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Metabolomics to Explore Imidacloprid-Induced Toxicity in the Central Nervous System of the Freshwater Snail *Lymnaea stagnalis*

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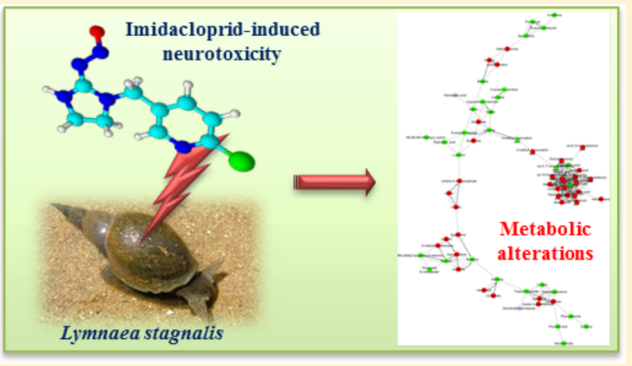
Supporting Information

ABSTRACT: Modern toxicology is seeking new testing methods to better understand toxicological effects. One of the most concerning chemicals is the neonicotinoid pesticide imidacloprid. Although imidacloprid is designed to target insects, recent studies have shown adverse effects on nontarget species. Metabolomics was applied to investigate imidacloprid-induced sublethal toxicity in the central nervous system of the freshwater snail *Lymnaea stagnalis*. The snails (n = 10 snails) were exposed for 10 days to increasing imidacloprid concentrations (0.1, 1, 10, and 100 μg/L). The comparison between control and exposure groups highlighted the involvement and perturbation of many biological pathways. The levels of several metabolites belonging to different metabolite classes were significantly changed by imidacloprid exposure. A change in the amino acids and nucleotide metabolites like tryptophan, proline, phenylalanine, uridine, and guanosine was found. Many fatty acids were down-regulated, and the levels of the polyamines, spermidine and putrescine, were found to be increased which is an indication of neuron cell injury. A turnover increase between choline and acetylcholine led us to hypothesize an increase in cholinergic gene expression to overcome imidacloprid binding to the nicotinic acetylcholine receptors. Metabolomics revealed imidacloprid induced metabolic changes at low and environmentally relevant concentration in a nontarget species and generated a novel mechanistic hypothesis.



INTRODUCTION

Over the past decades, the presence of manufactured chemicals in the environment has raised concerns because of their potentially lethal and sublethal effects on organisms, resulting in ecosystem functionality damages.[1] The environmental risk assessment guidelines (e.g., Water Framework Directive, 2000) are currently based on phenotypical end point effects, measured by acute and chronic lethal concentrations and with experiments focusing on effects such as mobility, ventilation, and reproduction (www.OECD.org). These types of toxicity testing are incapable of mimicking a realistic environmental exposure scenario and are failing to predict effects in anything other than the target species.[2]

To test the possible sublethal toxic effects of environmental pollutants in the past decades, several biochemical biomarkers have been developed.[3] Among these, enzymatic assays indicate toxic effects because the activity of these biomarker enzymes has been linked to oxidative stress.[4] One of these enzymatic tools is the Ellman's cholinesterase assay, which provides a simple colorimetric determination of acetylcholinesterase (AChE) activity. One commom method used to investigate toxic effects induced by pesticides is to apply the AChE bioassay; this is especially effective in determining the toxicity of organophosphate and carbamate pesticides because these compounds directly block AChE.[5]

Nowadays, after the introduction on the market of the neonicotinoid pesticide imidacloprid in 1991 by Bayer CropScience, the global insecticides market is dominated by this new class of pesticides.[6,7] Due to its extensive application and combined with the high persistency and leaching potential,[8,9] imidacloprid concentrations in water bodies have been found to exceed the regulatory norms in several countries.[10−13] Recently, this compound gained attention due to its significant ecotoxicological effects.[14] The pesticide is considered to be insect-specific, as it acts mainly as an agonist of the nicotinic acetylcholine receptors (nAChRs) on the postsynaptic membrane of neuronal cells of insects.[15,16] However, recent reports indicate a decline in nontarget species in surface waters contaminated with imidacloprid, demonstrating serious cascading effects of imidacloprid on aquatic and terrestrial ecosystem functionality.[10,17,18]

Due to the different mode of action of imidacloprid, an alternative strategy to the AChE bioassay should be found to warrant the investigation of imidacloprid-induced toxicity in nontarget species. To this extent, promising alternatives to

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traditional toxicity testing are found in the "omics" field.[19] Transcriptomics, proteomics, and metabolomics can measure changes in intracellular functioning upon exposure to toxicants at lower concentrations than traditional toxicity testing methods and can focus on numerous end points (genes, proteins, and metabolites) simultaneously.[20] The combination of these "omics" techniques in a system biology approach will enable a more accurate determination of the mechanism of action of toxicants, which may improve environmental risk assessment.[21,22] Compared to the other omics, metabolomics has the advantage that metabolites are more conserved across species and therefore this approach is more suited to determine conserved end points.[23] Metabolomics is adding to the base of knowledge on the ecotoxicological effects of compounds that are of immediate concern to environmental health.[22−24]

To further improve the base of evidence of imidacloprid toxicity, the effect of imidacloprid on nontarget species should be more thoroughly investigated. A promising species to study imidacloprid-induced sublethal effects is the freshwater snail *Lymnaea stagnalis*. This species is a globally distributed inhabitant of freshwater ecosystems and a model organism in environmental toxicology and neurobiology.[25,26] *L. stagnalis* has been applied as a model organism in the omics field, as shown by the increasing number of recent publications.[25,27−30]

In this study, a 10-day exposure to imidacloprid at environmentally relevant concentrations (0.1 and 1.0 μg/L) and higher concentrations (10 and 100 μg/L) was carried with *L. stagnalis*. Each exposure group comprised 10 snails, and along with the exposure experiment, a control group with 10 snails not exposed to imidacloprid was used. Effects on reproduction and on the activity of acetylcholine esterase (AChE) of the snails' central nervous systems (CNSs) were examined. To assess the toxicity of imidacloprid at the molecular level, multiple metabolomics approaches were applied and compared to traditional toxicity assessment methods. A metabolomics-targeted approach based on hydrophilic interaction liquid chromatography (HILIC) coupled to tandem mass spectrometry (MS) was performed to profile neurotransmitters in the CNSs of the exposed *L. stagnalis*. A nontargeted metabolomics strategy based on liquid chromatography (LC) and gas chromatography (GC) coupled to high-accuracy MS was used to investigate changes in hydrophilic and hydrophobic metabolites after imidacloprid exposure. Multivariate data analysis (MVDA) and multiple *t* test with false discovery rate (FDR) correction was employed to determine the metabolites contributing to the differences between the control group and exposed groups and potential biomarkers of exposure were identified. Biochemical networks were created to provide mechanistic insights into the metabolic pathways associated with imidacloprid toxicity.

MATERIALS AND METHODS

Reagents and Materials. Milli-Q water was obtained from a Millipore purification system (Waters-Millipore Corporation, Milford, MA). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were from JT Baker Chemical (Phillipsburg, NJ). MS-grade formic acid (98% purity) and sodium formate salt (purity ≥99%) were obtained from Fluka (Steinheim, Germany). Chloroform, hexane, and isooctane were obtained from Sigma-Aldrich (Schnelldorf, Germany).

Hydrophilic standards (amino acids, sugars, organic acids, neurotransmitters, and nucleotides) and the hydrophobic standard mixture, consisting of 37 fatty acid methyl esters, were purchased from Sigma-Aldrich. The hydrophilic metabolites were mixed in ACN/H_2O 90:10 v/v at a concentration of 1 mg/L, and the fatty acid methyl esters mixture was diluted to 1 mg/L in isooctane. These standards were used as quality control (QC) for the LC-MS and GC-MS analysis, respectively. The MS metabolite library of standards (MSMLS) was obtained from IROA Technologies (Ann Arbor, Michigan), and the mixtures of metabolites were prepared as described by the manufacturer. A list of all the analytical standards used can be found in Table S1 (Supporting Information). The stable isotope-labeled internal standards of 3-MT-d_4, acetylcholine-d_4, serotonin-d_4, 5-HIAA-d_5, L-tryptophan-d_3, and GABA-d_6 were from CND Isotopes (Quebec, Canada). DOPA-d_3, dopamine-d_4, L-tyrosine-d_4, epinephrine-$^{13}C_2$ ^{15}N, choline-d^{13} and glutamate-d_5 were obtained from Cambridge Isotope Laboratories (Andover, MA). Glutamine-^{13}C ^{15}N, norepinephrine-d_6 and 5-hydroxy-L-tryptophan-d_4 were bought from Toronto Research Chemicals (Toronto, Ontario, Canada). Imidacloprid analytical standard (99.9%) and imidacloprid-d_4 were purchased from Sigma-Aldrich.

***L. stagnalis* Selection and Exposure.** *L. stagnalis* snails used in our study were 16 weeks old, with an average shell length of 26.14 ± 0.69 mm, and from a synchronized population cultured at the VU University Amsterdam, The Netherlands. In the breeding facility, the snails were kept in a circulation system of copper-free freshwater (average water characteristics: hardness 1.48 mmol/L, pH 8.12, total organic carbon 1.9 mg/L) at 20 ± 1 °C in a 12 h light/12 h dark cycle and fed on lettuce leaves ad libitum.

The snails were individually exposed for 10 days to different concentrations of imidacloprid (control, 0.1, 1.0, 10, and 100 μg/L). The control and exposure groups were composed of 10 snails each. Two days prior to the exposure, the snails were acclimatized in glass beakers with copper-free water. Afterward, the glass beakers were filled with 150 mL of copper-free water spiked with different concentrations of imidacloprid, previously dissolved in copper-free water. The beakers were placed in a climate room at 20 °C in an 8:16-h light−dark cycle. A suspension of 250 μL TetraPhyll fish feed in copper-free water (133 g/L) was added daily.

Effects on reproduction were assessed by measuring the number of laid eggs and the dry weight of the egg clutches. Every other day, egg clutches were collected, and the eggs were counted using the cell counter plug-in of the image analysis software, ImageJ. The egg clutches were dried at 50 °C for 12 h and weighed (Supporting Information).

Sample Preparation. After the exposure experiment, the snails were sacrificed by snap freezing in liquid nitrogen. The CNSs were dissected, and the sample preparation was carried out following a two-step extraction with the Precellys24 Dual device (Bertin Technologies, France) operating at 6500 rpm for 2 cycles of 10 s with a 15 s break between cycles. The first extraction step was performed with Milli-Q water. From the aqueous homogenate, 10 and 15 μL were withdrawn from the homogenate to perform the Bradford and Ellman assays, respectively. Chloroform and a mixture of neurotransmitters stable isotope-labeled internal standards in MeOH was added to the homogenate and in order to reach the final solvent composition of 1:1:1 v/v/v H_2O/MeOH/$CHCl_3$ in the final volume of 500 μL. The homogenates were kept in ice for 10 min to allow the metabolite partitioning in the biphasic mixture. The samples were centrifuged in a precooled centrifuge (Heraeus Biofuge Stratos, Heraeus Instruments, Germany) at

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4 °C for 10 min at 17 000 rpm, and the hydrophilic fractions were dried in a Centrivap Concentrator (Labconco Co., Kansas City, MO) for 240 min at 20 °C. The residues were reconstituted in 100 μL of mobile phase, vortexed, and centrifuged again. The clear solutions were transferred to autosampler vials for analysis. The chloroform fractions containing the lipophilic metabolites were dried under a gentle flow of N_2 and then derivatized with 500 μL of BF_3 methanolic solution kept for 30 min at the temperature of 80 °C. After cooling, a liquid−liquid extraction was performed three times with 500 μL of hexane. The hexane fractions were reconstituted in an autosampler vial and evaporated until dryness with nitrogen, and finally, 200 μL of isooctane was added.

Acetylcholinesterase Activity Assay. The experimental setup of the AChE bioassay is described in the Supporting Information.

Imidaclopid Exposure Concentrations. The exposure media solutions were refreshed every second day. Samples of the exposure solutions were collected and analyzed with LC triple quadrupole (QqQ) mass spectrometer (QqQ) to determine actual exposure concentrations. The internal standard was added to the water samples in a final concentration of 5 ng/mL. Analyses were carried out with a Agilent (Palo Alto, CA) 1260 infinity binary liquid chromatography system coupled to an Agilent (Palo Alto, CA) QqQ 6410 series. A pentafluorophenyl column (100 × 2.1 mm 3.5 μm particle size) from Phenomenex was used at a flow rate of 0.2 mL/min. The mobile phase composition was H_2O 0,1% formic acid and MeOH and the elution was achieved with a gradient from 20 to 90% of MeOH in 6 min. The electrospray source (ESI) was operated in positive mode and the following parameters were set: gas temperature, 350 °C; gas flow, 6 L/min; nebulizer pressure, 40 PSI; and ESI capillary voltage, 3000 V. The MS data acquisition was carried out in multiple reaction monitoring (MRM) mode. The calibration line ranged from 0.05 μg/L (LOQ) to 1000 μg/L and was linear with a correlation coefficient (R^2) of >0.98. The MRM transitions monitored for imidacloprid were 256.1 → 175.1 (identification) and 256.1 → 209.1 (quantification) and for imidacloprid-d_4 were 260.1 → 213.1 (identification) and 260.1 → 179.2 (quantification). The fragmentor was set to 90 V, the collision energy was set to 30 eV, and the dwell time was set at 50 ms for all transitions. Data acquisition and analysis using the QqQ was performed with a MassHunter Workstation by Agilent. The actual concentrations of the 0.1 and 1 μg/L) groups were in the range of the nominal concentrations (Supporting Information, Figure S3). The actual concentrations of the 10 and 100 μg/L groups were about 3 times lower than the nominal concentrations. The stability of imidacloprid concentrations in the exposure media were assessed before carrying out the exposure experiment (Supporting Information).

Metabolomics. A targeted metabolomics analysis of neurotransmitters, precursors and metabolites was performed with MS/MS based on the method by Tufi et al.[31] A cross-platform nontargeted metabolomics approach, based on HILIC high resolution Time of Flight (ToF) and GC-HRToF coupled to atmospheric pressure chemical ionization (APCI), was used to profile the hydrophilic metabolites and the chloroform fraction respectively according to Tufi et al.[28]

Data Analysis. The data were normalized for the CNS protein content and outliers were removed using the Dixon's Q test. The analysis of variance (ANOVA) with post hoc Tukey's honestly significant difference (HSD) was performed with the software SPSS (IBM).

The high-resolution (HR) time-of-flight (ToF) chromatograms were analyzed with Compass DataAnalysis software (Bruker Daltonik, Bremen, Germany) where a mass accuracy below 5 ppm was assured by calibrating chromatograms using sodium nitrate calibration curves. The chromatograms were analyzed with the software DataAnalysis 4.0 (Bruker Daltonik, Bremen, Germany). The first data treatment step consisted of a internal calibration of the spectra using the enhanced quadratic mode. The peak intensities of the detected metabolites in all HILIC-ToF and GC-ToF data were normalized for the CNS protein content.

On the basis of the MSMLS, we created a target list, was performed a batch targeted analysis with the software PathwayScreener (Bruker Daltonik, Bremen, Germany). The acquired LC and GC HR-MS chromatograms were then automatically screened for the accurate masses of metabolites in the target list. The results were exported to ProfileAnalysis 2.1 (Bruker Daltonik) that performs exclusion of outliers based on the interquartile ranges and multiple comparison *t*-test with *p*-value adjustment based on false discovery rate (FDR) for the comparison between control and exposed groups. Fold changes were calculated by dividing the average of metabolites in exposed groups by the average of metabolites in the control group. Pathway over-representation analysis based on the web tool IMPaLA was performed to investigate which pathways were involved in imidacloprid exposure.[32] Biochemical network maps were generated using Metamapp[33] and visualized in the open source software platform Cytoscape.[34]

RESULTS AND DISCUSSION

Neuronal Metabolism Disruption. On the basis of imidacloprid mode of action, we carried out a biologically driven targeted metabolomics analysis. Because imidacloprid binds to the ACh receptor on the postsynaptic membrane of neuronal cells, the neuronal metabolism was investigated by quantifying the levels of the main neurotransmitters, their metabolites and precursors. Of the 12 quantified neurotransmitters, metabolites and precursors, the levels of 8 metabolites were significantly (p-value < 0.05, t test) changed by imidacloprid (Figure S4). These metabolites are choline, acetylcholine, glutamate, glutamine, serotonin, tryptophan, phenylalanine and histidine. Four of these metabolites were significantly different at more than two exposure concentrations: choline, acetylcholine, phenylalanine, and histidine.

Most of the changes appear indeed to be implicated with the cholinergic system in accordance with the mode of action of imidacloprid. Acetylcholine decreases and the observed increase of the choline/acetylcholine ratio (Figure 1) might indicate a possible increase in the cholinergic gene expression.

This mechanism might take place in the synaptic cleft to enhance the clearance of acetylcholine, which accumulates because of the binding of imidacloprid to the nACh receptors. This compensatory strategy would then lead to a feedback increase of acetylcholine esterase (AChE) that has been previously observed in association with acute stress and AChE inhibitors.[35] The CNS extracts of the exposed snails were tested in the AChE activity assay. A slight increase in the AChE activity related to increased exposure concentrations was observed (Figure S5). The group exposed to the highest imidacloprid concentration showed an average AChE activity of 156.2 ± 33.6% compared to the control group (unpaired t test,

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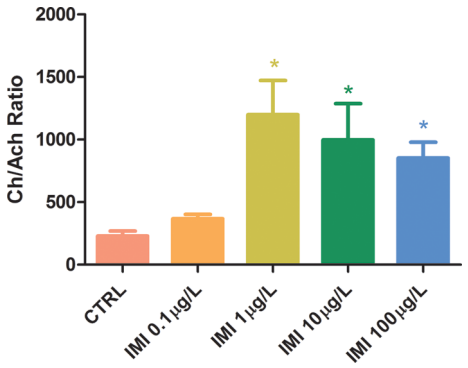

Figure 1. Choline (Ch)/ acetylcholine (ACh) ratio in *L. stagnalis* CNS exposed to increasing concentration of imidacloprid (IMI). Statistical significance (n = 10; error bars = SD; * = p-value <0.05, t test).

confidence interval 90%). Despite the large number of biological replicates, the coefficients of variation (CV%) in the exposed groups were above 30%. The incubation period of 10 days may have been too short to show any significant effects on AChE activity. However, a significant reduction of AChE activity after only 7 days of exposure at 25 μg/snail was previously reported in the land snail *Helix aspera*.[36] A significant inhibition of AChE activity was observed at 0.1 and 1.0 mg/L in exposed mussels gills.[37] In blood and brain of rats exposed to imidacloprid an enzyme inhibition was shown as well.[38,39] Phenotypical end points like locomotion and ventilation of *Chironomus riparius* Meigen larvae showed to be more sensitive to imidacloprid exposure than AChE activity.[40] On the contrary, an increase in AChE activity was observed in caged bees in response to imidacloprid exposure.[41] Even though AChE has been suggested as potential biomarker of imidacloprid exposure[38,41] the effect of imidacloprid on AChE activity appears to be species-specific or not significant. On the basis of these results, AChE cannot be used to assess imidacloprid-induced sublethal effects and is not suitable as biomarker for imidacloprid exposure.

Metabolome Perturbation. To further explore what metabolite levels were altered due to exposure of imidacloprid, the HILIC and GC chromatograms were screened for a larger number of metabolites. This approach allowed increasing the number of detected metabolites, and in combination with statistics, it enhanced the chances to discover exposure biomarkers. Many metabolites in our standard library (Table S1) were accurately identified on the basis of three quality parameters (mass accuracy, retention time, and isotopic pattern) and were found to be statistically significant (p-value < 0.05, t test FDR). For the exposure at environmental concentrations of 0.1 μg/L and 1 μg/L we have identified 22 and 25 significant metabolites, respectively. At 10 and 100 μg/L, 27 and 30 identified metabolites, respectively were significantly different (Table 1). The fold changes and p-values of all the identified metabolites in the different exposure groups are given in Table S2.

The fold changes for metabolites which were significantly changed in at least two exposure concentrations are shown in Figure 2. Decreased levels were found for tryptophan, linolelaidic acid, linoleic acid, cis-10-heptadecenoic acid, 3-methoxy-4-hydroxymandalate, uridine, stearic acid, oleic acid, inosine, heptadecanoic acid, guanosine, γ-linolenic acid, elaidic acid, acetylcholine, and 3-methyl-2-oxovaleric acid. An increase in the level of spermidine, proline, leucine, histidine, betaine, 5-methylthioadenosine, putrescine, 4-methyl-2-oxovaleric acid, valine, creatinine, 4-guanidino-butanoate, phenylalanine, choline, and carnitine was observed.

Table 1. Number of Metabolites Identified in the Different Exposure Groups, Number of Significantly Different Metabolites based on p-Values Corrected by False Discovery Rate, and Percentage of Significantly Different Metabolites of the Identified Metabolites

exposure group	no. of identified metabolites	no. of significant metabolites	significant metabolites of identified metabolites (%)
control vs 0.1 μg/L	71	22	31
control vs 1.0 μg/L	56	25	45
control vs 10 μg/L	61	27	44
control vs 100 μg/L	68	30	44

The significant biomarkers can be used as biomarkers of exposure. Several biomarkers for imidacloprid exposure have been determined, and their accuracy has been assessed by the receiver-operating characteristic (ROC) curve analysis. Of the 29 metabolites that showed a statistically significant change at least at two exposure concentrations (shown in Figure 2), 12 showed an area under the curve (AUC) above 0.8 (Table S3 and Figure S8). These metabolites are carnitine, elaidic acid, γ-linolenic acid, linolelaidic acid, stearic acid, 3-methyl-2-oxovaleric acid, acetylcholine, creatinine, guanosine, inosine, phenylalanine and tryptophan. Among these metabolites, acetylcholine is directly related to mode of action of imidacloprid. The profile of these metabolites can be used as a biomarker of imidacloprid exposure.

Biochemical networks were built to provide information on the mechanism of toxicity and the metabolic pathways affected. The networks were based on p-values and fold changes between the control group and exposed groups (Figure 3). Using biochemical network maps, the biological interpretation is facilitated since it allows the visualization of consistent changes among the exposure concentrations. Pathway over-represented analysis was performed with the web-tool IMPaLA on the list of significantly changed metabolites. This tool analyzes whether these metabolites are significantly associated with a particular pathway or set of pathways. The metabolic pathways in which significantly changed metabolites are involved are reported in Table 2. In this table ,the pathway name, the database source, the pathway size, the number of metabolites overlapping to the metabolic pathway, and the percentage of the pathway coverage are provided. In addition, the p-value and q-value corrected by FDR for each pathway are reported.

With an increase in the exposure concentration, a down-regulation was found for the fatty acids biosynthesis and the cholinergic system, whereas an increase was observed for many amino acids for which several amino acid biosynthesis pathways were involved.

The decrease in the levels of many fatty acids indicates a down-regulation of fatty acid biosynthesis and up-regulation of fatty acids degradation through the mechanism of β-oxidation. In this metabolic breakdown of long-chain fatty acids, carnitine and acylcarnitines play the key role of carriers that assist the transportation across the inner mitochondrial membrane. The acetyl-CoA generated in the β-oxidation enters the TCA cycle, where it is further oxidized to CO_2, producing more reduced energy carriers, NADH and $FADH_2$. Another destination of acetyl-CoA is the production of ketone bodies by the liver that

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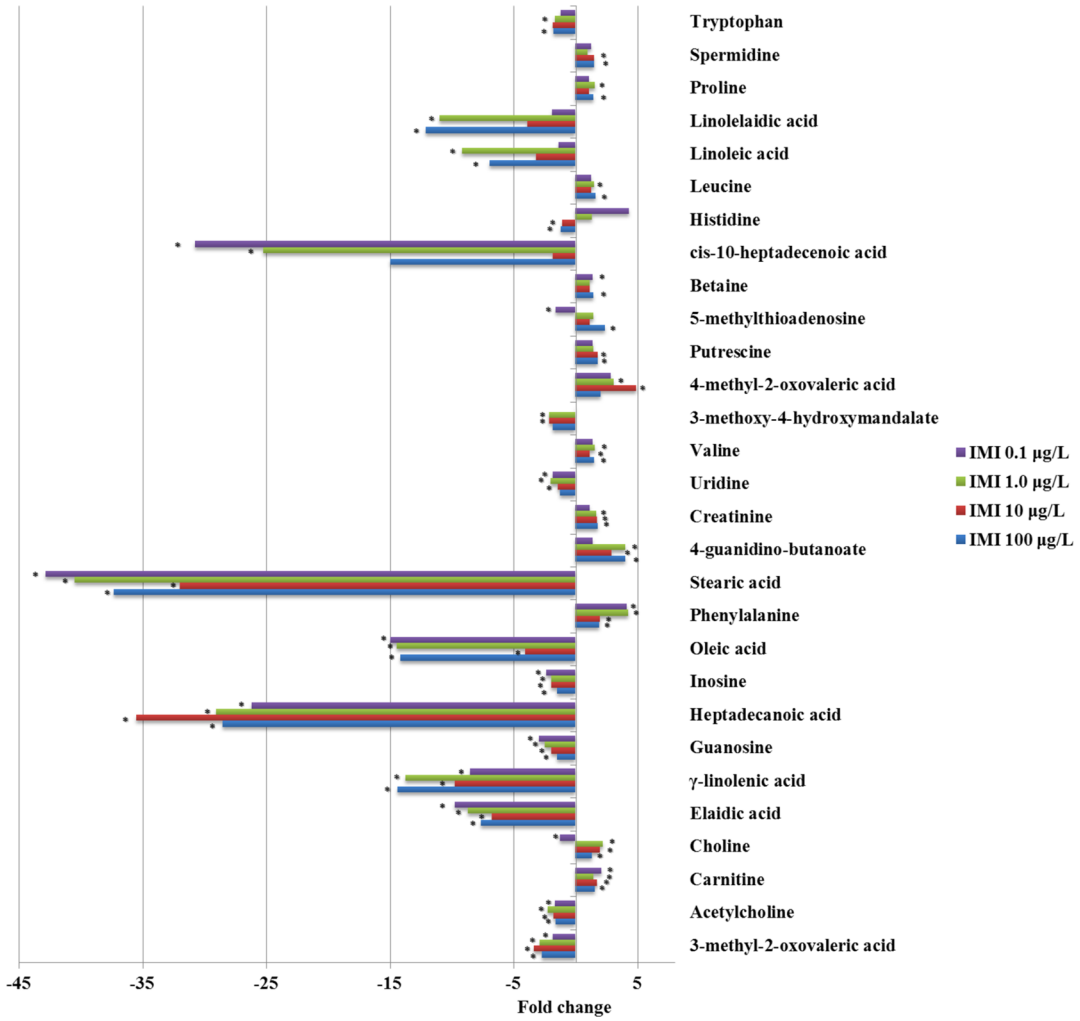

Figure 2. Fold changes of the significantly different metabolites (* p-value <0.05, t test FDR) for two or more imidacloprid (IMI) exposure concentrations.

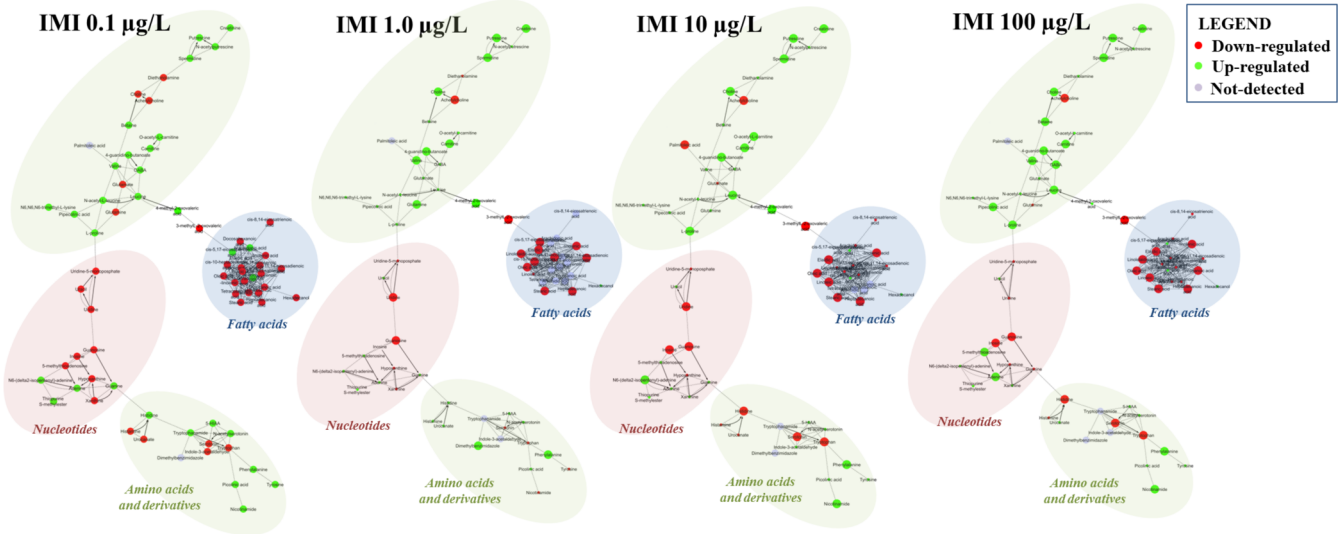

Figure 3. Biochemical network mapping for the comparison between control and exposed groups to increasing concentrations of imidacloprid (IMI). In the networks, the size of the nodes (metabolites) depends on the p-values. Statistically significant metabolites (p-value <0.05, t test FDR) are shown by bigger nodes, whereas not significant metabolites are represented by smaller nodes. The color of the node represents the fold change: (red) down-regulated, (green) up-regulated, and (gray) not detected metabolites. The clusters of the fatty acids metabolite class is shown in blue, the nucleotides are clustered within the red circle and in green the cluster comprising amino acids and derivatives is shown.

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Table 2. Pathway Over-Represented Analysis of the Significantly Changed Metabolites at More than Two Concentration Exposure of Imidacloprid and P and Q Values (FDR) for the Pathways

pathway name	pathway source	no. of overlapping metabolites	no. of all pathway metabolites	pathway coverage (%)	P value	Q value (FDR)
metabolism of amino acids and derivatives	Reactome	12	181	6.6	6.70×10^{-11}	5.90×10^{-08}
metabolic disorders of biological oxidation enzymes	Reactome	11	305	3.6	3.40×10^{-07}	7.20×10^{-05}
biological oxidations	Reactome	7	220	3.2	1.90×10^{-04}	6.80×10^{-03}
immune system	Reactome	6	87	6.9	7.70×10^{-06}	3.70×10^{-04}
urea cycle and metabolism of arginine, proline, glutamate, aspartate and asparagine	EHMN	6	125	4.8	6.20×10^{-05}	2.50×10^{-03}
Adaptive Immune System	Reactome	5	48	10.4	6.50×10^{-06}	3.30×10^{-04}
antigen processing-cross presentation	Reactome	5	29	17.2	4.80×10^{-07}	9.10×10^{-05}
arginine and proline metabolism	KEGG	5	91	5.5	1.50×10^{-04}	5.50×10^{-03}
glutathione conjugation	Reactome	5	38	13.2	2.00×10^{-06}	2.20×10^{-04}
glutathione synthesis and recycling	Reactome	5	30	16.7	5.80×10^{-07}	1.00×10^{-04}
leukotriene biosynthesis	HumanCyc	5	29	17.2	4.80×10^{-07}	9.10×10^{-05}
methionine metabolism	SMPDB	5	41	12.2	2.90×10^{-06}	2.20×10^{-04}
biosynthesis of unsaturated fatty acids	KEGG	4	54	7.4	2.40×10^{-04}	8.30×10^{-03}
valine, leucine, and isoleucine biosynthesis	KEGG	4	23	17.4	7.50×10^{-06}	3.70×10^{-04}
valine, leucine, and isoleucine degradation	KEGG	4	40	10	7.20×10^{-05}	2.80×10^{-03}
β-alanine metabolism	KEGG	3	31	9.7	7.20×10^{-04}	2.10×10^{-02}
biogenic amine synthesis	Wikipathways	3	17	17.6	1.10×10^{-04}	4.30×10^{-03}
branched-chain amino acid catabolism	Reactome	3	36	8.3	1.10×10^{-03}	3.20×10^{-02}
metabolism of polyamines	Reactome	3	30	10	6.50×10^{-04}	2.00×10^{-02}
nucleotide metabolism	Wikipathways	3	17	17.6	1.10×10^{-04}	4.30×10^{-03}
spermidine and spermine biosynthesis	SMPDB	3	17	17.6	1.10×10^{-04}	4.30×10^{-03}

are transported to tissues such as heart and brain tissue for energy. The observed increase in carnitine and acetyl-carnitine associated with the decrease in fatty acid levels suggest a possible alteration in mitochondrial metabolism, energy production, and acute oxidative stress.[42]

Pathway analysis revealed the involvement of metabolic pathways associated with biological oxidation, immune system and inflammation process. Glutathione metabolism is usually correlated to oxidative stress[43] and leukotriene biosynthesis is related to the occurrence of an inflammatory reaction in tissue injuries caused by xenobiotic.[44] A significant difference was found in the biogenic amine synthesis in which the main neurotransmitters are synthesized starting from their amino acid precursors, confirming the involvement of the neuronal metabolism.

An alteration in many amino acids was observed, such as arginine and proline metabolism, methionine metabolism, and β-alanine metabolism. Branched chained amino acids like valine, leucine, and isoleucine metabolism were also affected by imidacloprid exposure. Pathways of valine, leucine and isoleucine biosynthesis and degradation and branched-chain amino acid catabolism appear to be involved. The levels of the 3-methyl-2-oxovaleric acid, isoleucine alpha-keto acid and precursor were decreased whereas the levels of valine and leucine were enhanced, suggesting an increasing turnover of these amino acids biosynthesis. The levels of other amino acids like phenylalanine and proline were found to be increased whereas a decrease in tryptophan was found.

Nucleotides were involved as well, showing a decrease in the levels of inosine, uridine and guanosine.

Polyamine levels were also significantly changed and, as a consequence, the polyamine metabolism, spermidine levels, and spermine biosynthesis were dysregulated by imidacloprid exposure. Putrescine and spermidine had increased, showing an up-regulation in polyamine metabolism. The enzymatic reaction of spermidine synthetase catalyzes the production of spermidine from putrescine that is involved in the amino acid pathways such as arginine and proline metabolism, β-alanine metabolism, cysteine and methionine metabolism, and glutathione metabolism. Increases in putrescine levels have been related to cell injuries in the CNS associated with pathological conditions and neurotoxin exposure.[45,46]

To our knowledge, this is the first time a metabolomics study has been applied to investigate the metabolic alterations in the CNSs of the freshwater snail *L. stagnalis*. With this approach, the molecular mechanism of imidacloprid toxicity in a nontarget species was investigated. This quantitative and biologically driven approach was effective to single out metabolites whose levels were affected by the exposure of the snails to different levels of imidacloprid, showing the importance of the followed strategy. Because metabolomics focuses simultaneously on multiple endpoints our study found indications that, besides the binding of imidaclorpid to the AChE, this neonicotinoid can probably cause inflammation and neuron cell injury. This should be further investigated. Metabolomics was more sensitive than tradition toxicity testing because it enabled to determine metabolic alterations at low and environmentally relevant concentrations. The combination of metabolomics with statistical and visualization tools, such as biochemical networks and pathway analysis, facilitated the biological interpretation of the results and a better understanding of the undergoing toxicity mechanism. However, the proposed hypothesis of an increase in the cholinergic gene expression should be further studied by applying gene expression techniques and future research should investigate the validity of the exposure biomarkers also in other species.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b03282.

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Additional information as noted in the text. (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting Information

Metabolomics to explore imidacloprid induced toxicity in the central nervous system of the freshwater snail *Lymnaea stagnalis*

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Material and Methods

Protein content measurement. The protein content was determined by the Bradford colorimetric protein assay. For the calibration, bovine serum albumin (BSA) was used (Sigma-Aldrich). The Dye Reagent was purchased from Bio-Rad Laboratories (Richmond, CA, USA). The absorbance was measured with the SPECTRAmax 340PC 96 well-plate reader spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

Acetylcholinesterase activity assay. A potassium-phosphate buffer (0.1M KH_2PO_4/K_2HPO_4) (Sigma-Aldrich), 5mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (99%, Sigma-Aldrich), and 0.8 mM S-acetylthiocholine-iodide (ATC) (98%, Sigma-Aldrich) were prepared. The samples were prepared by mixing 15 µL of the CNS homogenate with 135 µL of MilliQ water. Subsequently, a 96-well plate was filled with 50 µL of 5 µM DTNB and 50 µL of 0.8 mM ATC. After 5 minutes of incubation, 50 µL of the CNS sample was added in triplicate to the 96-well plate. The plate was placed in a SPECTRAmax 340PC Spectrophotometer, where the absorbance of the wells was measured at 412 nm for 30 minutes with 20 second intervals. Finally, the maximum rate of change in absorbance (V_{max}) was analyzed with SoftmaxPro5.2. As positive control the AChE-inhibiting organophosphate pesticide chlorpyrifos (Sigma-Aldrich) was used.

Results

Imidaclopid exposure concentrations.

Before the exposure experiment, we have tested if imidacloprid concentrations were stable in cupper-free water under the experimental condition of the sequent exposure experiment.

The beakers were filled with 150mL of cupper-free water which were spiked with imidacloprid to reach the final concentration of 10 µg/L and 1000 µg/L. The experiment was carried out in triplicate and before injecting to the LC-MS/MS system, the IS was added to the samples. To

test if there was a significant decline in imidacloprid concentrations the analysis of variance (ANOVA) with post hoc Tukey's honestly significant difference (HSD) was performed with the software SPSS (IBM). No significant variations in imidacloprid concentrations were observed at both concentration of 10 µg/L (Figure S1) and 1000 µg/L (Figure S2).

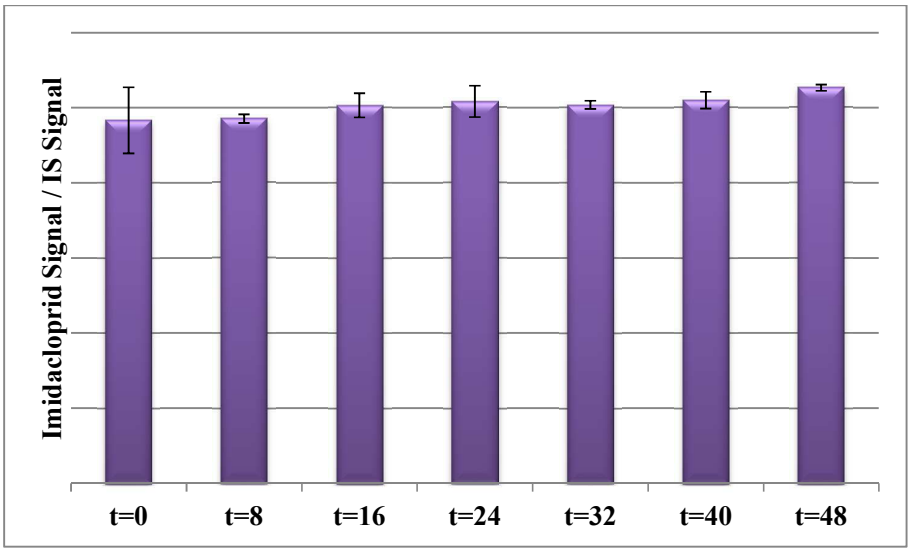


Figure S1 Concentrations of imidacloprid at 10 µg/L in cupper-free water every 8 hours of 48 subsequent hours. (n=3, error bars = SE, *=p<0.05).

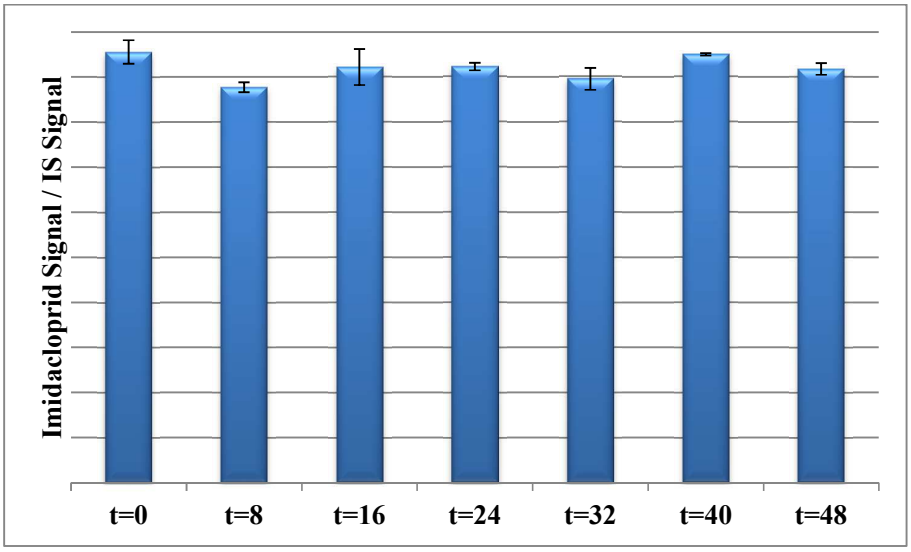


Figure S2 Concentrations of imidacloprid at 1000 µg/L in cupper-free water every 8 hours of 48 subsequent hours. (n=3, error bars = SE, *=p<0.05).

The concentration of imidacloprid in the exposure media were measured at two subsequent days of incubation (0, 24 and 48 h). The results are shown in Figure S3.

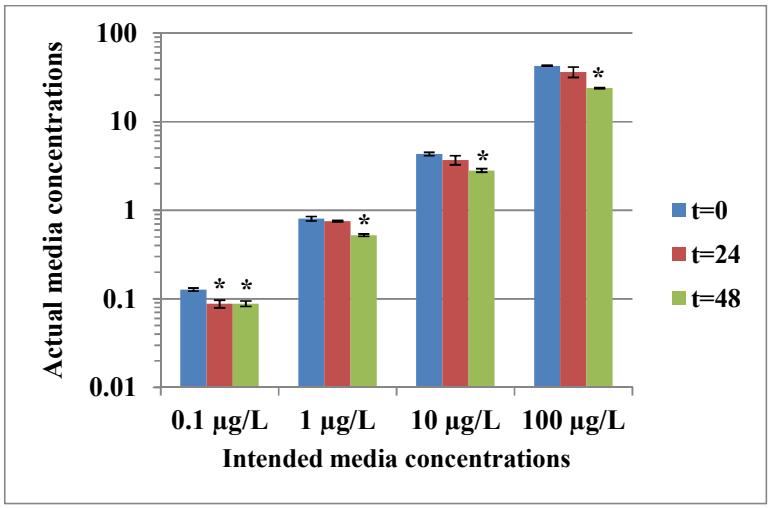


Figure S3 Actual measured imidacloprid concentrations per exposure group in µg/L in 48 subsequent hours. (n=5, error bars = SE, *=p<0.05).

The averaged actual concentrations of imidacloprid over 0, 24 and 48 hours of exposure were 0.10 ± 0.01 µg/L, 0.7 µg/ ± 0.09 µg/L, 3.6 ± 0.43 µg/L and 34.4 ± 5.6 µg/L for the exposure groups of 0.1 µg/L, 1 µg/L, 10 µg/L and 100 µg/L, respectively. The actual imidacloprid concentrations were in the range of the nominal concentration for the 0.1 and 1 µg/L groups. However for the higher dose groups (10 and 100 µg/L) the concentrations were about 3 times lower than the nominal concentration. For all exposure concentrations there was a significant decrease in imidacloprid concentration after 48 hours (ANOVA, *p-value* < 0.01). The deviation of the nominal concentrations for the two high dose groups could be due to bioaccumulation of imidacloprid in *L. stagnalis* which is also indicated by the decline of the concentrations in time. It has been found that the bioaccumulation factor of imidacloprid in snails is high[2].

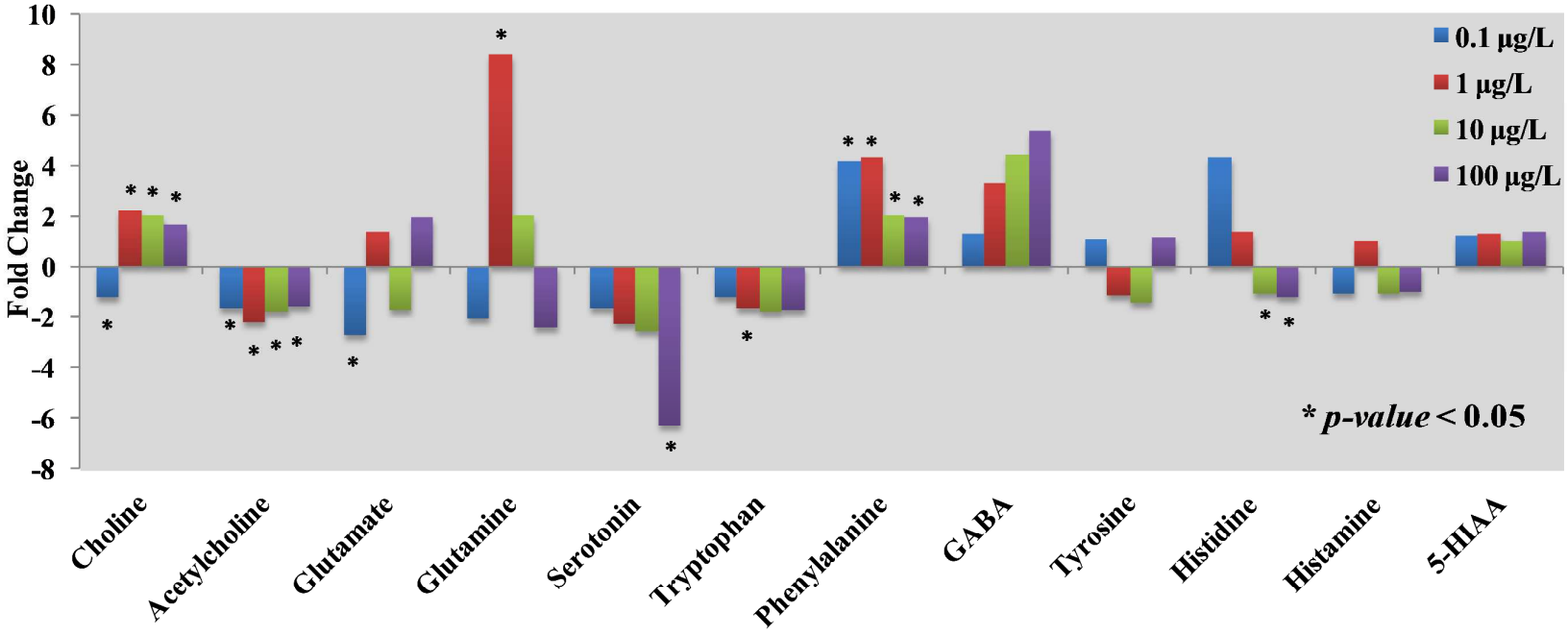


Figure S4 Fold changes for the quantified neurotransmitters, precursors and metabolites in *L. stagnalis* CNS exposed to different imidacloprid concentrations.

Traditional toxicological endpoints.

The AChE activity of *L. stagnalis* CNS exposed to different imidacloprid concentrations was tested. The average of V_{max} compared to the control group was calculated for all exposure groups (Figure S3).

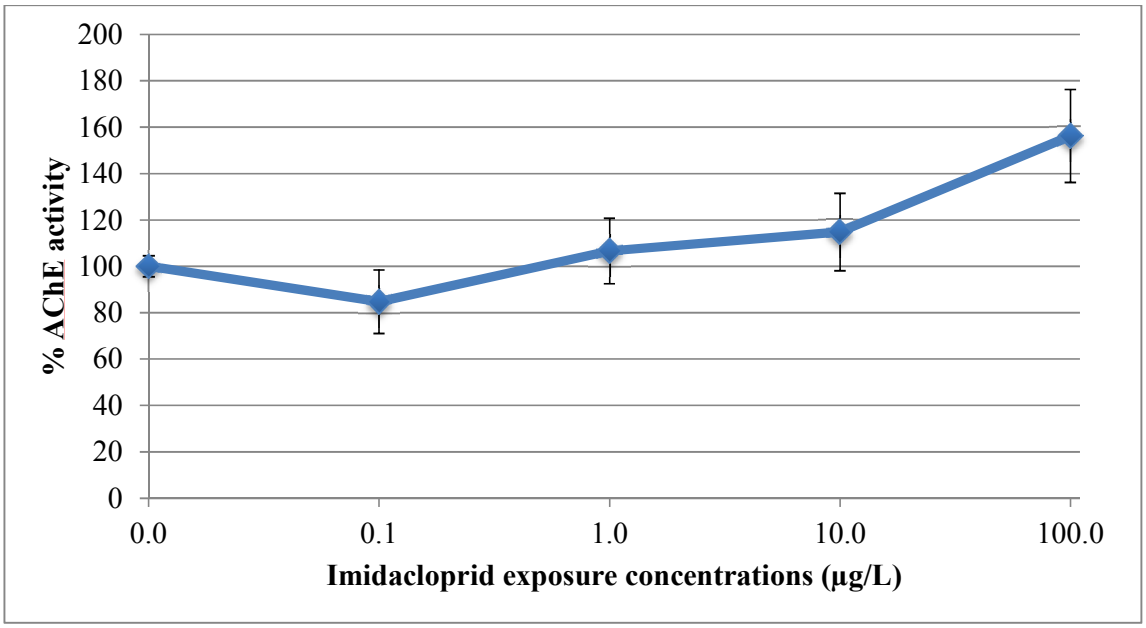


Figure S5 Average AChE activity in *L. stagnalis* CNS after 10 days of exposure to increasing concentrations of imidacloprid (n=10; error bars = SE).

The applicability of AChE bioassay to determine imidacloprid-induced toxic effects has been tested. The reliability was tested by incubating *L. stagnalis* CNS homogenate of unexposed snails with low concentrations of chlorpyrifos. Imidacloprid was tested to determine any intrinsic inhibiting effects on AChE. The protein contents, determined with the Bradford assay, were use to normalize for the heterogeneity in CNS sample sizes. The positive control chlorpyrifos showed a significantly lowered enzyme activity compared to the controls. This result confirmed the applicability of this assay to determine AChE activity in CNS samples of imidacloprid-exposed snails. Imidacloprid did, however, not significantly reduce the enzyme activity (Figure S4).

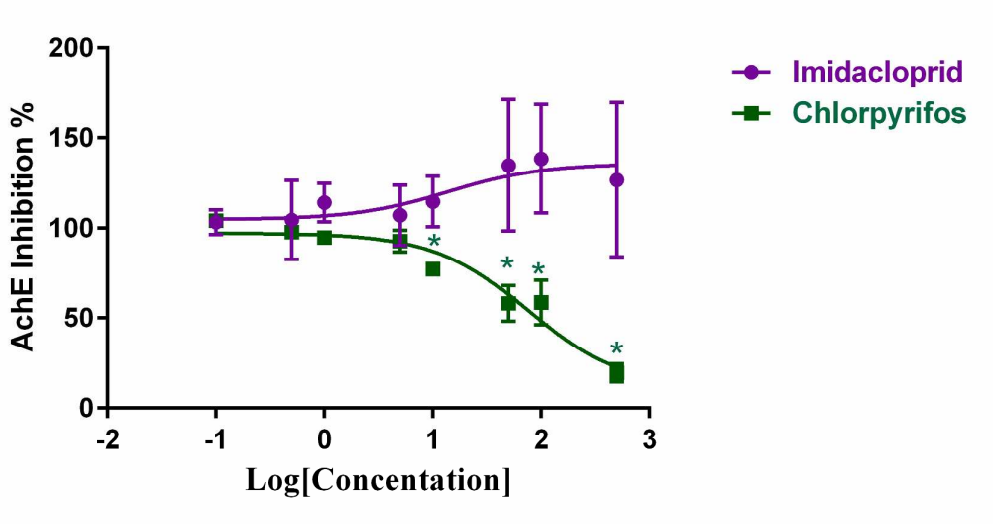
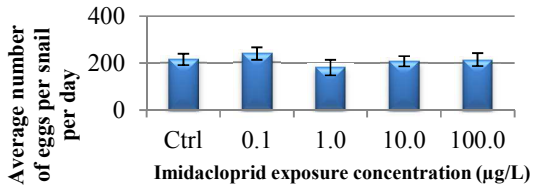
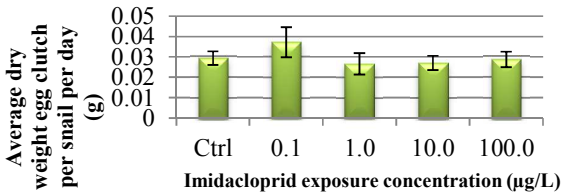


Figure S6 AChE activity inhibition % in CNS samples after 20 minutes of incubation to imidacloprid and chlorpyrifos, normalized for the controls. (n=3; error bars=SE; **p-value* <0.05).

No significant effects were observed on phenotypical endpoint of mortality and reproduction. *L. stagnalis* is able to reproduce through parthenogenesis and for this reason it is a well-suited species for isolated reproduction experiments[3]. Effects on the reproduction of *L. stagnalis* were tested by measuring the number of laid eggs and the dried weight of the egg clutches. The ANOVA performed on the egg count (Figure S5 (A)) and measurements of the dry weight of the egg clutches (Figure S5 (B)) of snails exposed to imidacloprid did not show significant differences between the exposure groups and the control. Even though reproductive outputs have been shown before to be a sensitive endpoint in *L. stagnalis*, the incubation time used in this study was probably too short to induce a significant effect on the snail reproduction[3]. Nevertheless, these results indicate that the lowest effect concentration (LOEC) for the analysed sublethal endpoints in *L. stagnalis* is higher than 34 µg/L for 10 days of exposure. This is in agreement with a high LOEC for imidacloprid found in another aquatic snail species: 25,000 µg/L caused heart rate effects in embryos of *Marisa cornuarietis* after 10 days of exposure[4]. Furthermore, Nyman et al.[2] found that the LC_{50} of *L. stagnalis* was about 50,000 µg/L with 4 days of exposure.



(A) Average number of eggs per snail per day within groups exposed to different concentrations of imidacloprid for ten days. (n=10, error bars = SE, *p<0.05).



(B) Average of the dry weight of egg clutches per snail per day within groups exposed to different concentrations of imidacloprid for ten days. (n=10, error bars = SE, * p<0.05)

Figure S7 Imidacloprid effect on the number of laid eggs (A) and dried egg clutches weights (B) of the freshwater snail *L. stagnalis*.

Tables and Figures legend:

Figure S1 Concentrations of imidacloprid at 10 µg/L in cupper-free water every 8 hours of 48 subsequent hours. (n=3, error bars = SE, *=p<0.05).

Figure S2 Concentrations of imidacloprid at 1000 µg/L in cupper-free water every 8 hours of 48 subsequent hours. (n=3, error bars = SE, *=p<0.05).

Figure S3 Actual measured imidacloprid concentrations per exposure group in 48 subsequent hours with their original intended imidacloprid concentrations. (*n*=5; error bars = SE; *=*p-value* <0.05).

Figure S4 Fold changes for the quantified neurotransmitters, precursors and metabolites in *L. stagnalis* CNS exposed to different imidacloprid concentrations.

Figure S5 Average AChE activity in *L. stagnalis* CNS after 10 days of exposure to increasing concentrations of imidacloprid (*n*=10; error bars = SE).

Figure S6 AChE activity inhibition % in CNS samples after 20 minutes of incubation to imidacloprid and chlorpyrifos, normalized for the controls. (*n*=3; error bars=SE; **p-value* <0.05).

Figure S7 Imidacloprid effect on the number of laid eggs (A) and dried egg clutches weights (B) of the freshwater snail *L. stagnalis*.

Table S1 Libraries of metabolite standards used for the target analysis.

Table S2 Metabolite identified in the different exposure groups. Fold change and p-values corrected by false discovery rate (FDR) are reported. Marked in red the metabolites significantly different for more three exposure concentrations and in green are marked the p-values below 0.05.

Table S3 Area under the ROC curve (AUC) for metabolite showing AUC > 0.8 in more than three exposure concentrations.

Figure S8 ROC curves for acetylcholine in the CNS of *L. stagnalis* exposed to increasing concentration of imidacloprid.

Table S1 Libraries of metabolite standards used for the target analysis.

METABOLITE NAME	Supplier	Analytical Platform	Detected in sample	m/z	Molecular Formula	RT	Mass Error (ppm)
3-METHYL-2-OXOVALERIC ACID	IROA MSMLS	Non-targeted (ToF)	√	130.0630	C6H10O3	2.1	<2ppm
4-GUANIDINO-BUTANOATE	IROA MSMLS	Non-targeted (ToF)	√	145.0851	C5H11N3O2	8.7	<2ppm
4-METHYL-2-OXOVALERIC ACID	IROA MSMLS	Non-targeted (ToF)	√	130.0630	C6H10O3	2.1	<2ppm
5-HIAA	Sigma-Aldrich	Targeted (QqQ)	√	191.0582	C10H9NO3	3.4	<2ppm
5'-METHYLTHIOADENOSINE	IROA MSMLS	Non-targeted (ToF)	√	297.0896	C11H15N5O3S	14.1	<2ppm
ACETYLCHOLINE	Sigma-Aldrich	Targeted (QqQ)	√	146.1176	C7H16NO2	3.0	<2ppm
ADENINE	IROA MSMLS	Non-targeted (ToF)	√	135.0545	C5H5N5	5.8	<2ppm
ALPHALINOLENIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	292.2402	C19H32O2	21.4	<2ppm
ARACHIDIC ACID	IROA MSMLS	Non-targeted (ToF)	√	326.3185	C21H42O2	25.1	<2ppm
ARACHIDONIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	318.2559	C21H34O2	24.1	<2ppm
BETAINE	IROA MSMLS	Non-targeted (ToF)	√	117.0790	C5H11NO2	9.2	<2ppm
CARNITINE	IROA MSMLS	Non-targeted (ToF)	√	161.1052	C7H15NO3	9.8	<2ppm
CHOLINE	Sigma-Aldrich	Targeted (QqQ)	√	104.1075	C5H14NO	6.3	<2ppm
CIS10HEPTADECENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	282.2560	C18H34O2	20.2	<2ppm
CIS1114EICOSADIENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	322.2872	C21H38O2	24.7	<2ppm
CIS1117EICOSATRIENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	320.2715	C21H36O2	24.3	<2ppm
CIS11EICOSENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	324.3028	C21H40O2	24.7	<2ppm
CIS1316DOCOSADIENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	350.3185	C23H42O2	27.4	<2ppm
CIS15TETRACOSENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	380.3654	C25H48O2	29.9	<2ppm
CIS517EICOSAPENTAENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	316.2402	C21H32O2	24.1	<2ppm

CIS814EICOSATRIENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	320.2715	C21H36O2	24.7	<2ppm
CREATININE	IROA MSMLS	Non-targeted (ToF)	√	113.0589	C4H7N3O	6.0	<2ppm
DIETHANOLAMINE	IROA MSMLS	Non-targeted (ToF)	√	105.0790	C4H11NO2	9.3	<2ppm
DIMETHYLBENZIMIDAZOLE	IROA MSMLS	Non-targeted (ToF)	√	146.0844	C9H10N2	8.9	<2ppm
DOCOSAHEXAENOIC ACID	IROA MSMLS	Non-targeted (ToF)	√	328.2402	C22H32O2	1.9	<2ppm
ELAIDIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	296.2715	C19H36O2	21.8	<2ppm
ELAIDIC ACID	IROA MSMLS	Non-targeted (ToF)	√	296.2715	C19H36O2	21.8	<2ppm
GABA	Sigma-Aldrich	Targeted (QqQ)	√	103.0633	C4H9NO2	12.1	<2ppm
GAMMALINOLENIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	292.2402	C19H32O2	21.7	<2ppm
GLUTAMATE	Sigma-Aldrich	Targeted (QqQ)	√	147.0532	C5H9NO4	13.6	<2ppm
GLUTAMINE	Sigma-Aldrich	Targeted (QqQ)	√	146.0691	C5H10N2O3	13.3	<2ppm
GUANINE	IROA MSMLS	Non-targeted (ToF)	√	151.0494	C5H5N5O	7.7	<2ppm
GUANOSINE	IROA MSMLS	Non-targeted (ToF)	√	283.0917	C10H13N5O5	9.0	<2ppm
HENICOSANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	340.3341	C22H44O2	26.5	<2ppm
HEPTADECANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	284.2715	C18H36O2	20.6	<2ppm
HISTAMINE	Sigma-Aldrich	Targeted (QqQ)	√	111.0797	C5H9N3	18.3	<2ppm
HISTIDINE	Sigma-Aldrich	Targeted (QqQ)	√	155.0695	C6H9N3O2	15.8	<2ppm
HYPOXANTHINE	IROA MSMLS	Non-targeted (ToF)	√	136.0385	C5H4N4O	6.0	<2ppm
INDOLE-3-ACETALDEHYDE	IROA MSMLS	Non-targeted (ToF)	√	175.0633	C10H9NO2	6.4	<2ppm
INOSINE	IROA MSMLS	Non-targeted (ToF)	√	268.0808	C10H12N4O5	8.3	<2ppm
LEUCINE	IROA MSMLS	Non-targeted (ToF)	√	131.0946	C6H13NO2	8.6	<2ppm
LINOLEIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	294.2559	C19H34O2	21.6	<2ppm
LINOLELAIDIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	294.2559	C19H34O2	21.8	<2ppm
L-TRYPTHOPHAN	Sigma-Aldrich	Targeted (QqQ)	√	204.0899	C11H12N2O2	10.7	<2ppm
L-TYROSINE	Sigma-Aldrich	Targeted (QqQ)	√	181.0739	C9H11NO3	12.1	<2ppm
N6-(DELTA2-ISOPENTENYL)-	IROA	Non-targeted (ToF)	√	203.1171	C10H13N5	8.8	<2ppm

ADENINE	MSMLS						
N-ACETYL-L-LEUCINE	IROA MSMLS	Non-targeted (ToF)	√	173.1052	C8H15NO3	1.4	<2ppm
N-ACETYLPUTRESCINE	IROA MSMLS	Non-targeted (ToF)	√	130.1106	C6H14N2O	8.8	<2ppm
N-ACETYLSEROTONIN	IROA MSMLS	Non-targeted (ToF)	√	218.1055	C12H14N2O2	5.0	<2ppm
NE,NE,NE-TRIMETHYLLYSINE	IROA MSMLS	Non-targeted (ToF)	√	188.1525	C9H20N2O2	12.7	<2ppm
NICOTINAMIDE	IROA MSMLS	Non-targeted (ToF)	√	122.0480	C6H6N2O	3.0	<2ppm
O-ACETYL-L-CARNITINE	IROA MSMLS	Non-targeted (ToF)	√	203.1158	C9H17NO4	8.8	<2ppm
OLEIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	296.2715	C19H36O2	21.6	<2ppm
PALMITOLEIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	268.2402	C17H32O2	18.6	<2ppm
PHENYLALANINE	Sigma-Aldrich	Targeted (QqQ)	√	165.0790	C9H11NO2	10.3	<2ppm
PICOLINIC ACID	IROA MSMLS	Non-targeted (ToF)	√	123.0320	C6H5NO2	5.0	<2ppm
PIPECOLINIC ACID	IROA MSMLS	Non-targeted (ToF)	√	129.0790	C6H11NO2	10.4	<2ppm
PROLINE	IROA MSMLS	Non-targeted (ToF)	√	115.0633	C5H9NO2	9.8	<2ppm
PUTRESCINE	IROA MSMLS	Non-targeted (ToF)	√	88.1001	C4H12N2	12.6	<2ppm
SEROTONIN	Sigma-Aldrich	Targeted (QqQ)	√	176.0950	C10H12N2O	10.5	<2ppm
SPERMIDINE	IROA MSMLS	Non-targeted (ToF)	√	145.1579	C7H19N3	15.2	<2ppm
STEARIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	298.2872	C19H38O2	22.2	<2ppm
TETRACOSANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	382.3811	C25H50O2	30.3	<2ppm
THIOPURINE S-METHYLESTER	IROA MSMLS	Non-targeted (ToF)	√	166.0313	C6H6N4S	1.5	<2ppm
TRICOSANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	√	368.3654	C24H48O2	29.1	<2ppm
TRYPTOPHANAMIDE	IROA MSMLS	Non-targeted (ToF)	√	203.1059	C11H13N3O	5.8	<2ppm
URACIL	IROA MSMLS	Non-targeted (ToF)	√	112.0273	C4H4N2O2	10.6	<2ppm
URIDINE	IROA MSMLS	Non-targeted (ToF)	√	244.0695	C9H12N2O6	6.0	<2ppm

URIDINE-5-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	√	324.0359	C9H13N2O9P	9.2	<2ppm
UROCANATE	IROA MSMLS	Non-targeted (ToF)	√	138.0429	C6H6N2O2	3.3	<2ppm
VALINE	IROA MSMLS	Non-targeted (ToF)	√	117.0790	C5H11NO2	3.1	<2ppm
XANTHINE	IROA MSMLS	Non-targeted (ToF)	√	152.0334	C5H4N4O2	15.0	<2ppm
5-AMINOIMIDAZOLE-4-CARBOXAMIDE-1-?-D-RIBOFURANOSYL 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x				
(2-AMINOETHYL)PHOSPHONATE	IROA MSMLS	Non-targeted (ToF)	x				
(R)-MALATE	IROA MSMLS	Non-targeted (ToF)	x				
(S)-1-PHENYLETHANOL	IROA MSMLS	Non-targeted (ToF)	x				
(S)-DIHYDROOROTATE	IROA MSMLS	Non-targeted (ToF)	x				
(S)-LACTATE	IROA MSMLS	Non-targeted (ToF)	x				
1,2-DIDECANOYL-SN-GLYCERO-3-PHOSPHOCHOLINE	IROA MSMLS	Non-targeted (ToF)	x				
1,2-DIPALMITOYL-RAC-GLYCERO-3-PHOSPHOETHANOLAMINE	IROA MSMLS	Non-targeted (ToF)	x				
1,2-DIPALMITOYL-SN-GLYCEROL	IROA MSMLS	Non-targeted (ToF)	x				
10-HYDROXYDECANOATE	IROA MSMLS	Non-targeted (ToF)	x				
12-HYDROXYDODECANOIC ACID	IROA MSMLS	Non-targeted (ToF)	x				
17A,21-DIHYDROXY-4-PREGNENE-3,20-DIONE	IROA MSMLS	Non-targeted (ToF)	x				
1-HYDROXY-2-NAPHTHOATE	IROA MSMLS	Non-targeted (ToF)	x				

1-METHYL-6,7-DIHYDROXY-1,2,3,4-TETRAHYDROISOQUINOLINE HYDROBROMIDE	IROA MSMLS	Non-targeted (ToF)	x
1-NAPHTHYLAMINE	IROA MSMLS	Non-targeted (ToF)	x
2,3-DIHYDROXYBENZOATE	IROA MSMLS	Non-targeted (ToF)	x
2,3-DIPHOSPHO-D-GLYCERIC ACID	IROA MSMLS	Non-targeted (ToF)	x
2',4'-DIHYDROXYACETOPHENONE	IROA MSMLS	Non-targeted (ToF)	x
2,4-DIHYDROXYPTERIDINE	IROA MSMLS	Non-targeted (ToF)	x
2,4-DIHYDROXYPYRIMIDINE-5-CARBOXYLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
2,5-DIHYDROXYBENZOATE	IROA MSMLS	Non-targeted (ToF)	x
2,5-DIMETHYLPYRAZINE	IROA MSMLS	Non-targeted (ToF)	x
2,6-DIHYDROXYPYRIDINE	IROA MSMLS	Non-targeted (ToF)	x
25-HYDROXYCHOLESTEROL	IROA MSMLS	Non-targeted (ToF)	x
2-ACETAMIDO-2-DEOXY-BETA-D-GLUCOSYLAMINE	IROA MSMLS	Non-targeted (ToF)	x
2-AMINO-2-METHYL-PROPANOATE	IROA MSMLS	Non-targeted (ToF)	x
2-AMINOETHYL DIHYDROGEN PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2-AMINOPHENOL	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYADENOSINE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYADENOSINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYADENOSINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYCYTIDINE 5'-	IROA	Non-targeted (ToF)	x

DIPHOSPHATE	MSMLS		
2'-DEOXYCYTIDINE 5-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2-DEOXY-D-GLUCOSE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYGUANOSINE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYGUANOSINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYGUANOSINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYGUANOSINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYURIDINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2'-DEOXYURIDINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
2-HYDROXY-4-(METHYLTHIO)BUTYRIC ACID	IROA MSMLS	Non-targeted (ToF)	x
2-HYDROXYBUTYRIC ACID	IROA MSMLS	Non-targeted (ToF)	x
2-HYDROXYPHENYLACETIC ACID	IROA MSMLS	Non-targeted (ToF)	x
2-HYDROXYPYRIDINE	IROA MSMLS	Non-targeted (ToF)	x
2-METHOXYETHANOL	IROA MSMLS	Non-targeted (ToF)	x
2-METHYLBUTANAL	IROA MSMLS	Non-targeted (ToF)	x
2-METHYLGLUTARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
2-METHYLMALEATE	IROA MSMLS	Non-targeted (ToF)	x
2-METHYLPROPANAL OXIME	IROA MSMLS	Non-targeted (ToF)	x
2-METHYLPROPANOATE	IROA MSMLS	Non-targeted (ToF)	x
2-OXOADIPATE	IROA	Non-targeted (ToF)	x

	MSMLS		
2-OXOBUTANOATE	IROA MSMLS	Non-targeted (ToF)	x
2-QUINOLINECARBOXYLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
3(2-HYDROXYPHENYL)PROPANOATE	IROA MSMLS	Non-targeted (ToF)	x
3(4-HYDROXYPHENYL)LACTATE	IROA MSMLS	Non-targeted (ToF)	x
3-(4-HYDROXYPHENYL)PYRUVATE	IROA MSMLS	Non-targeted (ToF)	x
3,3-DIAMINOPROPANE	IROA MSMLS	Non-targeted (ToF)	x
3,4-DIHYDROXY-1-PHENYLALANINE	IROA MSMLS	Non-targeted (ToF)	x
3,4-DIHYDROXYBENZOATE	IROA MSMLS	Non-targeted (ToF)	x
3,4-DIHYDROXYPHENYL GLYCOL	IROA MSMLS	Non-targeted (ToF)	x
3,4-DIHYDROXYPHENYLACETATE	IROA MSMLS	Non-targeted (ToF)	x
3',5'-CYCLIC AMP	IROA MSMLS	Non-targeted (ToF)	x
3,5-DIIODO-L-THYRONINE	IROA MSMLS	Non-targeted (ToF)	x
3,5-DIIODO-L-TYROSINE	IROA MSMLS	Non-targeted (ToF)	x
3ALPHA,11BETA,17ALPHA,21-TRETRAHYDROXY-5ALPHA-PREGNAN-20-ONE	IROA MSMLS	Non-targeted (ToF)	x
3ALPHA-HYDROXY-5-BETA-CHOLANATE	IROA MSMLS	Non-targeted (ToF)	x
3-AMINO-4-HYDROXYBENZOIC ACID	IROA MSMLS	Non-targeted (ToF)	x
3-AMINO-5-HYDROXYBENZOIC ACID	IROA MSMLS	Non-targeted (ToF)	x

3-AMINOISOBUTANOATE	IROA MSMLS	Non-targeted (ToF)	x
3'-CMP	IROA MSMLS	Non-targeted (ToF)	x
3-DEHYDROSHIKIMATE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXY-3-METHYLGLUTARATE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYANTHRANILATE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYBENZALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYBENZOATE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYBENZYL ALCOHOL	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYBUTANOIC ACID	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYKYNURENINE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYPHENYLACETATE	IROA MSMLS	Non-targeted (ToF)	x
3-HYDROXYPYRUVATE	IROA MSMLS	Non-targeted (ToF)	x
3-METHOXY-4-HYDROXYMANDELATE	IROA MSMLS	Non-targeted (ToF)	x
3-METHOXY-L-TYROSINE	IROA MSMLS	Non-targeted (ToF)	x
3-METHOXYTYRAMINE	IROA MSMLS	Non-targeted (ToF)	x
3-METHYL-2-OXINDOLE	IROA MSMLS	Non-targeted (ToF)	x
3-METHYLADENINE	IROA MSMLS	Non-targeted (ToF)	x
3-METHYLBUTANAL	IROA MSMLS	Non-targeted (ToF)	x
3-METHYLBUTANOL	IROA MSMLS	Non-targeted (ToF)	x
3-METHYLCROTONYL-COA	IROA	Non-targeted (ToF)	x

	MSMLS		
3-METHYLGLUTARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
3-METHYLHISTAMINE	IROA MSMLS	Non-targeted (ToF)	x
3-MT	Sigma-Aldrich	Targeted (QqQ)	x
3-SULFINO-L-ALANINE	IROA MSMLS	Non-targeted (ToF)	x
3-UREIDOPROPIONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
4-ACETAMIDOBUTANOATE	IROA MSMLS	Non-targeted (ToF)	x
4-AMINOBENZOATE	IROA MSMLS	Non-targeted (ToF)	x
4-AMINOBUTANOATE	IROA MSMLS	Non-targeted (ToF)	x
4-AMINOBUTANOIC ACID	IROA MSMLS	Non-targeted (ToF)	x
4-COUMARATE	IROA MSMLS	Non-targeted (ToF)	x
4-HYDROXY-3-METHOXYPHENYLGLYCOL	IROA MSMLS	Non-targeted (ToF)	x
4-HYDROXYBENZALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
4-HYDROXYBENZOATE	IROA MSMLS	Non-targeted (ToF)	x
4-HYDROXY-L-PHENYLGLYCINE	IROA MSMLS	Non-targeted (ToF)	x
4-HYDROXY-L-PROLINE	IROA MSMLS	Non-targeted (ToF)	x
4-HYDROXYPHENYLACETATE	IROA MSMLS	Non-targeted (ToF)	x
4-IMIDAZOLEACETIC ACID	IROA MSMLS	Non-targeted (ToF)	x
4-METHYL-2-OXO-PENTANOIC ACID	IROA MSMLS	Non-targeted (ToF)	x
4-METHYLCATECHOL	IROA MSMLS	Non-targeted (ToF)	x

4-PYRIDOXATE	IROA MSMLS	Non-targeted (ToF)	x
4-QUINOLINECARBOXYLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
5,6-DIHYDROURACIL	IROA MSMLS	Non-targeted (ToF)	x
5-AMINOLEVULINIC ACID	IROA MSMLS	Non-targeted (ToF)	x
5-AMINOPENTANOATE	IROA MSMLS	Non-targeted (ToF)	x
5'-DEOXYADENOSINE	IROA MSMLS	Non-targeted (ToF)	x
5-HYDROXYINDOLEACETATE	IROA MSMLS	Non-targeted (ToF)	x
5-HYDROXY-L-TRYPTOPHAN	Sigma-Aldrich	Targeted (QqQ)	x
5-HYDROXY-L-TRYPTOPHAN	IROA MSMLS	Non-targeted (ToF)	x
5-HYDROXYMETHYLURACIL	IROA MSMLS	Non-targeted (ToF)	x
5-METHYLCYTOSINE	IROA MSMLS	Non-targeted (ToF)	x
5-OXO-D-PROLINE	IROA MSMLS	Non-targeted (ToF)	x
5-OXO-L-PROLINE	IROA MSMLS	Non-targeted (ToF)	x
5-PHOSPHO-D-RIBOSE-1-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
5-VALEROLACTONE	IROA MSMLS	Non-targeted (ToF)	x
6-DEOXY-L-GALACTOSE	IROA MSMLS	Non-targeted (ToF)	x
6-HYDROXYDOPAMINE	IROA MSMLS	Non-targeted (ToF)	x
6-HYDROXYNICOTINATE	IROA MSMLS	Non-targeted (ToF)	x
6-PHOSPHOGLUCONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
ACETALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x

ACETOIN	IROA MSMLS	Non-targeted (ToF)	x
ACETYLCHOLINE CHLORIDE	IROA MSMLS	Non-targeted (ToF)	x
ACONITATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 2',3'-CYCLIC MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 3',5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 5'-DIPHOSPHORIBOSE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ADENOSINE-5'-DIPHOSPHOGLUCOSE	IROA MSMLS	Non-targeted (ToF)	x
A-D-GALACTOSE 1-PHOSPHATE DIPOTASSIUM SALT PENTAHYDRATE	IROA MSMLS	Non-targeted (ToF)	x
ADIPIC ACID	IROA MSMLS	Non-targeted (ToF)	x
AGMATINE SULFATE	IROA MSMLS	Non-targeted (ToF)	x
ALANINE	IROA MSMLS	Non-targeted (ToF)	x
ALANINE	IROA MSMLS	Non-targeted (ToF)	x
ALLANTOIN	IROA MSMLS	Non-targeted (ToF)	x

ALLOSE	IROA MSMLS	Non-targeted (ToF)	x
ALLOTHREONINE	IROA MSMLS	Non-targeted (ToF)	x
ALPHA-D-GLUCOSE 1-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
AMINOADIPATE	IROA MSMLS	Non-targeted (ToF)	x
AMYLOSE	IROA MSMLS	Non-targeted (ToF)	x
ANILINE	IROA MSMLS	Non-targeted (ToF)	x
ANILINE-2-SULFONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
ANTHRANILATE	IROA MSMLS	Non-targeted (ToF)	x
ARABINOSE	IROA MSMLS	Non-targeted (ToF)	x
ARABINOSE	IROA MSMLS	Non-targeted (ToF)	x
ARACHIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
ARGININE	IROA MSMLS	Non-targeted (ToF)	x
ASCORBATE	IROA MSMLS	Non-targeted (ToF)	x
ASPARAGINE	IROA MSMLS	Non-targeted (ToF)	x
ASPARTATE	IROA MSMLS	Non-targeted (ToF)	x
ASPARTATE	IROA MSMLS	Non-targeted (ToF)	x
AZELAIC ACID	IROA MSMLS	Non-targeted (ToF)	x
BENZALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
BENZOATE	IROA MSMLS	Non-targeted (ToF)	x
BENZYL ALCOHOL	IROA MSMLS	Non-targeted (ToF)	x

BENZYLAMINE	IROA MSMLS	Non-targeted (ToF)	x
BETA-ALANINE	IROA MSMLS	Non-targeted (ToF)	x
BETA-CAROTENE	IROA MSMLS	Non-targeted (ToF)	x
BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE 2'-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
BETA-NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
BILIRUBIN	IROA MSMLS	Non-targeted (ToF)	x
BILIVERDIN	IROA MSMLS	Non-targeted (ToF)	x
BIOTIN	IROA MSMLS	Non-targeted (ToF)	x
BIS(2-ETHYLHEXYL)PHTHALATE	IROA MSMLS	Non-targeted (ToF)	x
BIS(3-AMINOPROPYL)AMINE	IROA MSMLS	Non-targeted (ToF)	x
BUTANAL	IROA MSMLS	Non-targeted (ToF)	x
BUTANEDIOL	IROA MSMLS	Non-targeted (ToF)	x
BUTANOATE	IROA MSMLS	Non-targeted (ToF)	x
BUTYRIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
CADAVERINE	IROA MSMLS	Non-targeted (ToF)	x
CAFFEIC ACID	IROA MSMLS	Non-targeted (ToF)	x
CAFFEINE	IROA MSMLS	Non-targeted (ToF)	x
CAPRYLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
CARBAMOYL PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x

CARNOSINE	IROA MSMLS	Non-targeted (ToF)	x
CATECHOL	IROA MSMLS	Non-targeted (ToF)	x
CELLOBIOSE	IROA MSMLS	Non-targeted (ToF)	x
CHENODEOXYCHOLATE	IROA MSMLS	Non-targeted (ToF)	x
CHOLESTEROL PALMITATE	IROA MSMLS	Non-targeted (ToF)	x
CHOLESTERYL ACETATE	IROA MSMLS	Non-targeted (ToF)	x
CHOLESTRA-5,7-DIEN-3BETA-OL	IROA MSMLS	Non-targeted (ToF)	x
CHOLESTRYL OLEATE	IROA MSMLS	Non-targeted (ToF)	x
CHOLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
CHOLINE	IROA MSMLS	Non-targeted (ToF)	x
CINNAMALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
CINNAMATE	IROA MSMLS	Non-targeted (ToF)	x
CIS10PENTADECENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
CIS419DOCOSAHEXAENOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
CIS-4-HYDROXY-D-PROLINE	IROA MSMLS	Non-targeted (ToF)	x
CITRATE	IROA MSMLS	Non-targeted (ToF)	x
CITRULLINE	IROA MSMLS	Non-targeted (ToF)	x
CMP	IROA MSMLS	Non-targeted (ToF)	x
COENZYME A	IROA MSMLS	Non-targeted (ToF)	x
CORTICOSTERONE	IROA MSMLS	Non-targeted (ToF)	x

CORTISOL	IROA MSMLS	Non-targeted (ToF)	x
CORTISOL 21-ACETATE	IROA MSMLS	Non-targeted (ToF)	x
CORTISONE	IROA MSMLS	Non-targeted (ToF)	x
CREATINE	IROA MSMLS	Non-targeted (ToF)	x
CREATINE PHOSPHATE DIBASIC TETRAHYDRATE	IROA MSMLS	Non-targeted (ToF)	x
CYCLOPENTANONE	IROA MSMLS	Non-targeted (ToF)	x
CYS-GLY	IROA MSMLS	Non-targeted (ToF)	x
CYSTATHIONINE	IROA MSMLS	Non-targeted (ToF)	x
CYSTEAMINE	IROA MSMLS	Non-targeted (ToF)	x
CYSTEIC ACID	IROA MSMLS	Non-targeted (ToF)	x
CYSTEINE	IROA MSMLS	Non-targeted (ToF)	x
CYSTINE	IROA MSMLS	Non-targeted (ToF)	x
CYTIDINE	IROA MSMLS	Non-targeted (ToF)	x
CYTIDINE 2',3'-CYCLIC MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
CYTIDINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
CYTIDINE 5'-DIPHOSPHOCHOLINE	IROA MSMLS	Non-targeted (ToF)	x
CYTIDINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
CYTOCHROME C	IROA MSMLS	Non-targeted (ToF)	x
CYTOSINE	IROA MSMLS	Non-targeted (ToF)	x
D-(-)-3-PHOSPHO-GLYCERIC	IROA	Non-targeted (ToF)	x

ACID	MSMLS		
D-(+)-GALACTOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
D(+)-RAFFINOSE	IROA MSMLS	Non-targeted (ToF)	x
D-(+)-TREHALOSE	IROA MSMLS	Non-targeted (ToF)	x
DECANOATE	IROA MSMLS	Non-targeted (ToF)	x
DECANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
DEHYDROASCORBATE	IROA MSMLS	Non-targeted (ToF)	x
DEHYDRO-L-(+)-ASCORBIC ACID	IROA MSMLS	Non-targeted (ToF)	x
DEOXYADENOSINE MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
DEOXYCARNITINE	IROA MSMLS	Non-targeted (ToF)	x
DEOXYCHOLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
DEOXYCHOLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
DEOXYCORTICOSTERONE	IROA MSMLS	Non-targeted (ToF)	x
DEOXYCYTIDINE	IROA MSMLS	Non-targeted (ToF)	x
DEOXYRIBOSE	IROA MSMLS	Non-targeted (ToF)	x
DEOXYURIDINE	IROA MSMLS	Non-targeted (ToF)	x
DESMOSTEROL	IROA MSMLS	Non-targeted (ToF)	x
DETHIOBIOTIN	IROA MSMLS	Non-targeted (ToF)	x
D-FRUCTOSE 6-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
D-GLUCONO-1,5-LACTONE	IROA MSMLS	Non-targeted (ToF)	x

D-GLUCOSAMINE 6-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
D-GLUCOSAMINE 6-SULFATE	IROA MSMLS	Non-targeted (ToF)	x
D-GLUCOSE 6-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
D-GLUCOSE-6-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
DIACETYL	IROA MSMLS	Non-targeted (ToF)	x
DIETHYL-2-METHYL-3-OXOSUCCINATE	IROA MSMLS	Non-targeted (ToF)	x
DIHYDROFOLATE	IROA MSMLS	Non-targeted (ToF)	x
DIHYDROXYACETONE PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
DIHYDROXYFUMARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
DIHYDROXYMANDELIC ACID	IROA MSMLS	Non-targeted (ToF)	x
DIMETHYL SULFIDE	IROA MSMLS	Non-targeted (ToF)	x
DIMETHYLALLYL PYROPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
DL-5-HYDROXYLYSINE	IROA MSMLS	Non-targeted (ToF)	x
D-LACTOSE	IROA MSMLS	Non-targeted (ToF)	x
D-LYXOSE	IROA MSMLS	Non-targeted (ToF)	x
D-MANNOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
D-MANNOSE 6-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
DOCOSANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
DODECANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
DOPA	Sigma-Aldrich	Targeted (QqQ)	x
DOPAMINE	Sigma-Aldrich	Targeted (QqQ)	x

DOPAMINE	IROA MSMLS	Non-targeted (ToF)	x
D-ORNITHINE	IROA MSMLS	Non-targeted (ToF)	x
D-PANTOTHENIC ACID	IROA MSMLS	Non-targeted (ToF)	x
D-RIBOSE 5-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
DTMP	IROA MSMLS	Non-targeted (ToF)	x
EPINEPHRINE	IROA MSMLS	Non-targeted (ToF)	x
EPINEPRHINE	Sigma-Aldrich	Targeted (QqQ)	x
ERUCIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
ERUCIC ACID	IROA MSMLS	Non-targeted (ToF)	x
ERYTHRITOL	IROA MSMLS	Non-targeted (ToF)	x
ESTRADIOL-17ALPHA	IROA MSMLS	Non-targeted (ToF)	x
ETHANOLAMINE	IROA MSMLS	Non-targeted (ToF)	x
ETHANOLAMINE PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ETHYL-3-INDOLE-ACETATE	IROA MSMLS	Non-targeted (ToF)	x
ETHYL-3-UREIDOPROPIONATE	IROA MSMLS	Non-targeted (ToF)	x
ETHYLMALONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
FARNESYL DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
FERULATE	IROA MSMLS	Non-targeted (ToF)	x
FLAVIN ADENINE DINUCLEOTIDE	IROA MSMLS	Non-targeted (ToF)	x
FOLIC ACID	IROA MSMLS	Non-targeted (ToF)	x

FORMAMIDE	IROA MSMLS	Non-targeted (ToF)	x
FORMATE	IROA MSMLS	Non-targeted (ToF)	x
FORMYL-L-METHIONYL	IROA MSMLS	Non-targeted (ToF)	x
FRUCTOSE	IROA MSMLS	Non-targeted (ToF)	x
FRUCTOSE 1,6-BIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
FUMARATE	IROA MSMLS	Non-targeted (ToF)	x
GALACTARATE	IROA MSMLS	Non-targeted (ToF)	x
GALACTITOL	IROA MSMLS	Non-targeted (ToF)	x
GALACTOSE	IROA MSMLS	Non-targeted (ToF)	x
GALACTURONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GERANYL PYROPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GERANYLGERANYL PYROPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GLUCONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GLUCOSAMINATE	IROA MSMLS	Non-targeted (ToF)	x
GLUCOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
GLUCOSE	IROA MSMLS	Non-targeted (ToF)	x
GLUCURONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GLUCURONOLACTONE	IROA MSMLS	Non-targeted (ToF)	x
GLUTAMIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GLUTAMINE	IROA	Non-targeted (ToF)	x

	MSMLS		
GLUTARATE	IROA MSMLS	Non-targeted (ToF)	x
GLUTARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GLUTATHIONE	IROA MSMLS	Non-targeted (ToF)	x
GLYCERALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
GLYCERALDEHYDE 3-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GLYCERATE	IROA MSMLS	Non-targeted (ToF)	x
GLYCERIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GLYCEROL	IROA MSMLS	Non-targeted (ToF)	x
GLYCEROL 2-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GLYCERYL TRIMYRISTATE	IROA MSMLS	Non-targeted (ToF)	x
GLYCERYL TRIPALMITATE	IROA MSMLS	Non-targeted (ToF)	x
GLYCINE	IROA MSMLS	Non-targeted (ToF)	x
GLYCOCHOLATE	IROA MSMLS	Non-targeted (ToF)	x
GLYCOLALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
GLYCOLATE	IROA MSMLS	Non-targeted (ToF)	x
GLYOXYLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
GUAIACOL	IROA MSMLS	Non-targeted (ToF)	x
GUANIDINOACETATE	IROA MSMLS	Non-targeted (ToF)	x
GUANOSINE 3',5'-CYCLIC	IROA MSMLS	Non-targeted (ToF)	x

GUANOSINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GUANOSINE 5'-DIPHOSPHO-D-MANNOSE	IROA MSMLS	Non-targeted (ToF)	x
GUANOSINE 5'-DIPHOSPHOGLUCOSE	IROA MSMLS	Non-targeted (ToF)	x
GUANOSINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GUANOSINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
GULONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
HEPTANOIC ACID	IROA MSMLS	Non-targeted (ToF)	x
HEXADECANOL	IROA MSMLS	Non-targeted (ToF)	x
HEXANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
HIPPURATE	IROA MSMLS	Non-targeted (ToF)	x
HISTAMINE	IROA MSMLS	Non-targeted (ToF)	x
HISTIDINE	IROA MSMLS	Non-targeted (ToF)	x
HISTIDINE	IROA MSMLS	Non-targeted (ToF)	x
HISTIDINOL	IROA MSMLS	Non-targeted (ToF)	x
HOMOCYSTEINE	IROA MSMLS	Non-targeted (ToF)	x
HOMOCYSTEINE THIOLACTONE	IROA MSMLS	Non-targeted (ToF)	x
HOMOCYSTINE	IROA MSMLS	Non-targeted (ToF)	x
HOMOGENTISATE	IROA MSMLS	Non-targeted (ToF)	x
HOMOSERINE	IROA MSMLS	Non-targeted (ToF)	x
HOMOVANILLATE	IROA MSMLS	Non-targeted (ToF)	x

HYDROQUINONE	IROA MSMLS	Non-targeted (ToF)	x
HYDROXYISOBUTYRIC ACID	IROA MSMLS	Non-targeted (ToF)	x
HYPOTAURINE	IROA MSMLS	Non-targeted (ToF)	x
INDOLE	IROA MSMLS	Non-targeted (ToF)	x
INDOLE-3-ACETAMIDE	IROA MSMLS	Non-targeted (ToF)	x
INDOLE-3-ACETATE	IROA MSMLS	Non-targeted (ToF)	x
INDOLE-3-ACETIC ACID	IROA MSMLS	Non-targeted (ToF)	x
INDOLE-3-ETHANOL	IROA MSMLS	Non-targeted (ToF)	x
INDOLE-3-PYRUVIC ACID	IROA MSMLS	Non-targeted (ToF)	x
INDOXYL SULFATE	IROA MSMLS	Non-targeted (ToF)	x
INOSINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
INOSINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
INOSINE 5'-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
INOSINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ISOCITRIC ACID	IROA MSMLS	Non-targeted (ToF)	x
ISOLEUCINE	IROA MSMLS	Non-targeted (ToF)	x
ISOPENTENYL PYROPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ITACONATE	IROA MSMLS	Non-targeted (ToF)	x
JASMONATE	IROA MSMLS	Non-targeted (ToF)	x
KETOGLUTARIC ACID	IROA	Non-targeted (ToF)	x

	MSMLS		
KYNURENIC ACID	IROA MSMLS	Non-targeted (ToF)	x
KYNURENINE	IROA MSMLS	Non-targeted (ToF)	x
KYNURENINE	IROA MSMLS	Non-targeted (ToF)	x
L-2,3-DIAMINOPROPIONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
L-2-PHOSPHOGLYCERIC ACID	IROA MSMLS	Non-targeted (ToF)	x
L-AMINOCYCLOPROPANE-1-CARBOXYLATE	IROA MSMLS	Non-targeted (ToF)	x
LANOSTEROL	IROA MSMLS	Non-targeted (ToF)	x
L-ANSERINE	IROA MSMLS	Non-targeted (ToF)	x
L-ARABITOL	IROA MSMLS	Non-targeted (ToF)	x
LAURIC ACID	IROA MSMLS	Non-targeted (ToF)	x
LAUROYLCARNITINE	IROA MSMLS	Non-targeted (ToF)	x
LEUKOTRIENE B4	IROA MSMLS	Non-targeted (ToF)	x
LIPOAMIDE	IROA MSMLS	Non-targeted (ToF)	x
LITHIUM ACETOACETATE	IROA MSMLS	Non-targeted (ToF)	x
LITHIUM CARBAMOYLPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
LITHIUM POTASSIUM ACETYL PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
LL-2,6-DIAMINOHEPTANEDIOATE	IROA MSMLS	Non-targeted (ToF)	x
L-METHIONINE SULFOXIMINE	IROA MSMLS	Non-targeted (ToF)	x
L-METHYLADENOSINE	IROA MSMLS	Non-targeted (ToF)	x

L-METHYLNICOTINAMIDE	IROA MSMLS	Non-targeted (ToF)	x
L-OLEOYL-RAC-GLYCEROL	IROA MSMLS	Non-targeted (ToF)	x
LUMICHROME	IROA MSMLS	Non-targeted (ToF)	x
LYSINE	IROA MSMLS	Non-targeted (ToF)	x
LYSINE	IROA MSMLS	Non-targeted (ToF)	x
MALATE	IROA MSMLS	Non-targeted (ToF)	x
MALEAMATE	IROA MSMLS	Non-targeted (ToF)	x
MALEIC ACID	IROA MSMLS	Non-targeted (ToF)	x
MALEIMIDE	IROA MSMLS	Non-targeted (ToF)	x
MALONATE	IROA MSMLS	Non-targeted (ToF)	x
MALTOSE	IROA MSMLS	Non-targeted (ToF)	x
MANDELIC ACID	IROA MSMLS	Non-targeted (ToF)	x
MANNITOL	IROA MSMLS	Non-targeted (ToF)	x
MANNOSE	IROA MSMLS	Non-targeted (ToF)	x
MELANIN	IROA MSMLS	Non-targeted (ToF)	x
MELATONIN	IROA MSMLS	Non-targeted (ToF)	x
MELIBIOSE	IROA MSMLS	Non-targeted (ToF)	x
MENAQUINONE	IROA MSMLS	Non-targeted (ToF)	x
MERCAPTOPYRUVATE	IROA MSMLS	Non-targeted (ToF)	x
MESO-TARTARIC ACID	IROA	Non-targeted (ToF)	x

	MSMLS		
MESOXALATE	IROA MSMLS	Non-targeted (ToF)	x
METHIONINE	IROA MSMLS	Non-targeted (ToF)	x
METHYL ACETOACETATE	IROA MSMLS	Non-targeted (ToF)	x
METHYL BETA-D-GALACTOSIDE	IROA MSMLS	Non-targeted (ToF)	x
METHYL INDOLE-3-ACETATE	IROA MSMLS	Non-targeted (ToF)	x
METHYL VANILLATE	IROA MSMLS	Non-targeted (ToF)	x
METHYLGUANDINE	IROA MSMLS	Non-targeted (ToF)	x
METHYLMALONATE	IROA MSMLS	Non-targeted (ToF)	x
MEVALOLACTONE	IROA MSMLS	Non-targeted (ToF)	x
MONO-ETHYL MALONATE	IROA MSMLS	Non-targeted (ToF)	x
MONO-METHYL GLUTARATE	IROA MSMLS	Non-targeted (ToF)	x
MYO-INOSITOL	IROA MSMLS	Non-targeted (ToF)	x
MYRISTIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
MYRISTIC ACID	IROA MSMLS	Non-targeted (ToF)	x
MYRISTOLEIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
N ALPHA-ACETYL-L-LYSINE	IROA MSMLS	Non-targeted (ToF)	x
N(PAI)-METHYL-L-HISTIDINE	IROA MSMLS	Non-targeted (ToF)	x
N,N-DIMETHYL-1,4-PHENYLENEDIAMINE	IROA MSMLS	Non-targeted (ToF)	x
N,W-METHYLTRYPTAMINE	IROA MSMLS	Non-targeted (ToF)	x
N1-ACETYLSPERMINE	IROA	Non-targeted (ToF)	x

	MSMLS		
N-ACETYL-D-GALACTOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-D-GLUCOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-DL-GLUTAMIC ACID	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-DL-METHIONINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-DL-SERINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-D-MANNOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-D-TRYPTOPHAN	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYLGLYCINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-L-ALANINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-L-ASPARTIC ACID	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-L-CYSTEINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYL-L-PHENYLALANINE	IROA MSMLS	Non-targeted (ToF)	x
N-ACETYLNEURAMINATE	IROA MSMLS	Non-targeted (ToF)	x
NAD	IROA MSMLS	Non-targeted (ToF)	x
N-ALPHA-ACETYL-L-ASPARAGINE	IROA MSMLS	Non-targeted (ToF)	x
N-AMIDINO-ASPARTATE	IROA MSMLS	Non-targeted (ToF)	x
NERVONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
N-FORMYLGLYCINE	IROA MSMLS	Non-targeted (ToF)	x
NICOTINAMIDE HYPOXANTHINE	IROA MSMLS	Non-targeted (ToF)	x

DINUCLEOTIDE			
NICOTINAMIDE MONONUCLEOTIDE	IROA MSMLS	Non-targeted (ToF)	x
NICOTINATE	IROA MSMLS	Non-targeted (ToF)	x
NICOTINE	IROA MSMLS	Non-targeted (ToF)	x
NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
NITRO-L-TYROSINE	IROA MSMLS	Non-targeted (ToF)	x
N-METHYL-D-ASPARTIC ACID	IROA MSMLS	Non-targeted (ToF)	x
N-METHYL-L-GLUTARATE	IROA MSMLS	Non-targeted (ToF)	x
NONANOATE	IROA MSMLS	Non-targeted (ToF)	x
NORADRENALINE	IROA MSMLS	Non-targeted (ToF)	x
NOREPINEPRHINE	Sigma-Aldrich	Targeted (QqQ)	x
NORLEUCINE	IROA MSMLS	Non-targeted (ToF)	x
NORMETANEPHRINE	IROA MSMLS	Non-targeted (ToF)	x
NORMETANEPRHINE	Sigma-Aldrich	Targeted (QqQ)	x
NORVALINE	IROA MSMLS	Non-targeted (ToF)	x
O-ACETYL-L-SERINE	IROA MSMLS	Non-targeted (ToF)	x
OCTANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
OCTOPAMINE	IROA MSMLS	Non-targeted (ToF)	x
O-PHOSPHO-DL-SERINE	IROA MSMLS	Non-targeted (ToF)	x
O-PHOSPHO-L-SERINE	IROA MSMLS	Non-targeted (ToF)	x
OPHTHALMIC ACID	IROA MSMLS	Non-targeted (ToF)	x

ORNITHINE	IROA MSMLS	Non-targeted (ToF)	x
OROTATE	IROA MSMLS	Non-targeted (ToF)	x
OROTIC ACID	IROA MSMLS	Non-targeted (ToF)	x
O-SUCCINYL-L-HOMOSERINE	IROA MSMLS	Non-targeted (ToF)	x
OXALIC ACID	IROA MSMLS	Non-targeted (ToF)	x
OXALOACETATE	IROA MSMLS	Non-targeted (ToF)	x
OXALOMALIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PALATINOSE	IROA MSMLS	Non-targeted (ToF)	x
PALMITIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
PANTOLACTONE	IROA MSMLS	Non-targeted (ToF)	x
PARAXANTHINE	IROA MSMLS	Non-targeted (ToF)	x
PENTADECANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
PENTANOATE	IROA MSMLS	Non-targeted (ToF)	x
PETROSELINIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PHENETHYLAMINE	IROA MSMLS	Non-targeted (ToF)	x
PHENOL	IROA MSMLS	Non-targeted (ToF)	x
PHENYL ACETATE	IROA MSMLS	Non-targeted (ToF)	x
PHENYLACETALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
PHENYLACETIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PHENYLALANINE	IROA MSMLS	Non-targeted (ToF)	x

PHENYLETHANOLAMINE	IROA MSMLS	Non-targeted (ToF)	x
PHOSPHO(ENOL)PYRUVIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PHOSPHOCHOLINE CHLORIDE	IROA MSMLS	Non-targeted (ToF)	x
PHOSPHOCREATINE	IROA MSMLS	Non-targeted (ToF)	x
PHOSPHONOACETATE	IROA MSMLS	Non-targeted (ToF)	x
PHOSPO(ENOL)PYRUVIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PHYLLOQUINONE	IROA MSMLS	Non-targeted (ToF)	x
PHYTIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PIMELIC ACID	IROA MSMLS	Non-targeted (ToF)	x
PIPECOLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
POTASSIUM CITRAMALATE	IROA MSMLS	Non-targeted (ToF)	x
POTASSIUM SORBATE	IROA MSMLS	Non-targeted (ToF)	x
PRENOL	IROA MSMLS	Non-targeted (ToF)	x
PROPANAL	IROA MSMLS	Non-targeted (ToF)	x
PROPANOATE	IROA MSMLS	Non-targeted (ToF)	x
PROPENOATE	IROA MSMLS	Non-targeted (ToF)	x
PROPYNOATE	IROA MSMLS	Non-targeted (ToF)	x
PROTOPORPHYRIN	IROA MSMLS	Non-targeted (ToF)	x
PSICOSE	IROA MSMLS	Non-targeted (ToF)	x
PTERINE	IROA	Non-targeted (ToF)	x

	MSMLS		
PURINE	IROA MSMLS	Non-targeted (ToF)	x
PYRAZOLE	IROA MSMLS	Non-targeted (ToF)	x
PYRIDINE-2,3-DICARBOXYLATE	IROA MSMLS	Non-targeted (ToF)	x
PYRIDOXAL	IROA MSMLS	Non-targeted (ToF)	x
PYRIDOXAL 5'-PHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
PYRIDOXAMINE	IROA MSMLS	Non-targeted (ToF)	x
PYRIDOXINE	IROA MSMLS	Non-targeted (ToF)	x
PYRIMIDINE	IROA MSMLS	Non-targeted (ToF)	x
PYRROLE-2-CARBOXYLATE	IROA MSMLS	Non-targeted (ToF)	x
PYRUVATE	IROA MSMLS	Non-targeted (ToF)	x
PYRUVIC ALDEHYDE	IROA MSMLS	Non-targeted (ToF)	x
QUINATE	IROA MSMLS	Non-targeted (ToF)	x
QUINOLINE	IROA MSMLS	Non-targeted (ToF)	x
RAC-GLYCEROL-L-MYRISTATE	IROA MSMLS	Non-targeted (ToF)	x
RESORCINOL	IROA MSMLS	Non-targeted (ToF)	x
RETINOATE	IROA MSMLS	Non-targeted (ToF)	x
RETINOL	IROA MSMLS	Non-targeted (ToF)	x
RETINYL PALMITATE	IROA MSMLS	Non-targeted (ToF)	x
RHAMNOSE	IROA MSMLS	Non-targeted (ToF)	x

RIBITOL	IROA MSMLS	Non-targeted (ToF)	x
RIBOFLAVIN	IROA MSMLS	Non-targeted (ToF)	x
RIBOSE	IROA MSMLS	Non-targeted (ToF)	x
RIBULOSE 1,5-BISPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
ROSMARINIC ACID	IROA MSMLS	Non-targeted (ToF)	x
S-(5'-ADENOSYL)-L-HOMOCYSTEINE	IROA MSMLS	Non-targeted (ToF)	x
S-(5'-ADENOSYL)-L-METHIONINE	IROA MSMLS	Non-targeted (ToF)	x
SACCHARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
SALICYLAMIDE	IROA MSMLS	Non-targeted (ToF)	x
SALICYLIC ACID	IROA MSMLS	Non-targeted (ToF)	x
SARCOSINE	IROA MSMLS	Non-targeted (ToF)	x
S-CARBOXYMETHYL-L-CYSTEINE	IROA MSMLS	Non-targeted (ToF)	x
SELENOCYSTAMINE	IROA MSMLS	Non-targeted (ToF)	x
SELENOMETHIONINE	IROA MSMLS	Non-targeted (ToF)	x
SERINE	IROA MSMLS	Non-targeted (ToF)	x
SEROTONIN CREATININE COMPLEX	IROA MSMLS	Non-targeted (ToF)	x
SEROTONIN HYDROCHLORIDE	IROA MSMLS	Non-targeted (ToF)	x
S-HEXYL-GLUTATHIONE	IROA MSMLS	Non-targeted (ToF)	x
SHIKIMATE	IROA MSMLS	Non-targeted (ToF)	x
SN-GLYCEROL 3-PHOSPHATE	IROA	Non-targeted (ToF)	x

BIS (CYCLOHEXYLAMMONIUM)	MSMLS		
SN-GLYCEROL-3-PHOSPHOCHOLINE	IROA MSMLS	Non-targeted (ToF)	x
SODIUM BENZOATE	IROA MSMLS	Non-targeted (ToF)	x
SODIUM D-GLUCONATE	IROA MSMLS	Non-targeted (ToF)	x
SODIUM PHENYLPYRUVATE	IROA MSMLS	Non-targeted (ToF)	x
SODIUM PROPIONATE	IROA MSMLS	Non-targeted (ToF)	x
SODIUM TAUROLITHOCHOLATE	IROA MSMLS	Non-targeted (ToF)	x
SORBITOL	IROA MSMLS	Non-targeted (ToF)	x
SORBOSE	IROA MSMLS	Non-targeted (ToF)	x
SPERMIDINE	IROA MSMLS	Non-targeted (ToF)	x
SPERMINE	IROA MSMLS	Non-targeted (ToF)	x
SPHINGANINE	IROA MSMLS	Non-targeted (ToF)	x
SPHINGOMYELIN	IROA MSMLS	Non-targeted (ToF)	x
SQUALENE	IROA MSMLS	Non-targeted (ToF)	x
STACHYOSE HYDRATE	IROA MSMLS	Non-targeted (ToF)	x
SUBERIC ACID	IROA MSMLS	Non-targeted (ToF)	x
SUCCINATE	IROA MSMLS	Non-targeted (ToF)	x
SUCCINATE	IROA MSMLS	Non-targeted (ToF)	x
SUCROSE	IROA MSMLS	Non-targeted (ToF)	x
TAGATOSE	IROA	Non-targeted (ToF)	x

	MSMLS		
TARTARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
TARTARIC ACID	IROA MSMLS	Non-targeted (ToF)	x
TAURINE	IROA MSMLS	Non-targeted (ToF)	x
TETRAHYDROFOLATE	IROA MSMLS	Non-targeted (ToF)	x
THEOBROMINE	IROA MSMLS	Non-targeted (ToF)	x
THEOPHYLLINE	IROA MSMLS	Non-targeted (ToF)	x
THIAMINE	IROA MSMLS	Non-targeted (ToF)	x
THIAMINE MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
THIAMINE PYROPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
THIOACETATE	IROA MSMLS	Non-targeted (ToF)	x
THIOUREA	IROA MSMLS	Non-targeted (ToF)	x
THREONINE	IROA MSMLS	Non-targeted (ToF)	x
THYMIDINE	IROA MSMLS	Non-targeted (ToF)	x
THYMIDINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
THYMIDINE-5'-DIPHOSPHO-ALPHA-D-GLUCOSE	IROA MSMLS	Non-targeted (ToF)	x
THYMINE	IROA MSMLS	Non-targeted (ToF)	x
THYROTROPIN RELEASING HORMONE	IROA MSMLS	Non-targeted (ToF)	x
THYROXINE	IROA MSMLS	Non-targeted (ToF)	x
TOCOPHEROL	IROA MSMLS	Non-targeted (ToF)	x

TRANS-4-HYDROXYPROLINE	IROA MSMLS	Non-targeted (ToF)	x
TRANS-CYCLOHEXANE-1,2-DIOL	IROA MSMLS	Non-targeted (ToF)	x
TRIDECANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
TRIGONELLINE	IROA MSMLS	Non-targeted (ToF)	x
TRIIODOTHYRONINE	IROA MSMLS	Non-targeted (ToF)	x
TRIMETHYLAMINE	IROA MSMLS	Non-targeted (ToF)	x
TRYPTAMINE	IROA MSMLS	Non-targeted (ToF)	x
TRYPTOPHAN	IROA MSMLS	Non-targeted (ToF)	x
TRYPTOPHAN	IROA MSMLS	Non-targeted (ToF)	x
TYRAMINE	Sigma-Aldrich	Targeted (QqQ)	x
TYRAMINE	IROA MSMLS	Non-targeted (ToF)	x
TYROSINE	IROA MSMLS	Non-targeted (ToF)	x
UNDECANOIC ACID	Sigma-Aldrich	Non-targeted (ToF)	x
URATE	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-DIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-DIPHOSPHOGALACTOSE	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-DIPHOSPHOGLUCOSE	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-DIPHOSPHOGLUCURONIC ACID	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-DIPHOSPHO-N-ACETYLGALACTOSAMINE	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-DIPHOSPHO-N-ACETYLGLUCOSAMINE	IROA MSMLS	Non-targeted (ToF)	x

URIDINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
URIDINE 5'-TRIPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
VITAMIN B12	IROA MSMLS	Non-targeted (ToF)	x
VITAMIN D2	IROA MSMLS	Non-targeted (ToF)	x
XANTHOSINE	IROA MSMLS	Non-targeted (ToF)	x
XANTHOSINE 5'-MONOPHOSPHATE	IROA MSMLS	Non-targeted (ToF)	x
XANTHURENIC ACID	IROA MSMLS	Non-targeted (ToF)	x
XYLITOL	IROA MSMLS	Non-targeted (ToF)	x
XYLOSE	IROA MSMLS	Non-targeted (ToF)	x

Table S2 Metabolite identified in the different exposure groups. Fold change and *p*-values correc QC LC-ToF and Targeted QqQ analysis ted by false discovery rate (FDR) are reported. Marked in red the metabolites significantly different for more than three exposure concentrations and in gree QC LC-ToF and Targeted QqQ analysis n the *p*-values below 0.05 are marked.

METABOLITE	Fold change	*p*-value (FDR)	Fold change	p-value (FDR)	Fold change	p-value (FDR)	Fold change	p-value (FDR)
	ctrl vs 0.1 µg/L		Ctrl vs 1.0 µg/L		ctrl vs 10 µg/L		ctrl vs 100 µg/L	
3-methyl-2-oxovaleric acid	-1.81	0.028	-2.85	0.001	-3.31	0.000	-2.70	0.000
4-guanidino-butanoate	1.44	0.320	4.08	0.002	2.92	0.012	4.06	0.002
4-methyl-2-oxovaleric acid	2.86	0.074	3.09	0.049	4.91	0.022	2.09	0.127
5-HIAA	1.19	0.651	1.29	0.755	1.00	0.620	1.33	0.552
5-methylthioadenosine	-1.57	0.044	1.45	0.193	1.18	0.529	2.44	0.008
Acetylcholine	-1.66	0.026	-2.24	0.013	-1.75	0.043	-1.58	0.008
Adenine	1.11	0.807	1.39	0.259	1.15	0.572	2.07	0.012
Arachidic acid	-2.83	0.382	nd	nd	nd	nd	-6.04	0.063
Arachidonic acid	1.32	0.491	nd	nd	-1.40	0.216	-1.35	0.113
Betaine	1.42	0.009	1.18	0.136	1.18	0.114	1.45	0.002
Carnitine	2.10	0.000	1.47	0.002	1.76	0.000	1.61	0.003
Choline	-1.19	0.026	2.24	0.005	2.01	0.022	1.36	0.008
cis-10-heptadecenoic acid	-30.73	0.019	-25.19	0.007	-1.78	0.559	-14.95	0.099
cis-11,14-eicosadienoic acid	-1.43	0.138	nd	nd	-1.00	0.000	-1.34	0.262
cis-11,17-eicosatrienoic acid	-1.27	0.195	nd	nd	nd	nd	-1.02	0.988
cis-11-eicosenoic acid	-12.12	0.000	nd	nd	-1.00	0.000	-5.26	0.079
cis-13,16-docosadienoic acid	1.00	0.998	nd	nd	nd	nd	-1.07	0.926
cis-15-tetracosenoic acid	-1.03	0.983	nd	nd	nd	nd	1.32	0.429
cis-5,17-eicosapentaenoic acid	1.16	0.664	nd	nd	-1.00	0.000	-1.01	0.988
cis-8,14-eicosatrienoic acid	-2.76	0.071	nd	nd	nd	nd	-2.51	0.260
Creatinine	1.17	0.418	1.70	0.014	1.74	0.013	1.80	0.003

Diethanolamine	-1.89	0.053	-1.24	0.261	1.07	0.775	1.17	0.643
Dimethylbenzimidazole	nd	nd	5.25	0.002	nd	nd	nd	nd
Docosahexanoic acid	-2.39	0.005	nd	nd	nd	nd	1.06	0.843
Elaidic acid	-9.73	0.000	-8.68	0.000	-6.75	0.006	-7.61	0.001
Erucic acid	-1.01	0.998	nd	nd	nd	nd	-1.00	0.988
GABA	1.26	0.262	3.28	0.245	4.44	0.624	5.38	0.036
Glutamate	-2.72	0.041	1.35	0.442	-1.71	0.389	1.95	0.131
Glutamine	-2.06	0.078	8.36	0.023	2.04	0.143	-2.47	0.079
Guanine	1.10	0.807	1.60	0.293	1.16	0.756	-1.05	0.893
Guanosine	-2.92	0.002	-2.46	0.001	-1.94	0.020	-1.46	0.003
Henicosanoic acid	-1.26	0.195	nd	nd	nd	nd	-1.13	0.592
Heptadecanoic acid	-26.11	0.004	-29.01	0.000	-35.44	0.011	-28.48	0.000
Hexadecanol	-1.13	0.962	1.13	0.801	1.13	0.801	1.75	0.503
Histamine	-1.05	0.651	1.03	0.755	-1.09	0.620	-1.07	0.552
Histidine	4.34	0.761	1.37	0.685	-1.07	0.000	-1.19	0.001
Hypoxanthine	-1.90	0.011	-1.05	0.805	-1.73	0.097	-1.10	0.702
Indole-3-acetaldehyde	-1.14	0.807	nd	nd	1.39	0.475	nd	nd
Inosine	-2.33	0.004	-1.95	0.011	-1.93	0.011	-1.46	0.017
Leucine	1.31	0.145	1.52	0.001	1.31	0.174	1.67	0.002
Linoleic acid	-1.32	0.244	-9.13	0.000	-3.18	0.216	-6.89	0.049
Linolelaidic acid	-1.88	0.117	-10.94	0.005	-3.84	0.216	-12.04	0.012
L-proline	1.12	0.540	1.58	0.008	1.14	0.615	1.46	0.024
N6-(delta2-isopentenyl)-adenine	1.09	0.807	1.05	0.911	2.39	0.048	1.05	0.893
N6,N6,N6-trimethyl-L-lysine	1.75	0.222	2.16	0.067	1.89	0.123	1.91	0.090
N-acetyl-L-leucine	1.20	0.778	2.36	0.080	2.19	0.117	2.36	0.090
N-acetylputrescine	1.35	0.156	1.24	0.109	1.31	0.141	1.11	0.680
N-acetylserotonin	1.44	0.666	1.20	0.764	1.06	0.883	1.20	0.716
Nicotinamide	1.53	0.456	-1.04	0.956	5.29	0.048	nd	nd

O-acetyl-L-carnitine	1.27	0.540	1.06	0.911	2.37	0.040	1.20	0.680
Oleic acid	-14.94	0.000	-14.40	0.000	-4.06	0.005	-14.12	0.000
Palmitoleic acid	nd	nd	nd	nd	-1.47	0.028	nd	nd
Phenylalanine	4.17	0.005	4.27	0.002	2.01	0.005	1.93	0.018
Picolinic acid	1.20	0.456	1.21	0.342	1.16	0.484	1.40	0.085
Pipecolinic acid	1.22	0.415	1.25	0.293	1.34	0.114	1.94	0.006
Putrescine	1.43	0.105	1.47	0.067	1.85	0.022	1.84	0.008
Serotonin	-1.63	0.177	-2.29	0.103	-2.58	0.173	-6.28	0.036
Spermidine	1.28	0.136	1.03	0.911	1.55	0.022	1.54	0.024
Stearic acid	-42.80	0.000	-40.42	0.000	-31.92	0.001	-37.25	0.000
Tetracosanoic acid	-1.37	0.094	nd	nd	nd	nd	-1.02	0.988
Thiopurine S-methylester	1.08	0.807	1.03	0.931	1.04	0.846	1.04	0.893
Tricosanoic acid	-1.13	0.558	nd	nd	nd	nd	-1.00	0.000
Tryptophan	-1.18	0.446	-1.62	0.025	-1.81	0.094	-1.75	0.020
Tryptophanamide	1.36	0.412	nd	nd	nd	nd	nd	nd
Tyrosine	1.08	0.761	-1.13	0.521	-1.44	0.303	1.17	0.521
Uracil	-1.37	0.023	-1.20	0.081	1.15	0.550	1.11	0.680
Uridine	-1.78	0.002	-1.98	0.000	-1.42	0.011	-1.25	0.138
Uridine-5-monoposphate	-1.19	0.778	-2.25	0.062	-2.00	0.070	-1.51	0.552
Urocanate	-3.17	0.157	1.13	0.470	-2.63	0.080	1.10	0.684
Valine	1.39	0.016	1.60	0.049	1.18	0.146	1.52	0.002
Xanthine	-1.26	0.462	-1.10	0.291	1.08	0.608	1.15	0.371
α-linolenic acid	-1.97	0.162	nd	nd	-2.50	0.039	-1.84	0.072
γ-linolenic acid	-8.51	0.015	-13.73	0.005	-9.72	0.033	-14.38	0.000

Table S3 Area under the ROC curve (AUC ± Std. error) for metabolites > 0.8 in more than three exposure concentrations.

Metabolite	Imidacloprid exposure concentration (µg/L)			
	0.1	**1.0**	**10**	**100**
Carnitine	0.89 ± 0.07	0.86 ± 0.09	0.84 ± 0.11	0.90 ± 0.07
Elaidic acid	0.93 ± 0.07	1 ± 0.01	1 ± 0.01	0.86 ± 0.10
γ-linolenic acid	0.86 ± 0.10	1 ± 0.01	1 ± 0.01	0.83 ± 0.11
Linolelaidic acid	0.8 ± 0.15	0.94 ± 0.07	0.88 ± 0.12	0.9 ± 0.10
Stearic acid	0.93 ± 0.07	0.88 ± 0.12	0.88 ± 0.12	0.86 ± 0.12
3-methyl-2-oxovaleric acid	0.7 ± 0.12	0.97 ± 0.03	0.9 ± 0.08	1 ± 0.01
Acetylcholine	0.86 ± 0.11	0.96 ± 0.05	1 ± 0.00	0.7 ± 0.19
Creatinine	0.65 ± 0.12	0.88 ± 0.07	0.91 ± 0.06	0.95 ± 0.05
Guanosine	0.85 ± 0.10	0.95 ± 0.04	0.83 ± 0.09	0.79 ± 0.11
Inosine	0.81 ± 0.10	0.85 ± 0.08	0.84 ± 0.90	0.77 ± 0.11
Phenylalanine	0.59 ± 0.14	0.88 ± 0.07	0.84 ± 0.09	0.96 ± 0.04
Tryptophan	0.7 ± 0.16	0.93 ± 0.07	0.8 ± 0.19	1 ± 0.01

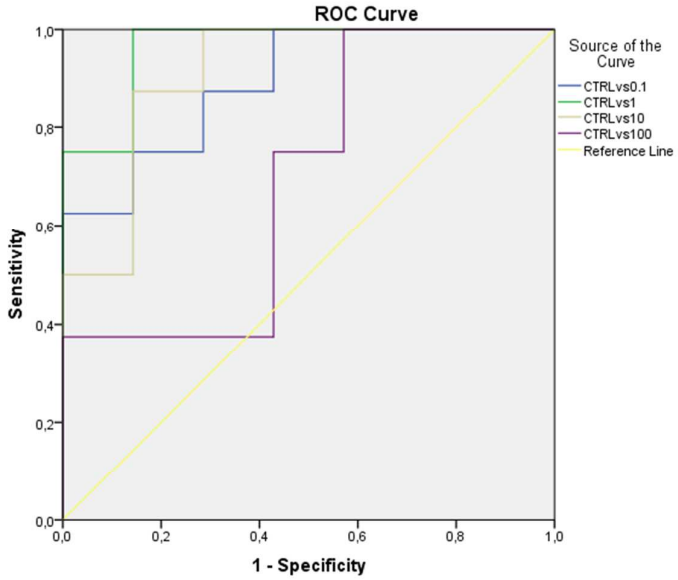


Figure S8 ROC curves for acetylcholine in the CNS of *L. stagnalis* exposed to increasing concentration of imidacloprid.

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