

Metabolism

International Edition: DOI: 10.1002/anie.201700736
German Edition: DOI: 10.1002/ange.201700736

Methylated Phenylarsenical Metabolites Discovered in Chicken Liver

Hanyong Peng, Bin Hu,* Qingqing Liu, Jinhua Li, Xing-Fang Li, Hongquan Zhang, and X. Chris Le*

Abstract: We report the discovery of three toxicologically relevant methylated phenylarsenical metabolites in the liver of chickens fed 3-nitro-4-hydroxyphenylarsonic acid (ROX), a feed additive in poultry production that is still in use in several countries. Methyl-3-nitro-4-hydroxyphenylarsonic acid (methyl-ROX), methyl-3-amino-4-hydroxyphenylarsonic acid (methyl-3-AHPAA), and methyl-3-acetamido-4-hydroxyphenylarsonic acid (or methyl-N-acetyl-ROX, methyl-N-AHPAA) were identified in such chicken livers, and the concentration of methyl-ROX was as high as $90 \mu\text{g kg}^{-1}$, even after a five-day clearance period. The formation of these newly discovered methylated metabolites from reactions involving trivalent phenylarsonous acid substrates, *S*-adenosylmethionine, and the arsenic (+3 oxidation state) methyltransferase enzyme As3MT suggests that these compounds are formed by addition of a methyl group to a trivalent phenylarsenical substrate in an enzymatic process. The IC_{50} values of the trivalent phenylarsenical compounds were 300–30 000 times lower than those of the pentavalent phenylarsenicals.

Arsenic consistently ranks first on the priority list of environmental contaminants because of the occurrence, persistence, and toxicity of various arsenic compounds. Chronic exposure to high concentrations of arsenic puts more than 100 million people around the world at risk of developing cancer and other adverse health effects.^[1] The general population is exposed to arsenic mainly through ingestion of water and food. The practice of feeding 3-nitro-4-hydroxyphenylarsonic acid (roxarsone, ROX; see the Supporting Information, Figure S1 for its structure) to poultry and swine lasted for more than 60 years^[2] before the European Union and the United States stopped its use. Many other countries continue to use phenylarsenicals in the poultry industry.^[3] Ingestion of such poultry meat and meat products results in exposure to residual arsenic. However, it remains unclear how

ROX may be metabolized and potentially produce new arsenic species of toxicological significance.

To gain an understanding of the possible metabolism of ROX, we have conducted a controlled feeding study that involved 1600 chickens of two common commercial strains. The chickens were given either a standard control feed or a ROX-supplemented feed. Chicken liver samples were collected for characterization of arsenic species. We previously identified eight arsenic species in chicken liver, breast meat, and waste.^[4] However, several arsenic-containing species were not identified, and their chemical nature remained unknown. We herein report the discovery of three methylated phenylarsenical metabolites of ROX in chicken livers and show the toxicological implications of these new arsenic metabolites because of the involvement of possible enzymatic methylation processes in the formation of these metabolites. Using chromatographic separation coupled with both elemental and molecular mass spectrometry techniques,^[5] we identified a group of new arsenic metabolites, namely methylated 3-nitro-4-hydroxyphenylarsonic acid (methyl-ROX), 3-amino-4-hydroxyphenylarsonic acid (methyl-3-AHPAA), and 3-acetamido-4-hydroxyphenylarsonic acid (methyl-N-AHPAA; see Figure S1), in liver samples of chickens that had been fed ROX. We further demonstrated the involvement of an arsenic methyltransferase (As3MT) in the methylation of the trivalent substrates ROX^{III} and $3\text{-AHPAA}^{\text{III}}$ to the corresponding methylated products of these phenylarsenicals. These intermediate trivalent arsenicals and methyl- ROX^{III} are much more cytotoxic than their pentavalent counterparts, with IC_{50} values in T24 cells that are lower by factors of 300–30 000. Therefore, the detection of these methylation metabolites and the implications of the trivalent intermediates are toxicologically significant in relation to human exposure to arsenic.

We first used HPLC and inductively coupled plasma mass spectrometry (ICP-MS) to identify arsenic species in the extracts of liver samples from chickens fed either a control diet or a ROX-supplemented diet. Representative chromatograms in Figure 1 show that ROX (Figure 1a) is present in the ROX-fed chicken (Figure 1c) and not in the control chicken (Figure 1b). Because of the selective ion monitoring of m/z 75 (As^+), the HPLC-ICP-MS analyses revealed the presence of eleven arsenic species in the ROX-fed chicken (Figure 1c) and traces of five arsenic species in the control chicken (Figure 1b). We then separately added a known amount of arsenobetaine (AsB), arsenite (As^{III}), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenate (As^{V}), 3-AHPAA, N-AHPAA, and ROX to aliquots of the chicken liver sample. Repeated HPLC-ICP-MS analyses of these spiked samples (Figure 1d–k) showed that the chromatographic retention times of these arsenic species match with

[*] Dr. H. Peng, Prof. B. Hu
Key Laboratory of Analytical Chemistry for Biology and Medicine
(Ministry of Education)
Department of Chemistry, Wuhan University
Wuhan, 430072 (China)
E-mail: binhu@whu.edu.cn

Dr. H. Peng, Dr. Q. Liu, J. Li, Prof. X.-F. Li, Prof. H. Zhang,
Prof. X. C. Le
Division of Analytical and Environmental Toxicology
Department of Laboratory Medicine and Pathology
Faculty of Medicine and Dentistry, University of Alberta
Edmonton, Alberta, T6G 2G3 (Canada)
E-mail: xc.le@ualberta.ca

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:
<https://doi.org/10.1002/anie.201700736>.

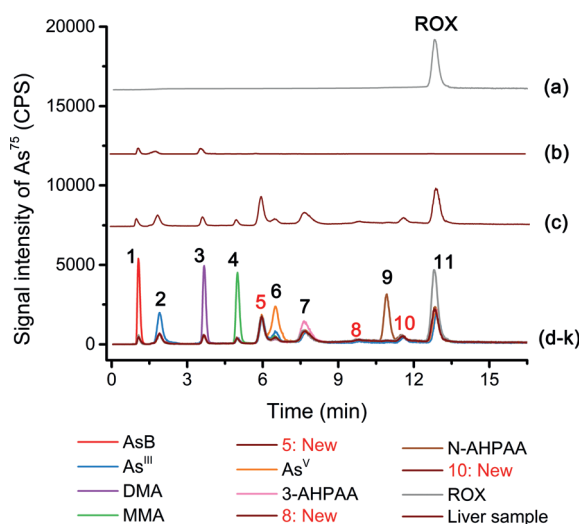


Figure 1. Chromatograms from HPLC-ICP-MS analyses of ROX and chicken liver samples from a ROX-fed chicken and a control chicken. a) ROX standard. b) A liver sample from a control chicken fed the basal diet. c) A liver sample from a chicken fed the ROX-containing diet. d–k) Analysis of the same chicken liver sample after replicate aliquots were separately spiked with AsB (d, peak 1), As^{III} (e, peak 2), DMA (f, peak 3), MMA (g, peak 4), As^V (h, peak 5), 3-AHPAA (i, peak 6), N-AHPAA (j, peak 7), 3-AHPAA (k, peak 8), N-AHPAA (l, peak 9), and ROX (m, peak 10). Peaks 5, 8, and 10 did not correspond to any available arsenic standards.

eight of the eleven arsenic species detected in the chicken liver sample. These results, which are in agreement with previous reports,^[4a,6] indicate the presence of 3-AHPAA, N-AHPAA, and ROX in ROX-fed chicken liver, in addition to the five arsenic species (AsB, As^{III}, DMA, MMA, and As^V) that are commonly present as background in both control and ROX-fed chicken liver samples. However, the retention times of three arsenic species (peaks 5, 8, and 10) did not match those of any available arsenic standards. These new arsenic species have thus not been reported previously. Therefore, we subsequently focused on characterizing these new arsenic metabolites arising from ROX.

To identify these new metabolites of unknown nature without standards, we developed a strategy that complemented HPLC-ICP-MS with a series of electrospray ionization mass spectrometry (ESI-MS) measurements (Figure S2 and Section S6). This approach made use of the characteristic fragment ion information of the known arsenic compounds (Table S1) to build a precursor ion scan method in the HPLC-ESI-MS analysis of the sample. Parallel HPLC-ICP-MS analysis of the same sample (Figure S3A) provided the retention time of the arsenic-containing peak. This approach allowed us to tentatively identify peak 10 in the HPLC-ICP-MS and HPLC-ESI-MS chromatograms as methyl-ROX. The arsenic-containing precursor ion at m/z 260 from the detection of peak 10 showed characteristic arsenic-containing fragment ions at m/z 91 (AsO[−]), 107 (AsO₂[−]), 121 (CH₂AsO₂[−]), and 123 (AsO₃[−]; Figure S3C).

We further complemented the molecular and fragment ion information with accurate mass measurements by high-resolution time-of-flight mass spectrometry (TOF-MS). Figure 2A shows a representative mass spectrum from the

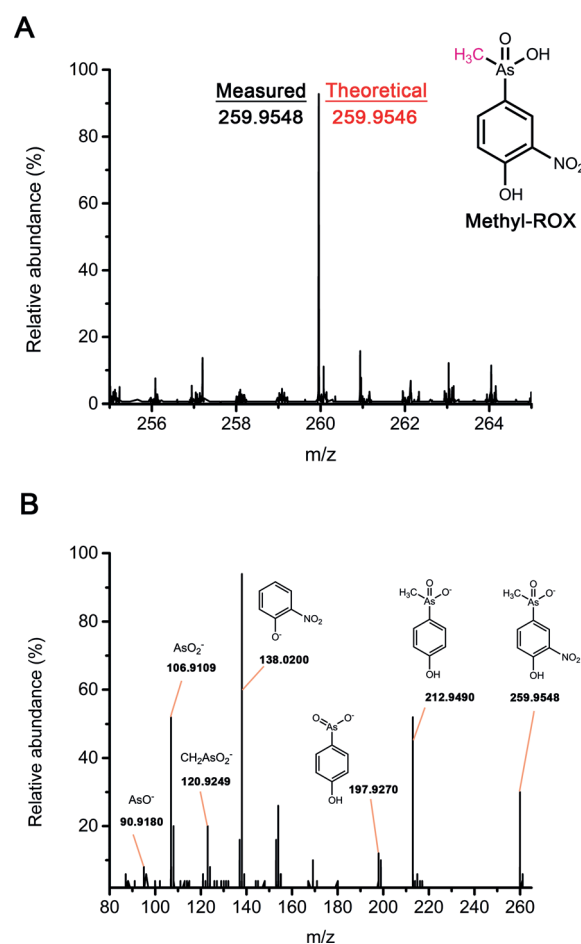


Figure 2. Identification of peak 10 in liver extracts using ESI-TOF-MS analysis. A) High-resolution TOF-MS analysis shows the accurate mass of peak 10 at m/z 259.9548, with a mass error of 0.8 ppm compared to the theoretical mass (259.9546) of methyl-ROX (C₇H₇NaAsO₃[−]). B) The product ion spectrum of m/z 259.9548 shows the specific fragment peaks.

detection of peak 10 (Figure S3) in the analysis of the chicken liver extract using HPLC-ESI-TOF-MS. The compound of interest [$M-1$][−] has an accurate mass of m/z 259.9548, which matches that of methyl-ROX. The measured value (259.9548) is in excellent agreement with the theoretical value (259.9546) for methyl-ROX, deviating by a very small mass error ($\Delta m/m$) of only 0.8 ppm. The spectrum (Figure 2B) generated from the precursor ion at m/z 259.9548 also showed expected characteristic fragment ions at m/z 90.9180 (AsO[−]), 106.9109 (AsO₂[−]), 120.9249 (CH₂AsO₂[−]), 138.0200 (C₆H₄NO₃[−]), and 212.9490 (C₇H₆AsO₃[−]), supporting the identification of methyl-ROX. The measured and theoretical values of these fragment ions are in good agreement (Table S2).

We then synthesized methyl-ROX (procedures shown in Section S7 and characterization shown in Figure S4 and Table S3) and used it to further confirm the detection of this compound in chicken liver. We combined HPLC separation with simultaneous detection by ICP-MS and ESI-MS (Figure 3A). Using this technique, we analyzed an extract of chicken liver as well as the same extract spiked with the synthesized methyl-ROX species. The element-specific ICP-

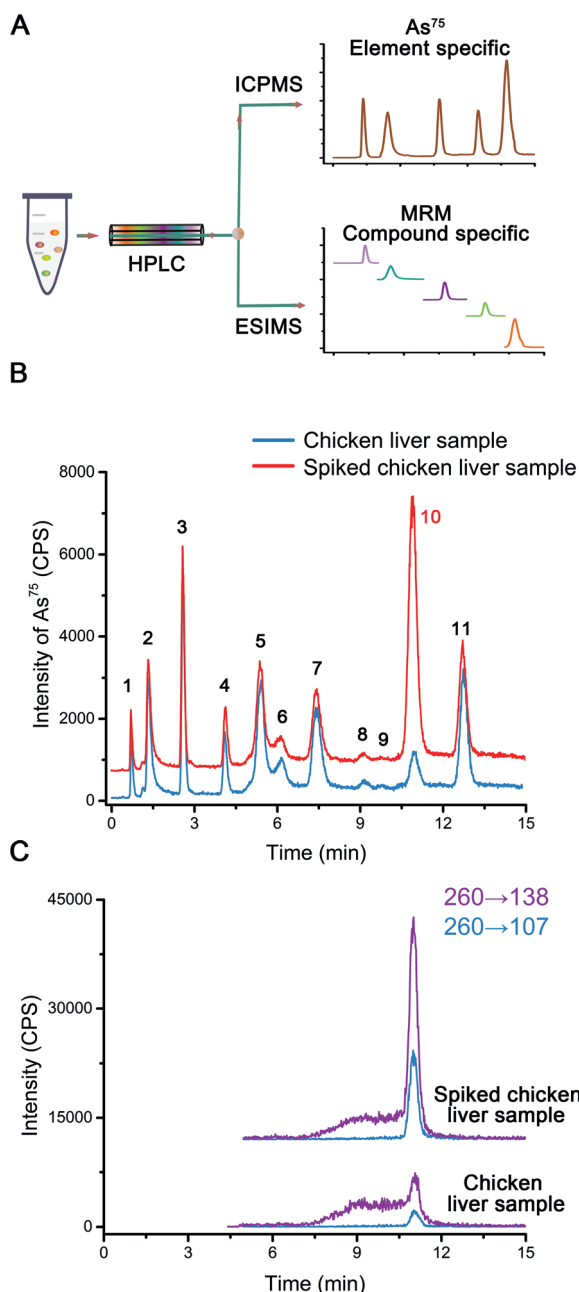


Figure 3. Identification of arsenic species by combining HPLC separation with simultaneous detection by both ICP-MS and ESI-MS. A) Features of ICP-MS and ESI-MS to provide complementary detection for HPLC. B) HPLC-ICP-MS analyses of a chicken liver sample and the same sample spiked with synthesized methyl-ROX. C) HPLC-ESI-MS analyses of a chicken liver sample and the same sample spiked with synthesized methyl-ROX. Two ion transitions (260/107 and 260/138) of methyl-ROX were monitored in the MRM mode.

MS detection of ^{75}As revealed the presence of eleven arsenic-containing compounds (Figure 3B). Analysis of the extract supplemented with the synthesized methyl-ROX shows an increase in peak 10, supporting that peak 10 is methyl-ROX. The simultaneous detection by ESI-MS (Figure 3C) revealed a consistent increase of both characteristic ion transitions (260/138 and 260/107; Table S4) in multiple reaction monitoring (MRM) mode. This peak has the same retention time as

peak 10 detected with ICP-MS (Figure 3B), further confirming the identity of peak 10 as methyl-ROX, a methylation metabolite of ROX.

Using the same strategy (Figure S2) and the complementary techniques, we also identified the other two new arsenic compounds as methyl-3-AHPAA (Figures S5–S8 and Tables S5 and S6) and methyl-*N*-AHPAA (Figures S9 and S10 and Table S7). Thus the three identified arsenic metabolites are a group of methylated analogues of ROX, 3-AHPAA, and *N*-AHPAA.

Having discovered the three new methylation metabolites of phenylarsenicals, we further investigated how these phenylarsenicals are methylated. Methylation of inorganic arsenic is known to involve arsenic (+3 oxidation state) methyltransferase (As3MT), which catalyzes the addition of a methyl group from *S*-adenosylmethionine (SAM, as the methyl donor) to trivalent arsenicals.^[7] As3MT has been shown to be responsible for the methylation of inorganic arsenic to methylarsenicals in experimental rats, mice, and algae.^[8] There is no report on the methylation of phenylarsenicals.

To test whether a similar pathway as for the methylation of inorganic arsenic can take place for the methylation of phenylarsenicals, we first incubated the trivalent phenylarsenicals as substrates with the As3MT enzyme and the methyl donor SAM, and monitored the formation of the methylated phenylarsenicals. Figure 4 shows experiments for testing the methylation of trivalent ROX^{III} to methyl-ROX (Figure 4A) and representative chromatograms from analyses of the treated reaction mixture at the beginning of the reaction and after 6 h (Figure 4B). Note that while the reaction used trivalent ROX^{III} as the substrate (Figure 4A), the chromatographic analysis involved pretreatment of the sample aliquots with 0.1% hydrogen peroxide. Thus the trivalent ROX^{III} was oxidized to the pentavalent ROX prior to analysis, and the peak of ROX in the chromatograms (Figure 4B) represents the trivalent ROX^{III} in the reaction mixture. HPLC analysis with both ICP-MS detection (Figure S11 A) and ESI-MS detection (Figure S11 B) showed the formation of methyl-ROX and its increasing amount over the 6 h period of reaction. The amount of methyl-ROX accounts for approximately 36% of the total arsenic in the reaction mixture (Figure 4C). Our positive control using MMA^{III} as a known substrate of As3MT^[9] provided the expected results. Approximately 50% of MMA^{III} was methylated to dimethylarsinic acid (DMA^V) in 3 h (Figure S12), which is consistent with previous results.^[10]

Similarly, the incubation of the trivalent 3-AHPAA^{III} substrate with the As3MT enzyme and the SAM methyl donor resulted in the formation of methyl-3-AHPAA (Figure S13). Both HPLC-ICP-MS and HPLC-ESI-MS measurements showed increasing amounts of methyl-3-AHPAA with increasing enzymatic reaction times from 0 to 6 h. More than 50% of the substrate 3-AHPAA^{III} had been converted into methyl-3-AHPAA after 6 h of the enzymatic reaction.

We further tested whether the pentavalent phenylarsenicals ROX and 3-AHPAA could serve as substrates for the As3MT-catalyzed methylation reaction. No methyl-ROX or methyl-3-AHPAA was detectable. Only when we added high

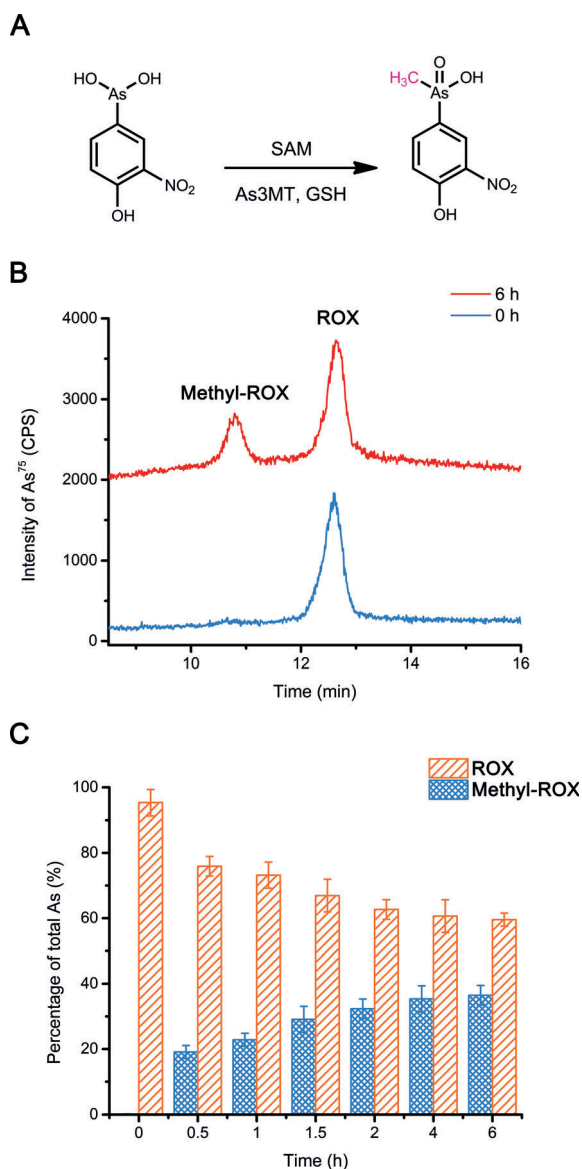


Figure 4. Methylation of the ROX^{III} substrate into methyl-ROX. A) Formation of methyl-ROX from ROX^{III}. B) Chromatograms from HPLC-ICP-MS analyses of a reaction mixture that contained the ROX^{III} substrate, the SAM methyl donor, and the As3MT enzyme. C) Percentage of methyl-ROX and ROX in the reaction mixture over the 6 h reaction period.

concentrations of reducing agents, such as 10 mM glutathione (GSH) and 1 mM tris(2-carboxyethyl)phosphine (TCEP), could we observe traces of methyl-ROX (Figure S14A) and methyl-3-AHPAA (Figure S14B). These results suggest that reduction of the pentavalent phenylarsenicals to the trivalent phenylarsenical intermediates is required, which in turn serve as the substrates for their enzymatic methylation. The finding that pentavalent arsenicals are reduced to the trivalent arsenicals followed by enzymatic methylation is consistent with the classic pathway for the biomethylation of inorganic arsenicals.^[10]

The implication that trivalent phenylarsenical intermediates are formed during the methylation process is toxicolog-

ically significant. Previous studies of other trivalent arsenicals have consistently shown that their toxicity is higher than that of the pentavalent arsenicals.^[12] Our toxicological tests with T24 human bladder carcinoma cells showed that the 24 h IC₅₀ values for the trivalent ROX^{III}, methyl-ROX^{III}, and 3-AHPAA^{III} species were 0.2 μ M, 0.4 μ M, and 22 μ M, respectively, whereas the IC₅₀ values for the respective pentavalent ROX, methyl-ROX, and 3-AHPAA compounds were 5700 μ M, 4700 μ M, and 680 μ M (Table S8). Thus the trivalent ROX^{III}, methyl-ROX^{III}, and 3-AHPAA^{III} compounds are approximately 300–30 000 times more cytotoxic than the pentavalent arsenic compounds ROX, methyl-ROX, and 3-AHPAA. This finding agrees with previous studies, which consistently reported higher toxicities for trivalent than for pentavalent methylarsenicals.^[12]

Chicken is the most consumed meat among all meat types in North America on a per capita basis,^[13] averaging 80 g per day. Although the European Union and the United States have discontinued the use of ROX in the poultry industry, many other countries continue to use ROX. Our analyses of liver samples from eight chickens fed ROX-supplemented food for 28 days showed $73 \pm 24 \mu\text{g kg}^{-1}$ methyl-ROX, $387 \pm 92 \mu\text{g kg}^{-1}$ methyl-3-AHPAA, and $32 \pm 4 \mu\text{g kg}^{-1}$ methyl-N-AHPAA (Table S9). Compared to the eight previously characterized arsenic species, the three methylated phenylarsenicals accounted for 42% of the total arsenic in these chicken liver samples. The standard practice in poultry industry requires a five-day clearance period after feeding ROX to chickens. We have determined the concentrations of arsenic species in liver samples collected from chickens ($n = 8$) five days after cessation of feeding ROX. The concentrations of the three methylated phenylarsenical metabolites were $92 \pm 23 \mu\text{g kg}^{-1}$, $29 \pm 8 \mu\text{g kg}^{-1}$, and $11 \pm 5 \mu\text{g kg}^{-1}$, for methyl-ROX, methyl-3-AHPAA, and methyl-N-AHPAA, respectively. These residual arsenic species in chicken liver are relevant to human exposure if chicken livers are consumed. In addition, the differences in toxicity among the ROX metabolites make the assessment of human exposure to phenylarsenicals important.

Acknowledgements

We thank Alberta Health, Alberta Innovates, the Canada Research Chairs Program, the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada, and the National Natural Science Foundation of China (21575107 and 21375097) for financial support. H.P., Q.L., and J.L. acknowledge support from the China Scholarship Council. We thank Dr. Martin J. Zuidhof for assistance in the chicken feeding experiments, Dr. David Thomas and Dr. Miroslav Styblo for providing the As3MT enzyme, and Dr. Rongfu Huang, Xiufen Lu, and Zonglin Yang for technical assistance.

Conflict of interest

The authors declare no conflict of interest.

Keywords: arsenic · cytotoxicity · metabolism · methylation · roxarsone

- [1] a) D. K. Nordstrom, *Science* **2002**, 296, 2143–2145; b) A. H. Smith, E. O. Lingas, M. Rahman, *Bull. World Health Org.* **2000**, 78, 1093–1193; c) M. F. Naujokas, B. Anderson, H. Ahsan, H. V. Aposhian, J. H. Graziano, C. Thompson, W. A. Suk, *Environ. Health Perspect.* **2013**, 121, 295–302.
- [2] a) H. D. Chapman, Z. B. Johnson, *Poult. Sci.* **2002**, 81, 356–364; b) F. T. Jones, *Poult. Sci.* **2007**, 86, 2–14; c) J. F. Stolz, E. Perera, B. Kilonzo, B. Kail, B. Crable, E. Fisher, M. Ranganathan, L. Wormer, P. Basu, *Environ. Sci. Technol.* **2007**, 41, 818–823; d) K. E. Nachman, P. A. Baron, G. Raber, K. A. Francesconi, A. Navas-Acien, D. C. Love, *Environ. Health Perspect.* **2013**, 121, 818–824; e) D. J. Fisher, L. T. Yonkos, K. W. Staver, *Environ. Sci. Technol.* **2015**, 49, 1999–2012.
- [3] a) S. Mafla, R. Moraga, C. G. Leon, V. G. Guzman-Fierro, J. Yanez, C. T. Smith, M. A. Mondaca, V. L. Campos, *World J. Microbiol. Biotechnol.* **2015**, 31, 1267–1277; b) T. Gul Kazi, A. Q. Shah, H. I. Afridi, N. A. Shah, M. B. Arain, *Ecotoxicol. Environ. Saf.* **2013**, 87, 120–123; c) K. E. Nachman, G. Raber, K. A. Francesconi, A. Navas-Acien, D. C. Love, *Sci. Total Environ.* **2012**, 417, 183–188; d) L. X. Yao, L. X. Huang, Z. H. He, C. M. Zhou, W. S. Lu, C. H. Bai, *Sci. Total Environ.* **2016**, 566, 1152–1158.
- [4] a) H. Peng, B. Hu, Q. Liu, Z. Yang, X. Lu, R. Huang, X.-F. Li, M. J. Zuidhof, X. C. Le, *J. Chromatogr. A* **2014**, 1370, 40–49; b) Q. Liu, H. Peng, X. Lu, M. J. Zuidhof, X.-F. Li, X. C. Le, *Environ. Health Perspect.* **2016**, 124, 1174–1181; c) Z. Yang, H. Peng, X. Lu, R. Huang, B. Hu, G. Kachanoski, M. J. Zuidhof, X. C. Le, *Environ. Sci. Technol.* **2016**, 50, 6737–6743.
- [5] a) S. A. Viczek, K. B. Jensen, K. A. Francesconi, *Angew. Chem. Int. Ed.* **2016**, 55, 5259–5262; *Angew. Chem.* **2016**, 128, 5345–5348; b) H. R. Hansen, R. Pickford, J. Thomas-Oates, M. Jaspars, J. Feldmann, *Angew. Chem. Int. Ed.* **2004**, 43, 337–340; *Angew. Chem.* **2004**, 116, 341–344; c) A. Rumpler, J. S. Edmonds, M. Katsu, K. B. Jensen, W. Goessler, G. Raber, H. Gunlaugsdottir, K. A. Francesconi, *Angew. Chem. Int. Ed.* **2008**, 47, 2665–2667; *Angew. Chem.* **2008**, 120, 2705–2707.
- [6] a) S. D. Conklin, N. Shockey, K. Kubachka, K. D. Howard, M. C. Carson, *J. Agric. Food Chem.* **2012**, 60, 9394–9404.
- [7] a) W. R. Cullen, K. J. Reimer, *Chem. Rev.* **1989**, 89, 713–764; b) M. Vahter, *Toxicology* **2002**, 181, 211–217; c) S. Lin, Q. Shi, F. B. Nix, M. Styblo, M. A. Beck, K. M. Herbin-Davin, L. L. Hall, J. B. Simeonsson, D. J. Thomas, *J. Biol. Chem.* **2002**, 277, 10795–10803; d) D. J. Thomas, J. X. Li, S. B. Waters, W. B. Xing, B. M. Adair, Z. Drobna, V. Devesa, M. Styblo, *Exp. Biol. Med.* **2007**, 232, 3–13; e) A. Ajees, B. P. Rosen, *Geomicrobiol. J.* **2015**, 32, 570–576.
- [8] a) J. Qin, C. R. Lehr, C. G. Yuan, X. C. Le, T. R. McDermott, B. P. Rosen, *Proc. Natl. Acad. Sci. USA* **2009**, 106, 5213–5217; b) S. Suzuki, L. L. Arnold, K. L. Pennington, B. W. Chen, H. Naranmandura, X. C. Le, S. M. Cohen, *Toxicol. Appl. Pharmacol.* **2010**, 244, 99–105; c) Z. Drobna, H. Naranmandura, K. M. Kubachka, B. C. Edwards, K. Herbin-Davis, M. Styblo, X. C. Le, J. T. Creed, N. Maeda, M. F. Hughes, D. J. Thomas, *Chem. Res. Toxicol.* **2009**, 22, 1713–1720; d) H. Naranmandura, K. Rehman, X. C. Le, D. J. Thomas, *Anal. Bioanal. Chem.* **2013**, 405, 1885–1891; e) J. M. Currier, C. Douillet, Z. Drobna, M. Styblo, *J. Environ. Sci.* **2016**, 49, 104–112.
- [9] a) M. Styblo, L. M. Del Razo, E. L. LeCluyse, G. A. Hamilton, C. Q. Wang, W. R. Cullen, D. J. Thomas, *Chem. Res. Toxicol.* **1999**, 12, 560–565.
- [10] L. Ding, R. J. Saunders, Z. Drobna, F. S. Walton, P. Xun, D. J. Thomas, M. Styblo, *Toxicol. Appl. Pharmacol.* **2012**, 264, 121–130.
- [11] a) F. Challenger, *Chem. Rev.* **1945**, 36, 315–361; b) W. R. Cullen, B. C. McBride, J. Reglinski, *J. Inorg. Biochem.* **1984**, 21, 45–60; c) W. R. Cullen, *Chem. Res. Toxicol.* **2014**, 27, 457–461.
- [12] a) M. J. Mass, A. Tennant, B. C. Roop, W. R. Cullen, M. Styblo, D. J. Thomas, A. D. Kligerman, *Chem. Res. Toxicol.* **2001**, 14, 355–361; b) J. S. Petrick, F. Ayala-Fierro, W. R. Cullen, D. E. Carter, H. V. Aposhian, *Toxicol. Appl. Pharmacol.* **2000**, 163, 203–207; c) T. Schwerdtle, I. Walter, I. Mackiw, A. Hartwig, *Carcinogenesis* **2003**, 24, 967–974; d) B. Moe, H. Peng, X. Lu, B. Chen, L. W. L. Chen, S. Gabos, X.-F. Li, X. C. Le, *J. Environ. Sci.* **2016**, 49, 113–124.
- [13] U. S. Department of Agriculture, Economic Research Service, 2014. Food availability (per capita) data system; <http://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/> [accessed January 20, 2017].

Manuscript received: January 21, 2017

Revised: March 16, 2017

Final Article published: ■ ■ ■ ■ ■ ■ ■ ■ ■ ■

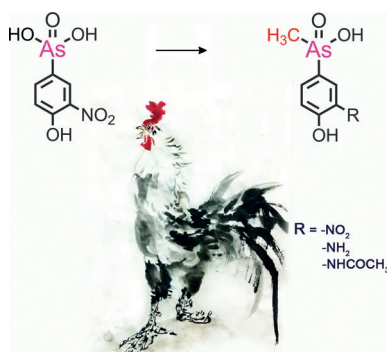
Communications



Metabolism

H. Peng, B. Hu,* Q. Liu, J. Li, X.-F. Li,
H. Zhang, X. C. Le* ——— ■■■■-■■■■

Methylated Phenylarsenical Metabolites
Discovered in Chicken Liver



Toxic chicken: Three toxicologically relevant methylated phenylarsenical metabolites were detected in the liver of chickens fed 3-nitro-4-hydroxyphenylarsonic acid (roxarsone), a feed additive in poultry production that is still in use in several countries. These compounds are likely formed by methylation of trivalent phenylarsonous acid substrates in the presence of an arsenic methyltransferase.

