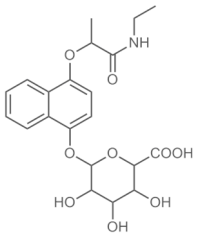
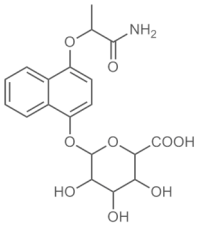
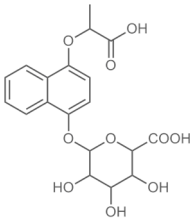
Extensive metabolism has been observed in mammals, where fifteen metabolites have been identified, the major of which are the glucuronide conjugates **4-OGlu-NPAM**, **4-OGlu-DE-NPAM**, **4-OGlu-NOPAM**, and **4-OGlu-NOPA** (Table 1). Such conjugations, where enzymatic hydroxylation is followed by glucuronidation, are common to a wide range of compounds and afford what are known as phase II metabolites.

Table 1 summarizes the metabolites reported by EFSA in 2010 as compounds of interest during the risk assessment and registration of napropamide.[5] Quality standards of these metabolites are therefore required to assess their toxicity profiles and meet EFSA requirements.



Scheme 5. GalChimia's synthetic route to Impurity 2.

Table 1 Napropamide metabolites identified by EFSA

Code (Clasical name)	Structure
NOPA (2-(Naphthalen-1-yloxy)propanoic acid)	
Hydroxy napropamide isomer 1 (*N*,*N*-Diethyl-2-(4-hydroxynaphthalen-1-yl) propanamide)	
Hydroxy napropamide isomer 2 (*N*,*N*-Diethyl-2-(1-hydroxynaphthalen-2-yl) propanamide)	
DEA (Diethylamine)	
Dimer (2,2'-(4,4'-Dihydroxy-1,1'-binaphthalene-3,3'-diyl)bis(*N*,*N*-diethylpropanamide))	
MNF (2-Methylnaphtho[1,2-b]furan-3(2H)-one)	
NQ (Naphthalene-1,4-dione)	
HNQ (2-Hydroxynaphthalene-1,4-dione)	
PA (Benzene-1,2-dicarboxylic acid)	
1-Naphtol (Naphthalen-1-ol)	
4-OGlu-NPAM (*N*-[(4-{[1-(Diethylamino)-1-oxopropan-2-yl]oxy}naphthalen-1-yl)oxy]glutamic acid)	
4-OGlu-DE-NPAM (*N*-[(4-{[1-(Ethylamino)-1-oxopropan-2-yl]oxy}naphthalen-1-yl)oxy]glutamic acid)	
4-OGlu-NOPAM (*N*-({4-[(1-Amino-1-oxopropan-2-yl)oxy]naphthalen-1-yl}oxy)glutamic acid)	
4-OGlu-NOPA (*N*-{[4-(1-carboxyethoxy) naphthalen-1-yl]oxy}glutamic acid)	

CASE STUDY

Synthesis of napropamide metabolites

With the aim of providing an added-value service to our customers and fulfill the needs of the agrochemical industry, GalChimia and Hypha Discovery decided to combine their efforts for the preparation of a series of metabolites of the herbicide napropamide.

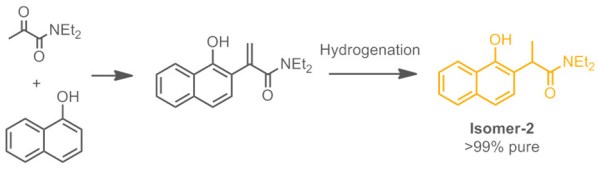
In the case of the napropamide metabolites included in Table 1, while the simplest molecules are commercially available (e.g., **1-naphthol**, **DEA**, **NQ**, or even **NOPA**), more complex metabolites such as **Hydroxy napropamide isomers 1** and **2**

and the glucuronidated derivatives require the design of customized synthetic routes. As such, we here present our experience in the synthesis of the more challenging **Hydroxy napropamide isomer 2** and the glucuronidated metabolites **4-OGlu-NPAM** and **4-OGlu-DE-NPAM**.

The first chosen metabolite was successfully prepared by organic synthesis in the laboratories of GalChimia in a rather straightforward manner. In contrast, the synthesis of glucuronidated compounds would involve selective and sequential hydroxylation and glucuronidation reactions, something particularly challenging by traditional organic synthesis techniques. In this instance, the experience of Hypha Discovery with microbial transformations provided a successful alternative. Microbial biotransformation can be an effective method to mimic mammalian, plant and soil metabolism, offering a scalable method for producing oxidized and conjugated metabolites.

i) Chemical synthesis

The synthetic route to **Hydroxy napropamide isomer 2** designed by GalChimia is outlined in Scheme 6. A different strategy was employed in this case, where *N*,*N*-diethyl-2-oxopropanamide was first prepared from 2-oxopropanoic acid and then reacted with 1-naphthol. The resulting intermediate was then reduced to afford the desired product as a white solid.



Scheme 6. GalChimia's synthetic route to Hydroxy napropamide isomer 2.

The product was fully characterized by ^{1}H-NMR spectroscopy and HPLC-MS, confirming a purity of over 99%. This metabolite is now available for purchase in the Chemical Catalog of GalChimia (Compound ID: GA02095).

Metabolite standards: Why hire a CRO?

As is usually the case with degradation products from agrochemicals, some can be prepared by chemical synthesis in a straightforward manner from commercially available raw materials or the active ingredient itself. However, some related compounds are phase II metabolites, where the initial molecules are conjugated with polar compounds. In those scenarios, biosynthesis has proven to be a more suited methodology.

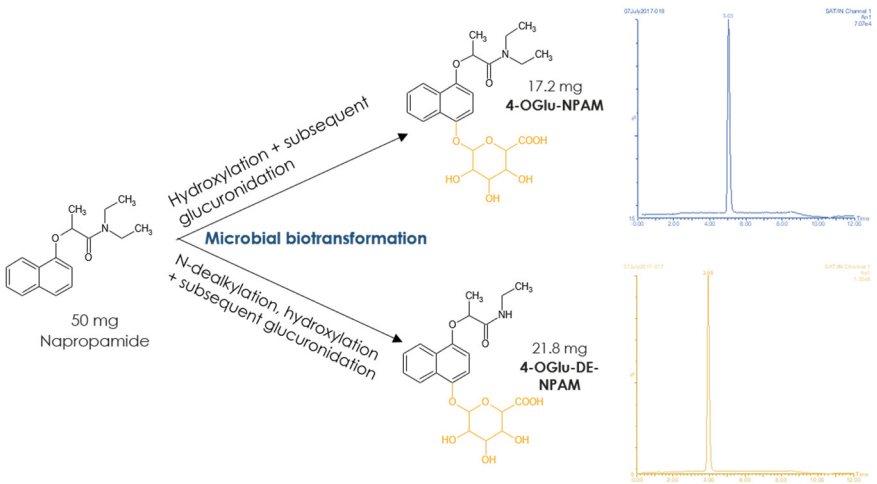
GalChimia and Hypha Discovery have established a productive collaboration to provide the best service to the agrochemical industry. GalChimia is specialized in organic and custom synthesis, while Hypha Discovery is vastly experienced in the preparation of complex metabolites by microbial biotransformation. Thus, the complementary expertises of GalChimia and Hypha Discovery are particularly well fitted for projects where a wide range of metabolites is required.

ii) Biosynthesis

Napropamide was screened against a panel of Hypha's biotransforming microbial strains with several hydroxylated and glucuronidated metabolites being detected by LC-MS.

One of the strains' products matched the LC-MS profiles of the **4-OGlu-NPAM** and **4-OGlu-DE-NPAM** metabolites. The reaction was scaled-up to 0.5 L scale, with 50 mg dosing of napropamide, yielding 17.2 mg of **4-OGlu-NPAM** and 21.8mg of **4-OGlu-DE-NPAM** (Scheme 7).

The metabolites were purified by prepHPLC to >95% purity and their structures confirmed by NMR spectroscopy.



Scheme 7. Glucuronidated metabolites obtained by Hypha Discovery through biotransformation.

4. Conclusions

EFSA's requirements for the registration of agrochemicals, whereby a range of compounds need to be identified, synthesized and characterized in a relatively short period of time, can become a great challenge for companies with limited capacity. In this sense, having access to a multidisciplinary team able to deliver on several fronts is of great appeal. It is with this aim that GalChimia and Hypha Discovery have established a productive collaboration, where the combined expertise of the two companies provides a synergistic benefit to our clients. That is the case with the present example of the herbicide napropamide, where microbial biotransformation was employed as a complement to traditional organic synthesis techniques to deliver a comprehensive family of napropamide derivatives of interest.

5. References

1. J. H. H. Chan, F. Walker, C. K. Tseng, D. R. Baker, and D. R. Arneklev, Synthesis and herbicidal activity of *N*,*N*-diethyl-2-(1-naphthyloxy)propionamide and its optical isomers. *J. Agric. Food Chem*. **1975**, *23*(5), 1008–1010.
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3. Regulation (EU) 283/2013.
4. C. K. Tseng, R. A. Simone, and F. H. Walker, Synthesis of aminobenzofurans and aminonaphtho[1,2-b]furans. *J. Org. Chem*. **1973**, *38*(9), 1746–1747
5. Conclusion on the peer review of the pesticide risk assessment of the active substance napropamide. EFSA Journal 2010, 8(4), 1565.

About GalChimia

GalChimia is a contract research organization offering specialized services in Discovery, Process, and Analytical Chemistry for the pharma, biotech, and agrochemical industries. In addition to the standards included in our catalogue, we can help you identify impurities and prepare metabolites on demand.

For more information, please visit our catalog
www.galchimia.com/chemical-catalog/
or contact us at hello@galchimia.com.

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About Hypha Discovery

Hypha Discovery is a UK-based CRO supporting pharmaceutical and agrochemical companies worldwide through the production of metabolites and new derivatives of drugs and agrochemicals in discovery and development. Hypha is an expert in the scalable synthesis and purification of drug metabolites and oxidised derivatives of lead compounds, and possesses a wealth of experience in the production, purification and structure elucidation of natural products.

For more information, contact us at
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