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Exploring Natural Models for the 'Rolling Unmasking Effect' of Downwind Odor Dispersion; Prairie Verbena, Prehensile-tailed Porcupine and Virginia Pepperweed

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ABSTRACT

As natural scale-models for community environmental odor issues, these odorant prioritization results illustrate an important consideration: … '*with respect to focusing an environmental odor issue, it is possible to look too closely at the source*'… Although simple odor dilution, as measured by odor concentration and intensity, certainly occurs during downwind dispersive migration from the source, these authors propose that the term ***dynamic dilution*** is limiting with respect to environmental odor impact. The results presented herein suggest that the odor character from an environmental source can vary dramatically, depending upon the distance of the human receptors from that source. It is further suggested that the process of downwind environmental odorant prioritization can best be characterized as a ***rolling unmasking effect*** or ***RUE***. The RUE is exhibited by the masking odors nearest the source sequentially 'falling away' with distance from the source, revealing a succession of increasingly simplified odor characteristic and composition. Because of scaling factors and meteorological unpredictability, the logistics involved in carrying-out odorant prioritization studies can be very challenging when targeting large-scale odor sources. However, for these authors' illustrative purposes, these challenges were reduced significantly by selecting natural, 'scale-model' odor-sources which represented significant reductions in the primary scaling factors; especially, reductions in the size of the odor sources and the distance of their downwind reach. Driven by odorant prioritization and the RUE, extremes of odor simplification-upon-dilution were demonstrated for two Central Texas plant varieties, prairie verbena and virginia pepperweed. Their 'odor frontal boundaries' were shown to be dominated by single, character-defining odorants; prairie verbena presenting with a p-cresol dominated 'barnyard' odor and virginia pepperweed with a benzyl mercaptan dominated 'burnt match' odor. Similar odor simplification was also shown for the South American prehensile-tailed porcupine (i.e. ***pt*** porcupine); its downwind 'odor frontal boundary' dominated by two potent, character-defining odorants (i.e. as yet unidentified): (**1**) **'onion' / 'body odor' odorant #1** and (**2**) **'onion' / 'grilled' odorant #2**. In contrast to their outer-boundary simplicities, each of these sources also presented, at the source, with odor compositions reflecting considerable complexity and corrresponding composite odor characters that were distinctly different from those reflected at their respective 'odor frontal boundaries'.

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INTRODUCTION

Although relatively intuitive and demonstrable, odorant prioritization does not appear to be widely recognized or referenced within the environmental odor field. With respect to contemporary environmental odor issues, it is still common to see an odor issue presented, roughly correlated to an extensive inventory listing of volatile chemicals which are shown to be emitting from a 'suspect' odor source. For example, one study, focused directly on odor emissions from sewage treatment facilities (Zarra, T. et.al., 2008), identified the following as typical, qualitative VOC / odorant emissions from one such facility: 39 VOCs distributed between: (a) 3 organic sulfides; (b) ketones; (c) aliphatic aldehydes; (d) aromatic aldehydes; (e) aromatic hydrocarbons; (e) terpenes; (f) alcohols; (g) 3 volatile fatty acids; (h) hydrocarbons; (i) chlorinated hydrocarbons and (j) aliphatic siloxanes. This obviously represents an extensive and complex emission 'soup'; potentially encompassing hundreds or thousands of individual VOCs and many chemical functionalities. Unfortunately, it often proves to be the case that these listings include a preponderance of volatile compounds and classes of compounds which have little, if any, downwind odor impact beyond the source fence-line. It is also often the case that these extensive inventory VOC listings fail to actually include the specific VOC odorant, or odorants, which are primarily responsible for the targeted at-distance odor. In one notable example from an odorant prioritization study to the rendering industry (Caraway et al; 2007), two odorants, trimethylamine and dimethylsulfide were identified as the impact-priority odorants downwind of a fish meal processing plant; a rendering facility specializing in the processing of fish and fish by-products. This finding stands in marked contrast to an earlier study (Luo et al, 1997), reporting ~300 organic compounds, 40 of which were odorous and stating that 'odorous compounds included alkanes, alkenes, ketones, hydrocarbons, alcohols, alkyl halides, fatty acids, amines, aromatics, aldehydes and epoxides'. It should be pointed out that this 300 compound listing did include trimethylamine and dimethylsulfide but those were not prioritized within this 300 compound inventory listing.

Strategies for mitigation of community environmental odor issues, can be improved by utilizing troubleshooting techniques originally developed for the food, beverage and consumer products industries. The experience of these authors in 25+ years of crisis-driven odor research and troubleshooting has shown that there is an odorant impact-priority ranking which is definable for virtually every odor source; whether of natural or man-made origin. The initial challenge is prioritization of environmental odor 'character' (defined as a descriptor of what it smells like), from the perspective of the impacted citizenry downwind.

Environmental odor issues are perfect examples of complicated problems that are confounded by many analytical, technological and socioeconomic factors. While considerable engineering know-how and technologies exist for mitigation of industrial odor, these technologies are often not adapted or adaptable for rural and agricultural odor

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(Maurer et al., 2016). The process of environmental odor dispersion has historically been described as 'downwind dilution' and monitored by standard techniques based upon dynamic dilution olfactometry (Parker et al. 2005; Jacobson et al., 2008; Akdeniz et al., 2012a). Major odor sample recovery problems have been identified (Koziel et al., 2005; Zhu et al., 2015). There is also wide recognition of an outstanding challenge to link specific, relatively easy to quantify compounds to resulting odor (Akdeniz et al., 2012b; Zhang et al., 2015). The use of the odor activity value (OAV) concept has been used with some degree of success to show that compound-specific odor detection thresholds (DTs) can be useful to explain why some compounds are more impactful odorants than others (Parker et al., 2012; Rice et al., 2015a, 2015b, 2015c). The use of simultaneous chemical and sensory analyses has also gained acceptance as a mature technology for isolating, ranking and prioritizing odor-causing compounds in a complex mixture of gases (Bulliner et al., 2006; Laor et al., 2008; Lo et al., 2008; Zhang et al., 2010).

Although simple odor dilution, as measured by odor concentration and intensity, certainly occurs during downwind dispersive migration from the source, this term is limiting with respect to environmental odor impact (Wright et al. 2006). Past experience has shown that the odor character from an environmental source can depend upon the downwind distance of the human receptors from that source; potentially radically different at locations nearest the source when compared to locations farther removed (Wright et al., 2005; Wright et al., 2006; Koziel et al., 2006).

In recent years, it has been proposed that the process of environmental odorant prioritization can better be described as a RUE (**Figure 1**). The RUE is exhibited by the priority masking odors nearest the source sequentially 'falling away' with distance from the source, revealing a succession of increasingly simplified odor characteristic and composition. This ends at the far downwind odor frontal boundary with an odor character, dominated by the impact-priority odorant (or odorants) from the complexly combined source emission. These are the odorant(s) reflecting the greatest downwind sensory reach relative to the source. For example, this has been shown for *p*-cresol, as a 'signature' downwind odor from animal feeding operations; recognizable at great distance from the source (Wright et al., 2005), in one case remaining the single, most offensive characteristic compound as far as 16 km away from the source (Koziel et al. 2006).

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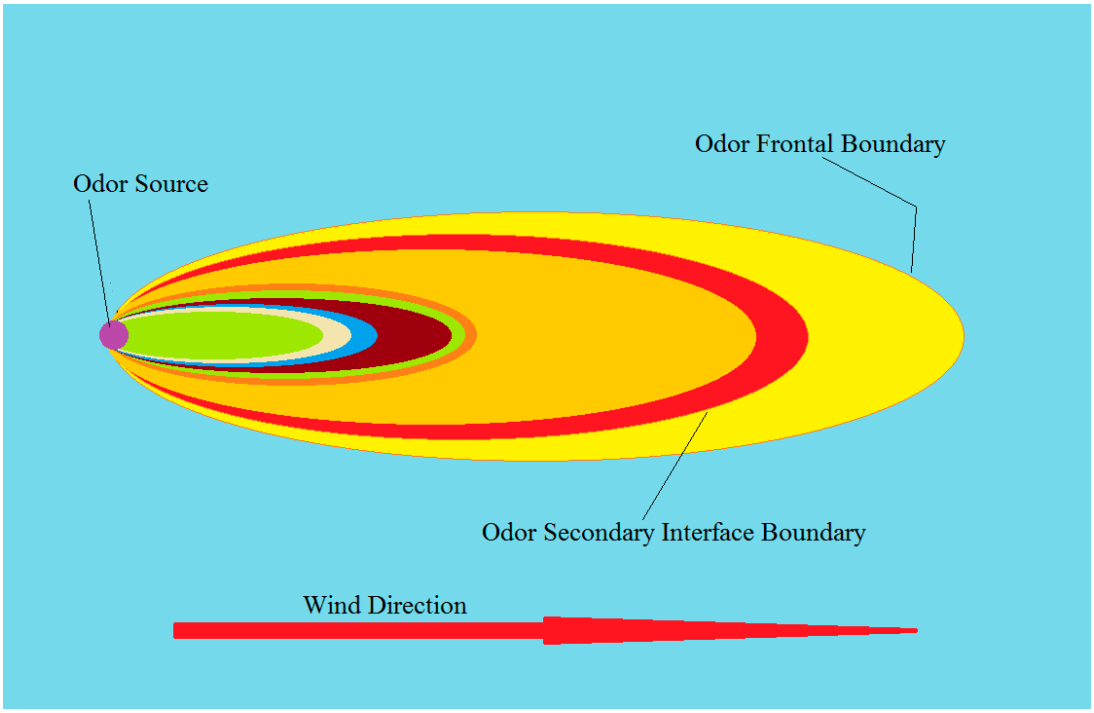

Fig. 1 Generic pictorial representation of 'rolling unmasking effect' (RUE). The odor frontal boundary represents the farthest downwind reach relative to the odor source while the internal bands represent the points of sequential odor unmasking as the secondary-impact odorants are diluted below their detection / masking concentration levels

Without conscious effort, the majority of the human population can make the association between characteristic environmental odors and specific chemicals primarily responsible for those odors. A mother's recognition of ammonia, without analytical confirmation, as the specific chemical odorant responsible for the 'ammonia' odor near an incubating pile of urine-soaked diapers is a simple manifestation of that innate ability.

The primary factor separating the general population from those who spend their careers deconstructing the chemical composition of diverse odors is the number and obscurity of such associations, which can be made in advance of analytical confirmation. Considering the thousands of odorous chemicals from which to select, the correct 'prediction' of a single, specific odorant responsible for a newly encountered, environmental odor represents strong evidence in support of that proposed odorant prioritization.

A number of natural examples of the RUE have been encountered and described by these authors over the past two decades. These have included, among others:

(1) the large colony of Mexican free-tailed bats (i.e. *Tadarida brasiliensis*) at Bracken Cave near San Antonio, Texas (Wright et al., 2006; Nielsen et al., 2006) and

(2) a large cattle feed-lot located near Amarillo, Texas (Wright et al., 2005).

With respect to the ancient Mexican free-tailed bat colony at Bracken Cave (Nielsen et. al., 2006); three distinct odor boundaries were definable relative to the cave source:

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(1) an overpowering 'ammonia' odor within the cave and for ~15 meters downwind of cave openings;
(2) emergence of a composite 'rat's nest' odor which was dominated by 4-methyl quinazoline (i.e. tentative identification) upon decline of the at-source masking of ammonia, and
(3) emergence of the characteristic 'bat cave', 'taco shell' odor, dominated by 2-amino acetophenone, upon approach to the outer 'odor frontal boundary' ~300 meters; enabled by the associated downwind decline of odor masking by 4-methyl quinazoline.

Similarly, with respect to the contrasting feedlot source near Amarillo, Texas, (Wright et al., 2005) at least two distinct odor boundaries were definable relative to the source:
(1) dominated by trimethylamine; a strong 'fishy', 'amine' odor within the feed-lot proper and for several hundred meters downwind, and
(2) the emergence of a composite 'barnyard' odor, dominated by *p*-cresol, upon approach to the outer 'odor frontal boundary'; enabled by the associated decline of downwind odor masking by trimethylamine.

In this new research, we aim at summarizing three new examples of RUE and discuss them more systematically. These new RUE examples involve both animal and plant-related odor. This current work focuses on three contrasting RUE examples from this combined field:
(1) prairie verbena (Verbenaceae *Glandularia bipinnatifida*),
(2) the South American prehensile-tailed porcupine (*Coendou prehensilis*),
(3) virginia pepperweed (Brassicaceae *Lepidium virginicum)*.
The ultimate significance of this approach is the illustration of naturally-occurring phenomena that can explain why some environmental odors and their sources are difficult to identify and mitigate.

METHODS and MATERIALS

Multidimensional Gas Chromatography--Mass Spectrometry-Olfactometry

MDGC-MS-Olfactometry is an integrated approach combining olfactometry and multidimensional GC separation techniques with conventional GCMS instrumentation. A commercial, integrated MultiDimensional-Gas Chromatography-Mass Spectrometry-Olfactometry (i.e. MDGC-MS-O) system was used for the odorant prioritization work as presented herein. The integrated system consisted of an Agilent 6890 Gas Chromatograph / 5975B Mass Spectrometer modified for MDGC-MS-O utilizing an AromaTrax™ control system from Volatile Analysis Corporation of Round Rock, Texas. Details regarding general hardware and AromaTrax™ operation have been described in detail in past publications (Wright et al., 1986; Lo et al.,, 2006) and are not restated here. Specific operational parameters utilized by the first author for the 3 targeted natural odor sources is summarized as follows: injection mode: split-less with Solid Phase Micro Extraction (SPME) sample collection and delivery; injection temperature: 250 °C; detector #1: Flame Ionization Detector (FID); detector #1 temperature: 280 °C; detector #2: Agilent 5975B MSD in MS-SCAN or MS-SIM acquisition mode**;** column # 1**:** 12 m x .53 mm ID BPX 5 - 1.0 µm film (pre-column from SGE); column # 2**:** 25 m x 0.53 mm ID BPX 20 - 1.0 µm film (analytical column from SGE); column temperature program

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(overview survey and MDGC-MS-O): 40 °C initial, 3 min hold, 7 °C/min., 220 °C final, 20 min hold.

MDGC parameters:
With regard to MDGC heart-cut isolation / clean-up of the 2 target 'onion' odorants for the ***pt*** porcupine; (**1**) optimal band for heart-cut #1 (i.e. **unknown 'onion' odorant #1**) was approximately 9.9 to 11.2 min; (**2**) optimal band for cryotrap #1 was approximately 9.4 to 11.5 min; (**3**) optimal band for heart-cut #2 (i.e. **unknown 'onion' odorant #2**) was approximately 14.4 to 15.8 min; (**4**) optimal band for cryotrap #2 was approximately 13.9 to 16.1 min; (**5**) long SPME collection of the whole urine headspace yielded overwhelming odor responses but NO obvious associated mass spectral ion detail for the critical 'onion' odorants. In contrast to the MDGC based heart-cut, isolation protocol, as applied to the 'onion' odorants for the urine, both the prairie verbena and virginia pepperweed headspace VOC / odor profiles were processed in overview survey mode; with total heart-cuts taken between 0.25 min to 32.0 min

Sampling:
Male *pt* porcupine urine (i.e. passive 'dirty collect' – with entrained fecal matter): The VOCs, odorous and otherwise, were collected from the equilibrated headspace formed within a 1 quart glass headspace vessel containing a few drops of the urine sample, injected onto a crumpled low-odor paper towel substrate. The sample was equilibrated, stored and sampled in an open-air laboratory environment which was maintained @ 24 degC. Direct comparison samples were collected utilizing a single, designated, 1 cm / 75 um Carboxen modified polydimethyl siloxane SPME fiber from Supelco. Headspace volatiles were collected by way of SPME fiber insertion through a pinhole placed in the vessel's PTFE disc closure. Volatiles loadings on the SPME fiber were varied by altering the length of time the fiber was exposed to the equilibrated headspace formed within the vessel.

Environmental air sample collections from *pt* porcupine exhibit: SPME fiber direct exposure: A series of direct environmental air samples were collected and analyzed in conjunction with this current effort, utilizing a direct SPME fiber exposure approach. The SPME fibers which were prepared for this segment of the project were: (1) preconditioned @260 degC by the first author; (2) transported, under dry-ice storage conditions, to the Moody Gardens Rainforest site on Feb 14, 2017 for execution of VOC collection by direct SPME fiber exposure within the ***pt*** porcupine indoor exhibit and (3) return transported by the first author, under dry-ice conditions back to Volatile Analysis Corporation laboratory; Round Rock, Texas; for execution of the odorant prioritization segment of the investigation. Preconditioned SPME samplers were secured onto a field-support fixture within the exhibit enclosure; the adsorbent coated fiber tips extended from their protective needle sheaths (i.e. exposed to the enclosure environment to effect VOC collection through surface adsorption). Volatiles loadings on the SPME fibers were varied by altering the length of time the SPME fibers were exposed to the air environments. Fiber exposures reflecting brief sampling intervals were executed for 7 and 9 minutes, respectively. Duplicate SPME fiber exposure intervals reflecting long exposure intervals were exposed for 15 hours. The 4 direct fiber collections were return

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transported, under dry-ice conditions, back to the laboratory for odorant prioritization assessment. At the time of sample collection, the odor, upon distance separation from the enclosure, was characterized, by the first author, as distinct 'grilled onion'; identical in character to that recalled for the two, MDGC-O isolated (but, as yet, unidentified) 'grilled onion' odorants from actual onion based odor profile studies.

Prairie Verbena: The qualitative odor profile assessment of the mature prairie verbena blossoms were processed in much the same manner as described above for the ***pt*** porcupine urine. The preconditioned 1 L glass headspace vessels were charged with freshly harvested blossoms; collected at peak maturity; from a dense natural cluster; located in the geographical vicinity of Georgetown, Texas on May 10, 2010. At the time of harvesting and analysis, the odor of the dense natural clusters, at the odor frontal boundary, was characterized, by the first author, as distinct 'barnyard'; identical in character to that recalled for the pure odorant, *p*-cresol.

Virginia Pepperweed: The qualitative odor profile assessment of the mature virginia pepperweed plant was processed in much the same manner as described above for the ***pt*** porcupine urine and prairie verbena blossoms. In this case, however, the preconditioned 1 L glass headspace vessels were charged with freshly harvested whole plants (i.e. stems + blossoms) reflecting either 'pristine' or 'crushed' conditions. This was necessary when it was determined that mechanical stressing of the plant was necessary before the characteristic 'burnt match' odor was released. The plants were harvested, for analysis, at peak maturity; from typically sparse / random distribution within lawn / field environments; from locations in the geographical vicinity of Georgetown, Texas in June of 2016. At the time of harvesting and analysis, the odor of the macerated whole plants, at the frontal boundary, was characterized, by the first author, as distinct 'burnt match'; identical in character to that recalled for the pure odorant, benzyl mercaptan.

Mass Spectrometry:

First Author: The Agilent 5975B mass spectrometer was operated in MS-SCAN mode for survey mode odorant identification. Under control of the Agilent Chemstation software, the mass range 35 amu to 400 amu was scanned at a rate of 3.84 scan / sec. The resulting spectra were imported into the Benchtop PBM library search protocol; referencing the Wiley 7 spectral library for best-match ranking of the unknown spectra against the Wiley 7 database. The first author retained final over-ride determination as to the likelihood of correctness of the best-match listings from the search routine. Spectra, adjudged by the first author, as not resulting in a good library match were listed as unknowns; unless overridden by other considerations (e.g. known retention time elution in combination with simultaneous odor character recognition at the olfactory detector). The proposed character-defining odorant identities for both the prairie verbena and virginia pepperweed (i.e. p-cresol and benzyl mercaptan, respectively) were confirmed through on-instrument retention time + odor character matching. Unfortunately, best efforts on the part of the first author failed to reveal the chemical identities of the two character-defining 'grilled-onion' odorants from the ***pt*** porcupine. In a further attempt to identify these unknowns, the first author engaged the collaborative services of two independent, highly experienced, research mass spectrometry experts in the food flavor /

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aroma field; Dr Thomas G. Hartman of Rutgers University and Anna Iwasinska of Volatile Analysis in Round Rock, Texas. Their approaches and results are summarized as follows:

Collaborative Investigator #2: (Thomas G. Hartman, Rutgers University). Beginning with about 2 ml of ***pt*** porcupine urine provided, it was saturated with NaCl; extracted into 5 ml of diethyl ether; centrifuged; lifted off the ether extract and then evaporated 1/2 of the ether extract into the purge & trap apparatus (in duplicate). The sample extract was then purged with nitrogen 30 minutes at 100C onto Tenax TA traps. The Tenax TA traps were thermally desorbed into the GC-MS at 250C/5 min. The GC was cryogenically temperature programmed from minus 20C (5 min) during desorption, followed by 10C/min. to 280C. Column was a single 60m x 0.32 mm id x 1.0 um film ZB5MS. The mass spectrometer, a Thermo-Finnigan Mat TSQ-7000, was set to scan the mass range 35-350 amu once per second. The data files were converted to HP Chemstation format using MassTransit software and burned onto a CD so they could be processed on the first author's Chemstation / Benchtop PBM system. The original data format was Thermo Xcaliber. A background plot was plotted (i.e. mass chromatogram 35_350-40-44) to background subtract argon and CO2 to clean up the chromatograms when viewing. The urine and ether extracts were sniffed before and after pre-concentration and confirmed to retain a strong odor in the extract delivered to the purge and trap apparatus. The Solid Sample Purge & Trap Collection System and Model TD-4 Short Path Thermal Desorber (i.e. both from Scientific Instrument Services; SIS, Ringoes, NJ) was the integrated system utilized for volatiles pre-concentration. Short Path Thermal Desorption (i.e. SPTD, a technology for which Dr. Hartman holds the utility patent) utilized Tenax TA packed traps to pre-concentrate the extracted VOCs. The samples were run in duplicate; extending one of the runs to get a few high boilers so there were 3 pre-concentrated runs total. The spectral data was picked through, scan by scan, but unfortunately, no sulfur compounds stood out; for either Dr. Hartman or for the first author in the subsequent data cross-check effort. Although these efforts failed to identify the two targeted 'grilled onion' odorants, they did serve as cross-check identity confirmation for key semi-volatile odorants of secondary 'barnyard' character-impact (e.g. p-cresol, 4-ethyl phenol, indole, skatole).

Collaborative Investigator #3: (Anna Iwasinska; Volatile Analysis Corporation). An Agilent 5975B based AromaTrax™ system (i.e. generally applied as described above for first author) was operated in MS-SCAN mode for targeted odorant identification / confirmation. Significant deviations from the parameters listed for the first author were: **Column # 1:** 30 meter x 0.53mm ID BPX 5 – 0.5µm film (pre-column, from SGE); **Column # 2:** 30 meter x 0.53mm ID SolgelWAX – 0.1µm film (analytical column, from SGE). The chromatographic data were acquired using Agilent MSD ChemStation Data Acquisition software (E.02.01.1177). The MSD scan mass range was: 35amu to 400amu at a rate of 3.84 scan / sec. The analysis of resulting chromatograms was performed using ChemStation Data Analysis software (F.01.03.2357) referencing the Wiley9-N08, NIST11 and FFNSC13 Mass Spectral libraries. The compound identities were reported based on best-match ranking of the experimental spectra against these databases.

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The samples of ***pt*** porcupine urine and virginia pepperweed (same batch of materials that was used by first author) were analyzed by this investigator in order to further explore identification of some leading odor notes. The headspace volatiles collection was conducted in a manner described above for the first author. The spectral data was reviewed in detail but, despite best efforts, failed to identify the two targeted 'onion' odorants from ***pt*** porcupine urine headspace. However, separate spectral interpretive efforts were also applied, by this investigator, to the prairie verbena data developed by the first author; serving as independent cross-check of the proposed VOC / odorant identity profile. This effort included, most notably, (**1**) the tentative identification of hyacinthin for the 'floral' note @18.0 min RT; (**2**) a 'possible' identification of __oxime for the isomer family carrying the 'ether' note @6.0 min RT and (**3**) independent cross-check confirmation of benzyl mercaptan as the character-defining 'burnt-match' odorant in virginia pepperweed.

Collaborator #4: (Paula Kolvig; Moody Gardens Rainforest Exhibit). At the request of the first author, the Moody Gardens Rainforest Exhibit staff carried out a passive, external urine collection from Bono, the 10 year old male half of the Moody Gardens breeder pair. The urine sample collection was carried out on April 12, 2017 utilizing a passive / 'dirty' collect procedure which allowed for some mixing of fecal solids with the liquid urine sample. As explained to the Rainforest Exhibit staff, this procedure enabled the first-author to execute a qualitative odor profile assessment of the combined waste from the male prehensile-tailed porcupine; although the urine was projected as the major reservoir for the targeted 'onion' odor.

RESULTS and DISCUSSION

The odorant prioritization process is the same regardless of whether the environmental odor source is a 50,000 head feedlot with a 16 km downwind reach, a colony of 2+ million Mexican Free-tails bats with several hundred meters downwind reach or a dense cluster of fragrant flowers with a downwind reach of only 50 meters. In each case the total VOC emission profile, monitored at the source, is often extremely complex.

With respect to natural sources, this complexity is often reflected in hundreds of discrete VOCs, dozens of which are likely odorous and, therefore, carry the potential for odor-impact significance at-distance from the source. It is the natural dilution process, in dispersive migration outward, which drives the simplification of the at-source or near-source odor complexity. In the outward progression from the source to the 'odor frontal boundary,' simplification of the priority odorant subset is a dynamic process; often reflected in a number of changes in odor character and corresponding odorant priority rankings in spanning the boundary extremes.

Simplification can be reflected in both the composition of the priority odorant subset as well as the total number of odorants which are essential for inclusion in that subset. As a result of scaling factors, the logistics involved in carrying-out an odorant prioritization study can be very challenging when targeting large odor sources. This is especially true with respect to large industrial or CAFO sources which can carry downwind odor reach

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of several kilometers. However, for illustrative study, these challenges can be significantly reduced by selecting natural odor-source 'scale-models' which represent significant reductions in the primary scaling factors; especially, reductions in the size of the odor sources and the distance of their downwind reach.

It is for this reason that the authors have selected three, relative small-scale natural environmental odor sources to illustrate downwind odor dispersion effects, as well as procedural aspects of the MDGC-MS-Olfactometry based odorant prioritization process. In this study, the downwind odor impact of the model, environmental odor sources are used to draw important contrasts and parallels to a typical large-scale, industrial or CAFO odor source.

Case Study #1: Prairie Verbena

With regard to odorant prioritization by MDGC-MS-Olfactometry, the relative absence of expected odorants from a targeted odor source can be as telling as the identities of those odorants which are shown to be present. Case Study #1 serves as an excellent illustration of this concept. It focuses on one unique species from the very large Verbena family of flowering perennials. There are reportedly as many as 250 different varieties of this ancient plant and these are widely distributed throughout the world. As is the case within most plant families reflecting such diversity, there is considerable variation in the physical characteristics represented among its members. This variation includes differences in physical size, shape, flower structure, appearance, flower color and, most importantly for current illustration purposes, odor characteristics.

The wonderful aroma characteristics of verbena flowers have been extolled by writers throughout *modern* history…. *According to William Faulkner, in his short story 'An Odor of Verbena' "vervain (verbena) is the only scent that can be smelled above the scent of horses and courage."* One species of verbena, the lemon verbena, has acquired something of a cultural icon status for its use in perfumery as a result of references in Margaret Mitchell's '*Gone With the Wind*' and the '*Little House on the Prairie'* book series by Laura Ingalls Wilder.

The reality, however, is that there is a wide range of odor characteristics reflected among the individual species and varieties of this extensive family. This range spans the extremes between virtually odorless (i.e., especially among many of the commercial hybrids) to pleasantly fragrant such as in the lemon-scented verbenas to remarkably unpleasant such as in the case of the regional species discussed herein. Adding to this, the odor character for a particular species can change significantly, depending upon the seasonal stage of the flowering cycle.

The focus of this case study is the prairie verbena (i.e., Verbenaceae, *Glandularia bipinnatifida*); specifically, the regionally isolated species of verbena which is native to the boundary region between adjacent Blackland Prairie and Edwards Plateau ecological regions near Georgetown, Texas. At the peak of its mid-spring flowering cycle (i.e. before peak decline), this particular plant presents with a familiar and surprising odor

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character which has been described by the first author, and others, as 'barnyard' or 'hog-truck'. The mid-spring odor emission from these plants can be surprisingly intense and, as a result, large natural clusters can present with a surprisingly distant downwind reach.

Focusing upon this characteristic, the premise which guided the execution of this study can be summarized as follows: '*as a result of the remarkable similarity in downwind odor characteristics, there is assumed to be some commonality between the minimum priority odorant subsets for the prairie verbena and the more typical, 'agrarian', sources of 'barnyard' odors'*. Therefore, the driving 'questions' which become the basis for the study, reported below, are the following:

(1) Are there character-impact odorants which are common to both prairie verbena and swine CAFO sources which account for the striking similarity in composite odor at their respective odor frontal boundaries?
(2) What is the level of overall agreement between the two contrasting sources when comparing their minimum priority odorant subsets?
(3) What is the level of overall agreement between the two when comparing their full background odorant profiles?
(4) What is the level of overall agreement between the two when comparing general headspace volatiles profiles (i.e., odorous and otherwise)?

Through analytical testing of the guiding premise, the authors demonstrate the odorant prioritization process through a 'cradle-to-grave' odor profile assessment of the regional prairie verbena; comparing and contrasting the odorant composition results to those previously developed relative to a true 'barnyard' odor source, a large commercial swine barn.

Composite Odor Assessment: All MDGC-MS-O based odorant prioritization studies began with the performance of a composite odor assessment. The first author was the individual tasked with making the initial critical correlations between the characteristic downwind odor of the source and the impact-priority odorants which are primarily responsible for that odor. With this in mind, the introduction of the first author to the prairie verbena as a 'model' environmental odor source is believed to be illustrative. The first conscious introduction to the dense natural clusters of prairie verbena was a chance encounter in an overgrown field adjacent to a local hiking route (i.e. **Figure #2**).

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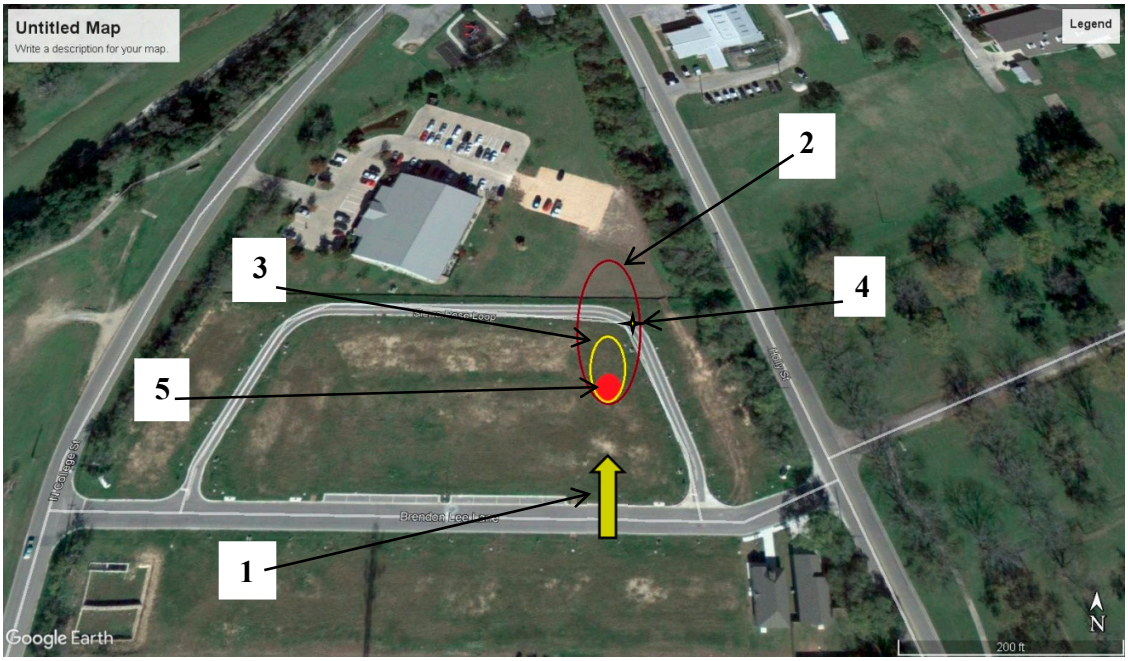

Figure #2; Google Earth Georgetown, Texas Image; prairie verbena 'barnyard' odor encounter; Showing: (1) approximate wind direction; (2) approximate 'barnyard' odor frontal boundary; (3) approximate 'floral' secondary (near-source) boundary; (4) this investigator's approximate location upon initial encounter in Georgetown, Texas in May, 2010 and (5) approximate location of odor source cluster of native prairie verbena.

Upon entering the verbena's odor frontal boundary the first author was immediately struck by its surprising intensity and, more importantly, its very familiar 'barnyard' odor character. This was an odor with which the first author had become very familiar as a result of past studies targeting several species of mammals as environmental odor sources (Parker et. al., 2005; Wright et. al., 2005; Wright et. al., 2005). The degree of similarity between the encountered odor and that of other common 'barnyard' odor sources was so close, in fact, that its connection to the flowering verbena clusters was not immediately apparent. Rather, initial attempts were made to connect it to a more obvious, agrarian source; a nearby barnyard fixture such as a livestock trailer, corral or the like. It wasn't until after eliminating these, more obvious, sources that a closer inspection of the prairie verbena colonies was made and their connection, as source, to the 'barnyard' odor was confirmed.

One more consideration from the initial composite odor assessment would prove to be significant relative to this encounter. This was the prediction, by the first author, in advance of analytical confirmation, that *p*-cresol would emerge as the character-defining odorant which is primarily responsible for the surprising 'barnyard' odor character of the prairie verbena clusters. This prediction is significant; considering, to ultimately be proven correct, requires the first author to have recognized and correctly identified a single odorous chemical from hundreds, or perhaps thousands, of other possible odorous chemicals and to have done so before any analytical work has been initiated. This 'blind' recognition is the definition of character-defining impact; a single, characteristic odorant rising above the complex background 'noise' emitting from the source.

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Odorant Prioritization: The ultimate goal of the odorant prioritization process is the correlation of an environmental odor of interest with the individual chemical odorants most responsible for that odor. With completion of the initial characterization of the prairie verbena composite odor as 'barnyard', the odor assessment enters the analytical phase with focus switching to the plant's complex VOC emission. A sense of the complexity of the plant's volatiles emissions are reflected in the overview VOC and odorant profiles as shown in **Figures 3** and **4** below. **Figure 3** is the total ion chromatogram (i.e., ms- SCAN TIC) reflecting the total VOC survey profile which was acquired in ms-SCAN acquisition mode; scanning the mass range between 35 and 400 amu. The sensory complexity is reflected in the corresponding **Figure 4** aromagram which was generated in parallel; with the first author acting in the capacity of human 'sensor'.

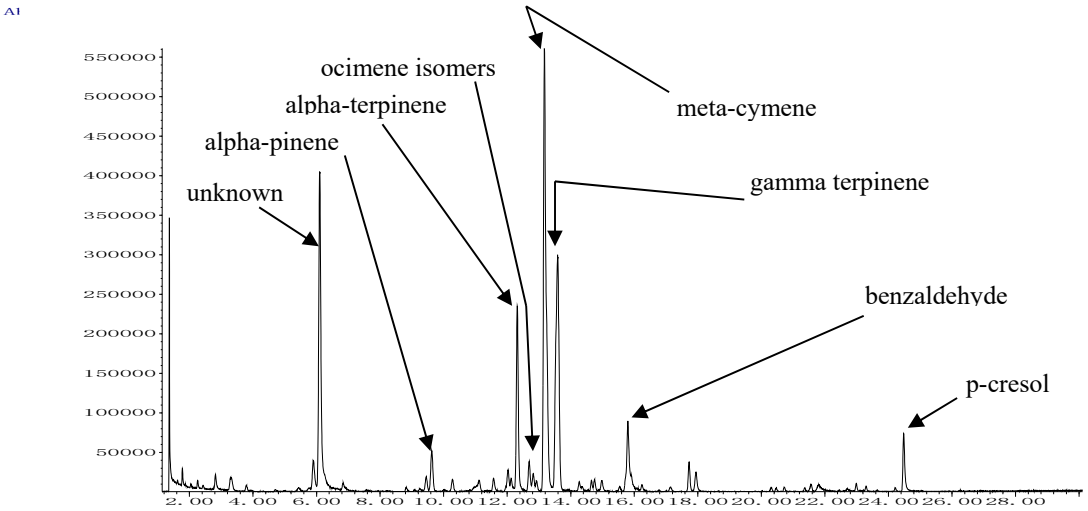

Figure #3 Overview of the Central Texas prairie verbena headspace volatiles; TIC overview VOC profile, generated in ms-SCAN acquisition mode. Volatiles collection by 70 min SPME fiber exposure.

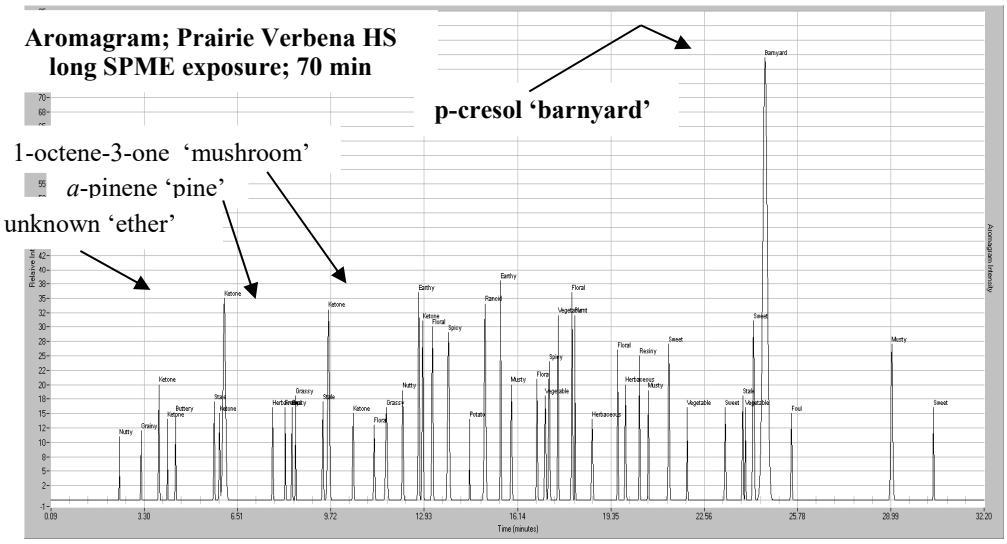

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Figure #4 Overview odor profile of the Central Texas prairie verbena headspace volatiles; aromagram odor profile, generated by GC-Olfactometry. Volatiles collection by 70 min SPME fiber exposure.

With respect to compositional complexity, it is believed noteworthy that the total ms-SCAN TIC volatiles profile in **Figure 3** approaches 100 discrete components; in spite of the fact that the initial analytical parameters were selected to favor relatively gross composition. Complexity of the prairie verbena headspace is also reflected in the range of chemical functionalities represented; including terpenes, hydrocarbons (saturated and unsaturated), ketones (aliphatic and aromatic), alcohols (aliphatic and aromatic), esters (aliphatic and aromatic) and phenolics. As a result of the gross composition format, the smallest of the peaks in this survey profile represent approximate concentrations in the high ppt to low ppb range. Likewise, with respect to sensory complexity, it is believed noteworthy that the total odor profile in **Figure 4** approaches 50 discrete odor 'notes'; in spite of the initial conditions favoring relatively gross composition.

In considering available strategies for odor assessment and monitoring, the two extremes are bounded by sensory-only and instrument-only approaches. The challenge for those taking an instrumental approach is immediately evident from the complexity of the ms-SCAN TIC chromatogram in **Figure 3**. Given that the ~100 discrete peaks in this trace actually represent relatively gross headspace composition for the prairie verbena, this 'modest' number is certain to increase several fold if pre-concentration efforts are required to reduce the detection limits from the low ppb to low ppt and below. Without direct sensory correlation, the analyst taking the instrument-only approach is faced with the daunting task of identifying, within this enormous field, all possible odorants representing potential high-impact and inferring which ultimately could constitute a character-impact subset. Not surprisingly, this effort will typically end in disappointment since the highest-impact odorants are typically at trace concentration levels; often 'buried' deep within an overwhelming background matrix.

If the instrument-only approach is flawed by a dearth of sensory correlation data, at the opposite extreme, the sensory-only approach is hobbled by the lack of critical compositional data. As stated above regarding the ms-SCAN TIC chromatogram, the ~50 discrete odor 'notes' shown in the corresponding **Figure 4** aromagram were developed under sampling parameters targeting relatively gross composition. It is fully expected that this number will increase dramatically as volatiles pre-concentration efforts are applied to reduce the limits of electronic detection from low ppb to ppt and below. Such trace-level concentrations have been shown to be 'odor-significant' for many members of the family of odorous VOCs.

Interestingly, among the arguments used for necessitating a sensory-only approach to environmental odor assessment and monitoring is the complexity such as shown in the **Figure 4** aromagram. It is often argued that the human olfactory response is inherently complex and, as a result, the composite odor from such complex mixtures is inevitably the 'combined effect' of many, if not most, of the odorous VOCs making up the complex emission field. Thus, with respect to the prairie verbena, it might be argued that the characteristic 'barnyard' odor, perceived downwind of the source colony, is the combined

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response to the ~50 odorants which are profiled in the **Figure 4** aromagram. However, the MDGC-GC-Olfactometry based odorant prioritization results below indicate that this is not the case at all. The data suggests that the characteristic 'barnyard' odor, downwind of this particular source, is remarkably simple; traceable to a single odorant from the 100+ VOC total source emission field.

After completion of the overview VOC and odorant survey profiles shown in **Figures 3** and **4**, a series of odorant prioritization runs were carried out under SPME sampling conditions; emulating a serial dilution process. The dilution process was emulated through a 7-step sequential reduction in the duration of the SPME fiber exposure interval to the equilibrated headspace formed above the prairie verbena flower clusters (i.e., 70 min 'undiluted' reference, 15 min, 5 min, 102 sec, 33 sec, 11 sec and 3 sec). Three of the VOC / aroma profiles from the incremental 3-fold 'dilution' series are shown in **Figures 5 and 6** below; reflecting, in turn, the 15 min versus 102 sec and 102 sec versus 11 sec 'dilution' steps; displayed in mirror-image format.

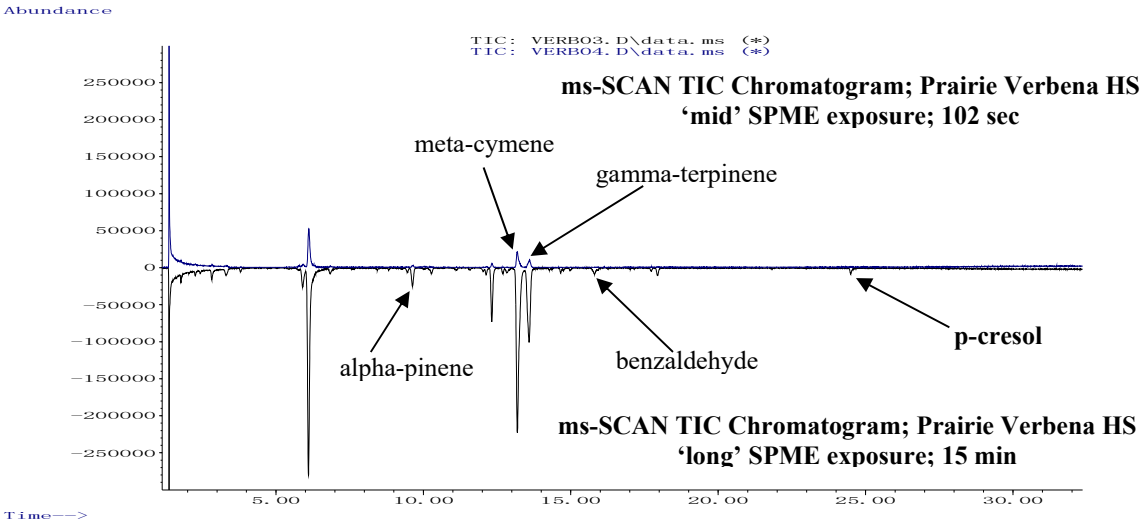

Figure #5 Serial dilution comparisons of the Central Texas prairie verbena headspace volatiles; TIC VOC profiles, generated in ms-SCAN acquisition mode. Contrasting volatiles collections of 15 min and 102 sec SPME fiber exposure. TIC chromatograms displayed in mirror-image format.

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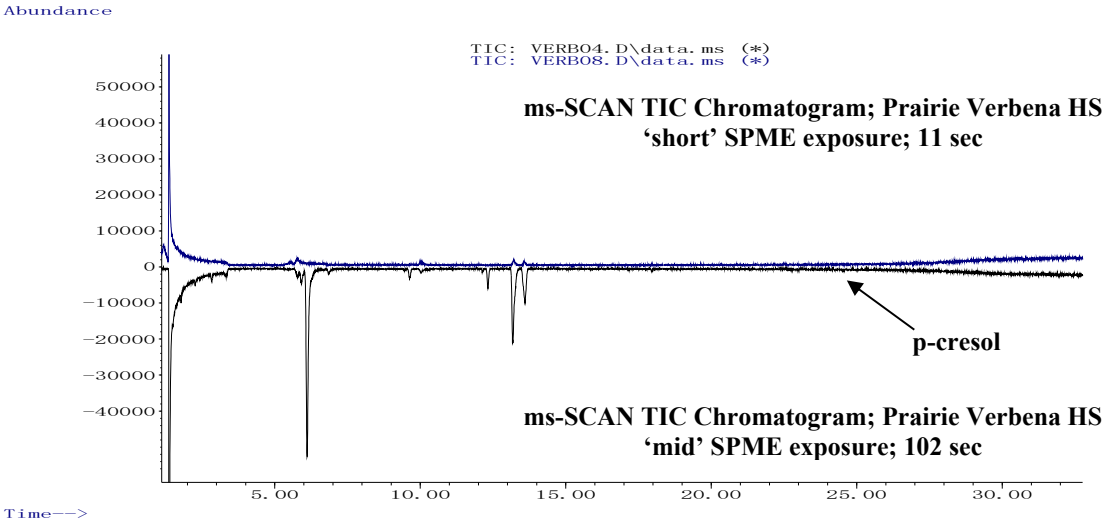

Figure #6 Serial dilution comparisons of the Central Texas prairie verbena headspace volatiles; TIC VOC profiles, generated in ms-SCAN acquisition mode. Contrasting volatiles collections of 102 sec and 11 sec SPME fiber exposure. TIC chromatograms displayed in mirror-image format.

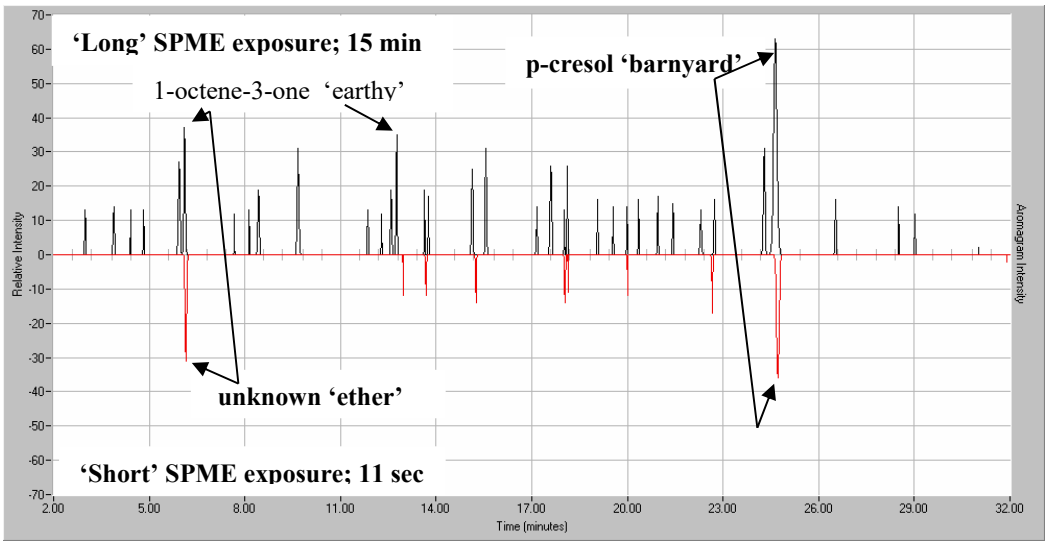

Figure #7 Serial dilution comparisons of the Central Texas prairie verbena headspace odorants; aromagram odor profiles, generated by GC-Olfactometry. Contrasting odorant collections of 15 min and 11 sec SPME fiber exposure. Aromagrams displayed in mirror-image format.;

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These profiles are representative of the simplification-on-dilution effect. Subset simplification is reflected in an approximate 10-fold reduction in total VOCs and corresponding 4-fold reduction in odorant responses in spanning the 15 min to 11 sec exposure-interval extremes. A six component, priority odorant subset was extracted from this series and is presented as a first-pass-approximation in the bolded entries in the **Table 1** listing below.

The approximate odorant priority subset for the prairie verbena can be summarized as: **(1) p-cresol (character-defining 'barnyard' or 'hog-truck' at the odor frontal boundary)**; **(2) –oxime isomers** (i.e. possible identification) @ 6.0 min with their 'ether' or 'ketone' odor character; **(3)** trans-**calamanene** @24.2 with its 'spicy' 'baked bean' odor character; **(4) alpha-pinene** with its 'pine oil' odor character; **(5) hycinthin** @ 18.0 min RT with its 'floral' odor character and **(6) 1-octene-3-one** with its 'earthy' or 'mushroom' odor character. This six component priority odorant subset represents a considerable simplification when compared to the 50 component total odorant field reflected in the 70 min profile (i.e., undiluted reference). If the goal of the study had been to prioritize the odorants responsible for the at-source or near-source composite odor of the prairie verbena colonies, these 6 odorants would carry the potential for odor character-impact significance. However, since the current interest is specifically focused on the 'barnyard' odor character at the odor frontal boundary, this six odorant subset is still considerably more complex than is necessary.

The initial odorant prioritization results suggest that the 'barnyard' composite odor at-distance relative to the prairie verbena clusters is carried, almost exclusively, by the single, character-defining odorant, *p*-cresol. Key supporting evidence for this conclusion includes the fact that: **(1)** the 'barnyard' odor character, as perceived at the olfactory detector for the isolated *p*-cresol peak, was virtually identical to the composite 'barnyard' odor character as perceived by the first author at the odor frontal boundary, downwind relative to the prairie verbena clusters; **(2)** the *p*-cresol 'barnyard' odorant was the last detectable odorant response under the maximum dilution, 3 sec SPME fiber exposure interval and **(3)** the *p*-cresol 'barnyard' response was the last detectable odorant response, in spite of the extremely brief exposure period; a condition known, under SPME sampling, to bias against compounds of such limited volatility. Taken together, this evidence constitutes a strong 'eureka moment' with respect to the odorant prioritization process. If this investigation had been tied to an actual environmental odor management issue, this evidence would be sufficiently conclusive to justify proceeding with subsequent validation and instrument-based monitoring protocol development. These follow-up efforts would focus on *p*-cresol as the target 'marker' odorant for odor monitoring and remediation efficacy assessment purposes. However, since this exercise is for the purpose of procedural illustration, it is instructive to look at the interpretive process with respect to the balance of the of the six component priority subset.

Secondary priority impact was assigned to an odorant, eluting @ 6.0 min, which carries a distinct 'ether' or 'ketone' odor. Initial mass spectral fragmentation pattern data suggests that this unknown could be a series of –oxime isomers but this prospect remains speculative, at this juncture. Key supporting evidence for the secondary priority ranking

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includes: **(1)** a relatively strong response at the 11 second SPME fiber exposure condition; presenting with a lower odor intensity in comparison to the strong 'barnyard' response for p-cresol but a considerably higher odor intensity than the balance of the priority subset field and **(2)** the fact that this odorant continued to present with a relatively high-intensity response in spite of relatively lengthy SPME fiber exposure intervals; a condition potentially biasing against compounds carrying higher volatilities.

Third priority-impact status was tentatively assigned to trans-calamanene for the 'spicy', 'sweet' or 'baked bean' aroma note @ 24.2 min RT. Key supporting evidence for this tertiary priority ranking includes: **(1)** trans-calamanene presents with a relatively strong response at the 102 sec SPME fiber exposure interval; a considerably lower odor intensity in comparison to the strong 'barnyard' response for *p*-cresol and 'ether' response for the unknown odorant @ 6.0 min RT but a considerably higher intensity in comparison to the remaining 3 components of the priority odorant subset and **(2)** the fact that the trans-calamanene 'spicy' or 'sweet' odorant was among the last remaining priority odorant responses upon dilution, in spite of the relatively short 102 sec exposure period; a condition known to bias against compounds reflecting such limited volatility.

Table 1. Comparative Impact-Priority Odorants; Prairie Verbena vs Swine Barn

Verbena Odorants* (priority odorants bolded)	**Priority Odorants* Common**	**Swine Barn Odorants*** (priority odorants bolded)
odor character = 'barnyard'	**'barnyard' odor**	**odor character = 'barnyard'**
p-cresol	**p-cresol**	**p-cresol**
-oxime unk 'ether' @ 6.0 min		**butyric acid**
trans-calamanene (tentative ID)		**isovaleric acid**
alpha-pinene 'pine'		**2-amino acetophenone**
hycinthin 'floral' @ 18.0 min		**4-ethyl phenol**
1-octene-3-one 'earthy'		**4-methyl quinazoline**
		skatole
		indole
Terpenes		**Sulfides**
beta-thujene		dimethyltrisulfide
camphene		methyl mercaptan
beta-myrcene		dimethyl sulfide
delta-3-carene		propyl mercaptan
I-phellandrene		dimethyl disulfide
alpha-terpinene		hydrogen sulfide
d-limonene		
o-cymene isomer		**Fatty Acids**
gamma-terpinene		valeric acid
delta-2-carene??		hexanoic acid
Trans-ocimene		propanoic acid
alpha-copaene		acetic acid
		heptanoic acid

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alpha-farnesene		
gamma-murrolene		**Amines**
sabinene		trimethylamine
beta-cymene		diethylamine
		1-pyrroline
Aromatics		**Aromatics**
guaiacol	guaiacol	guaiacol
benzaldehyde	benzaldehyde	benzaldehyde
benzyl isobutanoate		4-ethyl phenol
benzyl-2-methyl butyrate		phenol
benzyl-3-methyl butyrate		4-methyl-2-nitrophenol
benzyl alcohol		para-vinyl phenol
		benzoic acid
		phenyl acetic acid
		2-amino butophenone
Ketones		**Ketones**
acetone		2-octanone
2-butanone		6-methyl-5-heptene-2-one
		2-undecanone
		pentadecanone
		diacetyl
	1-octene-3-one	1-octene-3-one
Esters		
cis-carvyl acetate		**Aldehydes**
3-hexenyl-2-methyl butanoate		hexanal
methyl salicylate		nonanal
		methional
		undecanal
Alcohols		**Alcohols**
benzyl alcohol		1-octene-3-ol
		3-octanol
		1-heptene-3-ol
		trans-farnesol
		maltol
		geosmin
Miscellaneous		**Miscellaneous**
2-methyl furan	2-methyl furan	2-methyl furan
1,3-pentadiene		2-pentyl furan
2-methyl-1,3-pentadiene		dimethyl pyrazine
4,8-dimethyl-1,3,7-nonatriene		acetamide
1-methoxy-1,3,5-cycloheptatriene		4-methyl pyridine
tridecane		propanamide
		6-heptyltetrahydro-2H-pyran-2-one

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		butanamide
		3-methyl-phenyl acetate
		phenyl ethyl alcohol
		pentamide
		2-pyrrolidinone
		hexadecane
		valerolactam
		5-methyl-2,4-imidazolidinedione

Notes: * - many chemical identifications, beyond the impact-priority compounds, should be considered as tentative; they are the product of best-match efforts from Wiley and NIST mass spectral libraries matching. Many listed character-defining and character-impact odorants have been confirmed through on-instrument retention time and odor character matching.

The ranking order of the balance of the priority subset becomes much less definitive, beyond the first three priority ranking positions, and is therefore treated as a combined third tier grouping. This being said, the incentive for inclusion of the balance of the field includes: **(1)** alpha-pinene @ 9.6 min RT with its 'pine oil' odor character and hycanthin, a 'floral' note @ 18.0 min RT, both presenting with relatively strong responses at the 102 sec fiber exposure interval and **(2)** 1-octene-3-one with its 'earthy' or 'mushroom' odor @ 12.8 min RT presenting with a relatively strong response between 102 sec and 5 min fiber exposure intervals.

In summarizing the implications of the above results; it appears that ~44 of the initial ~50 odorant field likely constitute little more than background noise with respect to the at-source or near-source odor character of the prairie verbena clusters. Likewise, with respect to environmental odor impact, it appears likely that five of the initial six odorant priority subset contribute little more than background noise to the at-distance odor character of the source clusters. Finally, starting from a relatively complex VOC emission field at the source, it appears that the single, character-defining odorant, *p*-cresol, carries primary responsibility for the 'barnyard' odor character for the prairie verbena clusters, at-distance from the source. This result takes on added significance given the first author's prediction, before compositional analysis, that *p*-cresol would emerge as the character-defining odorant relative to the prairie verbena source; a clear example of prioritized odor impact simplicity arising from background complexity.

Contrasting downwind odorant prioritizations; the Central Texas prairie verbena versus a North Texas swine-barn: The character-defining status of p-cresol relative to the frontal boundary odor-character of the prairie verbena clusters forms an interesting contrast to some of the more conventional, 'agrarian' sources of 'barnyard' odors. This is clearly reflected in the **Table 1** parallel odorant prioritization listings for the prairie verbena clusters versus a representative North Texas commercial swine barn. Clearly, both of these profiles are very complex; as many as several hundred discrete VOCs, odorous and otherwise, have been previously identified as volatiles emissions from swine CAFOs (Schiffman, et.al., 2001). Interestingly, from the standpoint of at-distance odor impact, it is clearly evident in comparing the two listings that these two sources have little in common with respect to VOC emission profiles; in spite of sharing a virtually identical, at-distance odor character. Most importantly, they share no common priority

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odorants beyond *p*-cresol, the single, highest impact and character-defining component for both. Likewise, as has been previously stated, the relative absence of expected odorants from a targeted odor source can be as telling as the identities of those odorants which are confirmed to be present. In that respect, it is believed noteworthy that the prairie verbena profiles are virtually free of significant odor contribution from reduced sulfurs, free-fatty acids, saturated amines, indolics or phenolics (i.e., beyond *p*-cresol); all of which factor heavily in CAFO emission profiles. The absence of odor contribution from these odorous volatiles serves to magnify the impact significance of p-cresol and its singular correlation to the characteristic at-distance 'barnyard' odor; whether prairie verbena or swine barn sourced.

Case Study #2: Prehensile-tailed Porcupine

Composite Odor Assessment: The odorant prioritization experience, as outlined above for the fragrant prairie verbena clusters, was repeated, very closely, upon the first author's first encounter with the South American ***pt*** porcupine (i.e., *Coendou prehensilis*). This is a particularly odorous animal and an excellent natural model for the 'rolling unmasking effect'. Within the zoo population, the odor of the ***pt*** porcupine is often described as particularly 'foul'. However, the first author's initial encounter with the odor plume from an outdoor ***pt*** porcupine enclosure did not mesh, completely, with this negative description. The first encounter, in December, 2015, was an unplanned event while visiting the Moody Gardens Rainforest Exhibit in Galveston, Texas. The focus of this encounter and the odorant prioritization results which follow was the male half of the facility's ***pt*** porcupine breeder pair. The odor first encountered at the downwind odor frontal boundary relative to the outdoor exhibit (i.e. **Figure #8**); reflected a very distinct and familiar 'grilled onion' character.

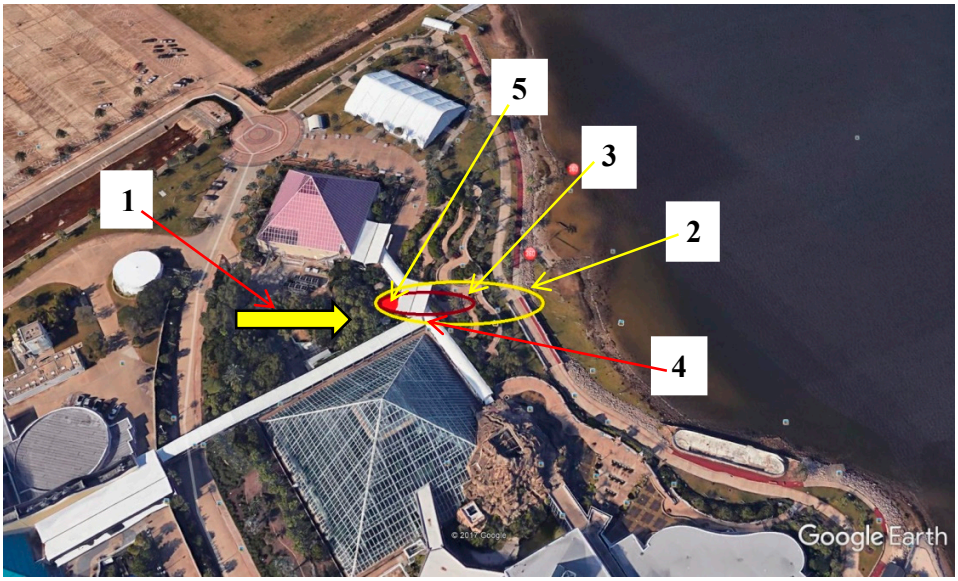

Figure #8; Google Earth Moody Gardens Rainforest Pyramid; ***pt*** **porcupine encounter showing: (1) approximate wind direction; (2) approximate odor frontal boundary; (3) approximate secondary (near-source) boundary; (4) this investigator's approximate location upon initial encounter in**

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December, 2015 and (5) approximate location of outdoor enclosure odor source for Bono, the male *pt* porcupine;

This was so much the case that upon first encounter with the odor frontal boundary, the first author actually began looking upwind of that location, trying to determine where the food court must be located; certain that that must be the source. The odor was surprisingly strong and reminiscent of 'grilled onion' or, as described by the first author at the time, '50s hamburger joint'. However, upon walking a bit deeper into the odor plume, there was encountered, almost simultaneously, an intense foul odor and an associated permanent exhibit display sign which read; 'What is that Foul Odor?'. The sign heading was followed by a descriptive paragraph bringing attention to the ***pt*** porcupine exhibit as the surprising odor source. In contrast to the relatively pleasant aroma of grilling hamburgers at the odor frontal boundary, the odor encountered deeper into the plume core was perceived as 'phenolic', 'industrial' and 'foul'. The dramatic difference in odor character between these two discrete boundaries was particularly surprising considering that only a few paces separated the pleasant 'grilled onion' character at the odor frontal boundary and the 'foul' odor character deeper into the plume core. The first author was immediately drawn to this encounter due to: **(1)** the fact that, as with the prairie verbena cluster encounter, he was fairly certain that he recognized, before analytical workup, the specific odorant / odorant family which subsequent odorant prioritization analysis would prove to be responsible for the odor, at the frontal boundary and **(2)** the ***pt*** porcupine source appeared to represent an almost perfect scale model / demonstration of the RUE which had been proposed in recent years to describe qualitative environmental odor dispersion dynamics.

Odorant Prioritization: Follow-up MDGC-MS-Olfactometry based odorant prioritization efforts, associated with the second encounter in December, 2016 confirmed the pre-analysis prediction that the impact-priority odorant would be found to be traceable, partially or exclusively, to a specific homolog from the extensive 'onion' odor carrier allylic-polysulfide family (Bleiler, et.al. 2014). The predicted '50s hamburger joint' odor note had previously been chromatographically isolated and described (i.e. chromatographic retention time and 'sniff port' detector basis only) in prior onion sourced odorant prioritization studies. Upon close inspection relative to prehensile porcupine urine headspace it appears that the 'grilled onion' odor note elutes a few seconds prior to dipropyl trisulfide and earlier still than the propyl – propenyl trisulfide isomer series. Odorant prioritization by MDGC-MS-Olfactometry also quickly confirmed that, accompanying the predicted '50s hamburger joint' odor note (i.e. unknown 'grilled onion' @20.8 min RT), was a second, earlier eluting 'onion' note (i.e. unknown 'body odor onion' @13.9 min RT) with a similar odor character. Remarkably, beyond these two character-defining odor notes, the extremely complex headspace odor profile appeared to be free of other members from the onion-sourced allylic-polysulfide family. As shown in **Figure 9** and **10** below, these two character-defining 'onion' odorants were shown to emerge from an extremely large and complex odorous VOC field; a compositional field previously shown to be common to mammalian waste in general (Roze et al., 2010; Soso et al., 2014, 2016, 2017). This result takes on added significance given the first author's prediction, before compositional analysis, that the specific unknown 'grilled onion' odorant would emerge as character-defining relative to

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the ***pt*** porcupine source; a clear example of prioritized odor impact simplicity arising from background compositional complexity.

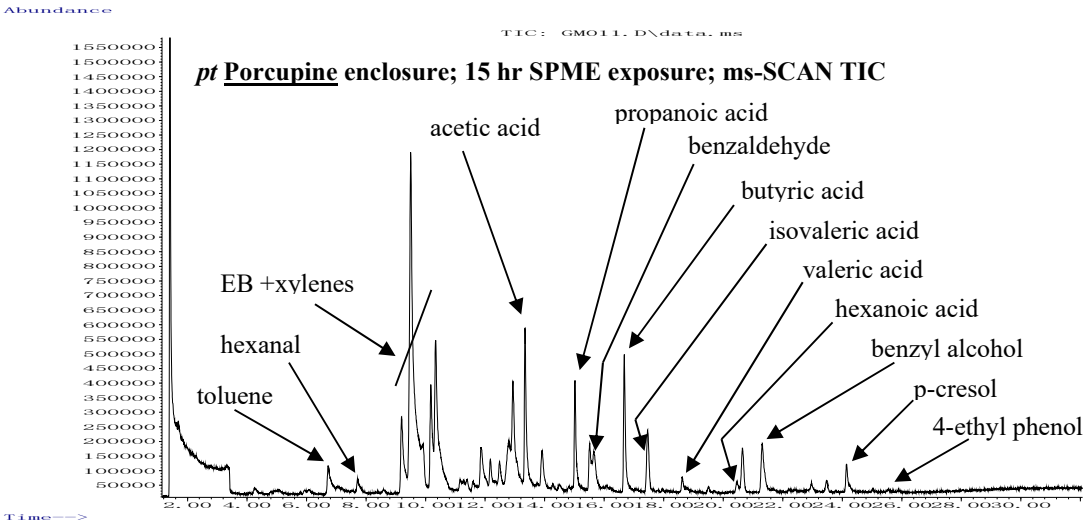

Figure #9 TIC Chromatogram of the *pt* porcupine indoor exhibit chamber VOCs; total ion overview VOC profile, generated in ms-SCAN acquisition mode. Volatiles collection by 15 hr. SPME fiber exposure to chamber environment.

***pt* Porcupine enclosure; 15 hr SPME exposure; Aromagram**

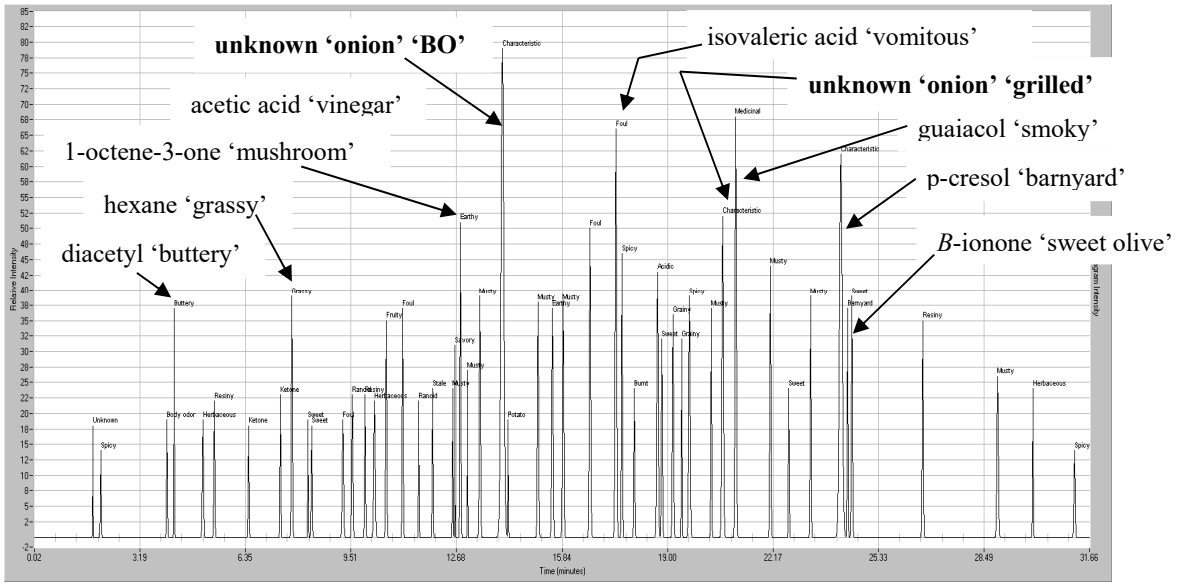

Figure #10 Aromagram odor profile of the *pt* porcupine indoor exhibit chamber odorants; overview odor profile, generated by GC-Olfactometry. Volatiles collection by 15 hr. SPME fiber exposure to the chamber environment.

Much of the analytical effort, to-date, relative to odor profiling of the male ***pt*** porcupine urine has been directed at chemical identification of the two, character-defining, 'onion' odor carrier compounds. This has included, relative to male ***pt*** porcupine urine headspace source: (**1**) the first author's MDGC-MS-Olfactometry based chromatographic

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isolation / clean-up from SPME headspace volatiles collection (**Figure 11, 12 and 13**; (**2**) Short Path Thermal Desorption based volatiles pre-concentration / single column GCMS ms-SCAN mode screening performed at Rutgers University and (**3**) independent MDGC-MS-Olfactometry based chromatographic isolation / clean-up from SPME headspace volatiles collection performed by Volatile Analysis Corporation. Despite these considerable efforts, the chemical identifications of the two character-defining 'onion' odor carrier compounds remain elusive, at the time of this writing. The primary factor accounting for this difficulty is the extreme trace concentration levels and odor potencies of these two odorants. Work to date suggests that the targeted unknown 'onion' carrier compounds appear to be unrelated to specific polysulfide odorants which have been previously reported as being responsible for 'grilled onion' and 'fried onion' odor character (Boelens, et.al., 1993; McGorrin. 2007; May-Chien Kuo, et.al. 1990). Work continues which targets chemical identification of the two critical unknown 'onion' odor carrier VOCs; odor compounds clearly critical to the characteristic odor at the odor frontal boundary relative to the ***pt*** porcupine as well as, possibly, the characteristic aroma associated with the grilling of onions.

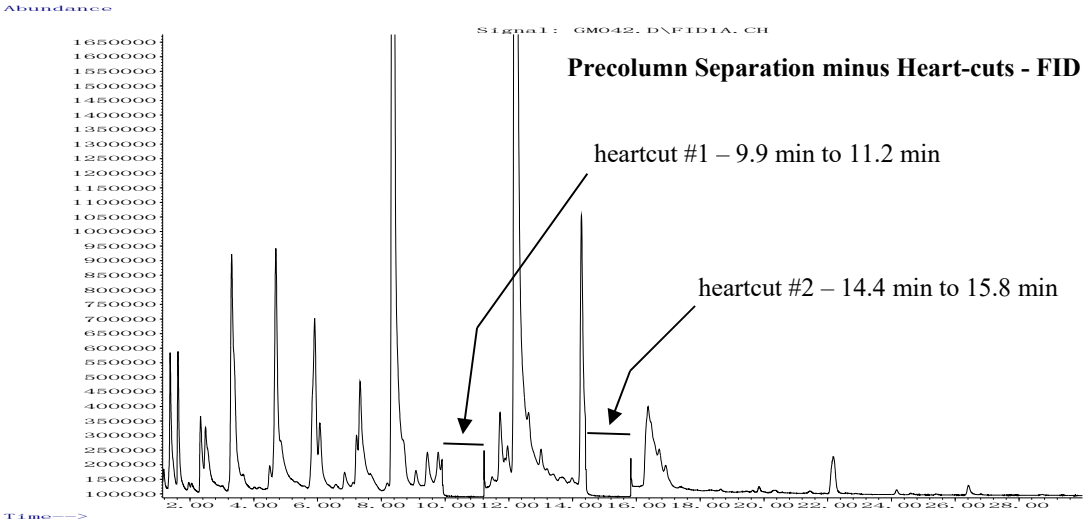

Fig. 11 FID Chromatogram of male *pt* porcupine urine headspace VOCs; pre-column overview VOC profile minus two 'onion' carrier target heart-cut isolation bands. Volatiles collection by 10 min SPME fiber exposure.

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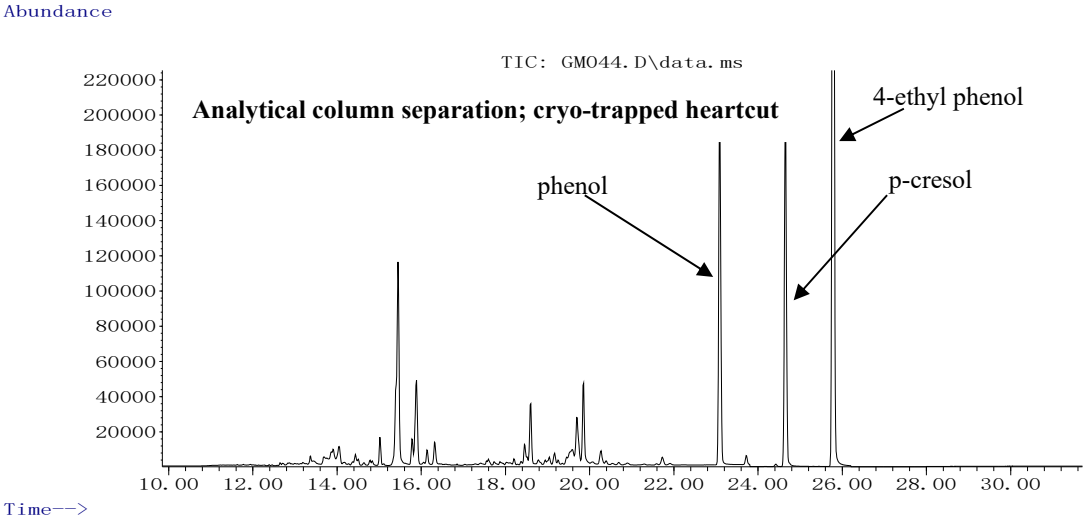

Fig. 12 ms-TIC Chromatogram of male *pt* porcupine urine headspace VOCs; analytical column separation of two 'onion' carrier target cryo-trapped heart-cut isolation bands. Volatiles collection by 60 min SPME fiber exposure.

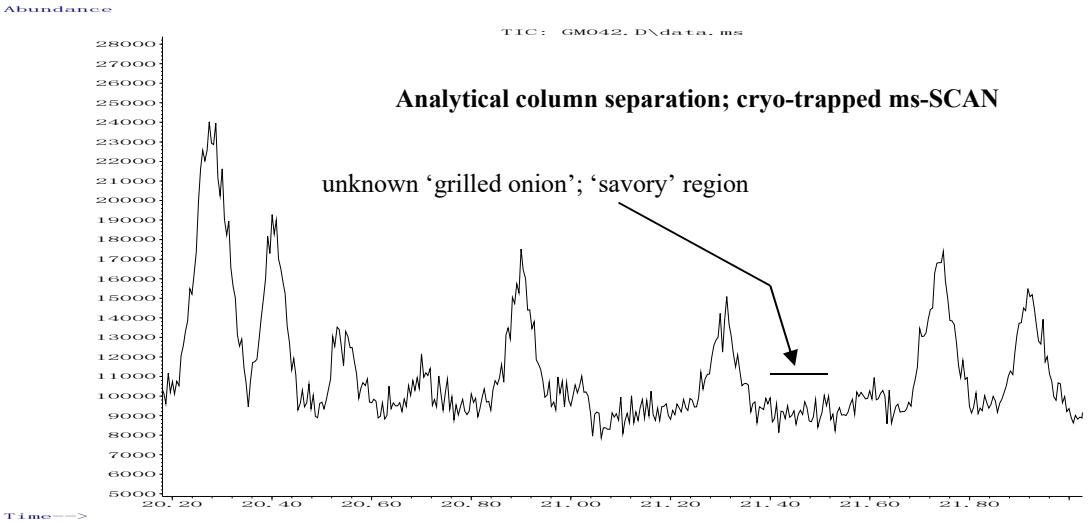

Fig. 13 ms-TIC Chromatogram of male *pt* porcupine urine headspace VOCs; analytical column separation focused on the second of two 'onion' carrier target cryo-trapped heart-cut isolation bands. Volatiles collection by 10 min SPME fiber exposure.

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838 **Table 2. Comparative Impact-Priority Odorants; *pt* Porcupine vs Swine Barn**

Prehensile Porcupine VOCs & Odorants* (priority odorants bolded)	**Priority Odorants* Common** italics = minor	**Swine Barn VOCs & Odorants*** (priority odorants bolded)
odor character = 'grilled onion'		**odor character = 'barnyard'**
unknown 'onion' @13.9 min		
unknown 'onion' @20.6 min		
p-cresol	**p-cresol**	**p-cresol**
butyric acid	**butyric acid**	**butyric acid**
isovaleric acid	**isovaleric acid**	**isovaleric acid**
		2-amino acetophenone
4-ethyl phenol	**4-ethyl phenol**	**4-ethyl phenol**
		4-methyl quinazoline
skatole	skatole	skatole
indole	indole	indole
Sulfides		**Sulfides**
dimethyltrisulfide	dimethyltrisulfide	dimethyltrisulfide
methyl mercaptan	methyl mercaptan	methyl mercaptan
dimethyl sulfide	dimethyl sulfide	dimethyl sulfide
propyl mercaptan		propyl mercaptan
dimethyl disulfide	dimethyl disulfide	dimethyl disulfide
hydrogen sulfide	hydrogen sulfide	hydrogen sulfide
Fatty Acids		**Fatty Acids**
valeric acid	valeric acid	valeric acid
hexanoic acid	hexanoic acid	hexanoic acid
propanoic acid	propanoic acid	propanoic acid
acetic acid	acetic acid	acetic acid
heptanoic acid	heptanoic acid	heptanoic acid
Amines		**Amines**
trimethylamine		trimethylamine
diethylamine		diethylamine
1-pyrroline		1-pyrroline
Aromatics		**Aromatics**
guaiacol	guaiacol	guaiacol
benzaldehyde	benzaldehyde	benzaldehyde
4-ethyl phenol	4-ethyl phenol	4-ethyl phenol
phenol	phenol	phenol
4-methyl-2-nitrophenol		4-methyl-2-nitrophenol
para-vinyl phenol		para-vinyl phenol
benzoic acid		benzoic acid
phenyl acetic acid		phenyl acetic acid
benzyl alcohol	benzyl alcohol	benzyl alcohol

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Ketones		**Ketones**
2-octanone		2-octanone
		6-methyl-5-heptene-2-one
		2-undecanone
		pentadecanone
diacetyl	diacetyl	diacetyl
acetone	acetone	acetone
Aldehydes		**Aldehydes**
hexanal	hexanal	hexanal
nonanal	nonanal	nonanal
methional	methional	methional
		undecanal
Alcohols		**Alcohols**
		1-octene-3-ol
		3-octanol
		1-heptene-3-ol
		trans-farnesol
		maltol
		geosmin
Miscellaneous		**Miscellaneous**
2-methyl furan	2-methyl furan	2-methyl furan
1,3-pentadiene		2-pentyl furan
dimethyl pyrazine		dimethyl pyrazine
4,8-dimethyl-1,3,7-nonatriene		acetamide
1-methoxy-1,3,5-cycloheptatriene		4-methyl pyridine
tridecane		propanamide
		6-heptyltetrahydro-2H-pyran-2-one
		butanamide
		3-methyl-phenyl acetate
		phenyl ethyl alcohol
		pentamide
		2-pyrrolidinone
		hexadecane
		valerolactam
		5-methyl-2,4-imidazolidinedione

Notes: * - many chemical identifications, beyond the impact-priority compounds, should be considered as tentative; they are the product of best-match efforts from Wiley and NIST mass spectral libraries matching. Many listed character-defining and character-impact odorants have been confirmed through on-instrument retention time matching.

Contrasting downwind odorant prioritizations; the South American *pt* porcupine versus a North Texas swine-barn: The character-defining status of the unknown 'onion' odorants relative to the odor frontal boundary of the ***pt*** porcupine forms an

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interesting contrast to the VOC compositional profile of the North Texas commercial swine barn. This is clearly reflected in comparing the **Table 1** and **Table 2** parallel odorant prioritization listings. In contrast to the prairie verbena versus swine barn comparisons in **Table 1**, it is clearly evident that while there was little VOC compositional commonality between the prairie verbena and the swine barn emissions, the ***pt*** porcupine and swine barn emissions present with much in common. This is especially the case with respect to their 'suspect' high-impact odorant subsets. In that respect, it is believed noteworthy that the ***pt*** porcupine presents with significant emission loadings of the reduced sulfurs, free-fatty acids, indolics and phenolics (i.e., including, in particular, *p*-cresol); all of which factor heavily in CAFO emission profiles. The absence of apparent odor contribution from these odorous VOCs, at the ***pt*** porcupine's odor frontal boundary, serves to magnify the impact significance of the two unknown 'onion' odorants and their apparent singular correlation to the characteristic frontal boundary 'grilled onion' odor. This is particularly interesting, considering that the ***pt*** porcupine and swine barn sources present with odor characteristics at their respective odor frontal boundaries which are distinctly different. Stated another way, these two sources share much in common with respect to odorous VOC emission profiles; in spite of exhibiting at-distance odor characteristics which appear unrelated!

Case Study #3: Virginia Pepperweed

Composite Odor Assessment: As described above for the prairie verbena and ***pt*** porcupine, the experience of the first author with the virginia pepperweed as a 'model' environmental odor source is also believed to be illustrative. The first conscious introduction of the first author to the natural clusters of virginia pepperweed was a chance encounter with small invasive pocket of the ubiquitous weed while mowing a Central Texas lawn. In cutting over the small clusters and approaching the odor frontal boundary, a very familiar odor was detected from his position on the lawn tractor. Approaching odor extinction at the frontal boundary, the first author was struck by the surprising intensity of the odor and its very distinct 'burnt match' odor character. This was an odor which the first author had become very familiar with as a result of past studies targeting a variety of environmental odor sources (Wright, et.al., 2008). As described previously for the prairie verbena and *pt* porcupine sources, the odorant responsible for the unique and characteristic odor from the mechanically macerated virginia pepperweed clusters could be confidently predicted at the time of that first encounter. This prediction, in advance of analytical confirmation, was that benzyl mercaptan would emerge as the character-defining odorant, primarily responsible for the distinct 'burnt match' odor character of the macerated virginia pepperweed clusters. As stated above, this prediction was significant; considering, to be ultimately proven correct, requires the first author to have recognized and correctly identified a single odorous chemical from the hundreds, or perhaps thousands, of other possible odorous chemicals and to have done so before any analytical work had been conducted.

Odorant Prioritization: Follow-up MDGC-MS-Olfactometry based odorant prioritization efforts, associated with this first encounter in June, 2016, confirmed the pre-analysis prediction that the character-defining odorant would be found to be

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traceable, dominantly or exclusively, to benzyl mercaptan. As shown below in **Figure 14** the mechanical stressing of this plant (i.e. crushing, cutting or chopping) is the critical factor which drives the release of the odorous VOC emission This is shown in the contrasting ms-SCAN TIC chromatograms as displayed below in mirror-image format; profiling ***pristine*** versus ***crushed*** VOC emission profiles.

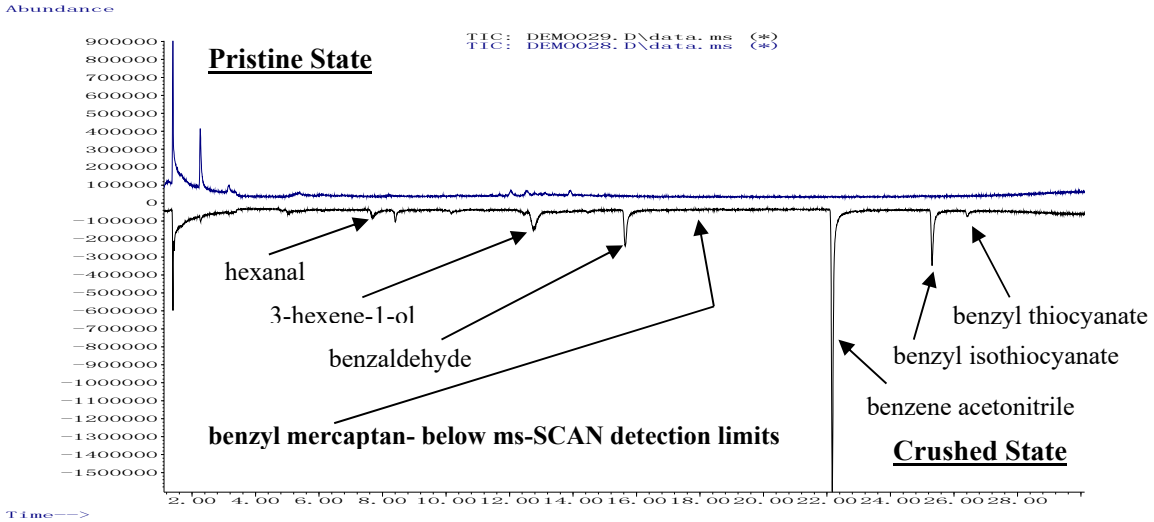

Figure #14 ms-SCAN TIC Chromatogram of the Central Texas virginia pepperweed headspace volatiles. Total ion overview profiles, generated in ms-SCAN acquisition mode and displayed in mirror-image format; reflecting pristine versus crushed states. Volatiles collection by SPME fiber exposure.

It is believed noteworthy that, while the odor at the frontal boundary presents as remarkably simple (i.e. benzyl mercaptan alone) the overall odorous VOC emission profile from the crushed plant is relatively complex. Under gross analysis conditions this complexity includes: (**1**) benzyl thiocyanate; (**2**) benzyl isothiocyanate; (**3**) hexanal; (**4**) 3-hexene-1-ol; (**5**) benzaldehyde and (**6**) benzene acetonitrile, among others. Interestingly, the character-defining benzyl mercaptan peak was not detectable under the initial gross loading / ms-SCAN survey acquisition parameters. Initial chemical identity confirmation for benzyl mercaptan was enabled by matching the known chromatographic retention time and known distinctive odor response for benzyl mercaptan at the olfactory detector. The corresponding odor profiles for the ***pristine*** versus ***crushed*** VOC emission profiles are shown in **Figure 15** below.

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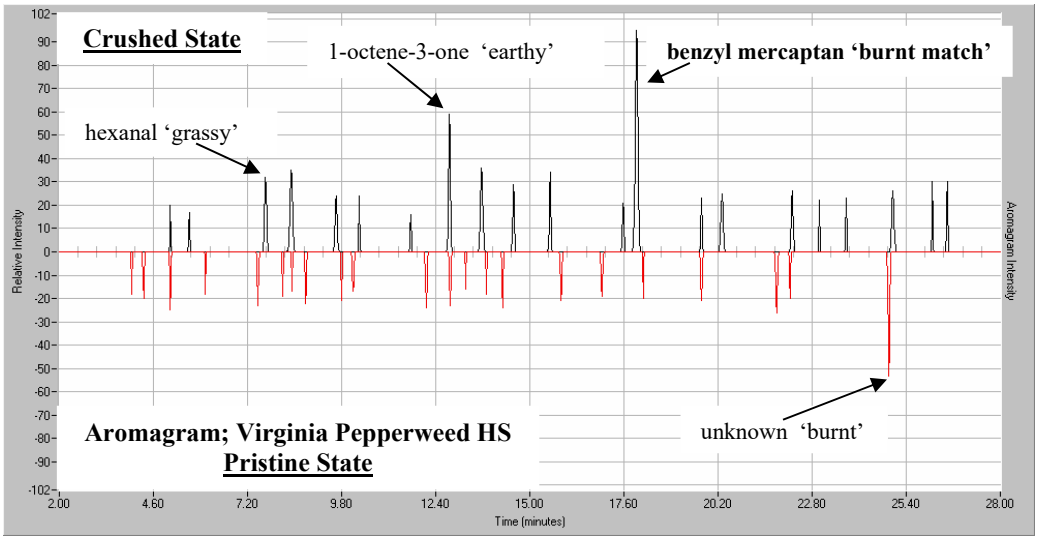

Figure #15 Odor profile aromagram of the Central Texas virginia pepperweed headspace odorants. Overview odor profiles, generated by GC-Olfactometry and reflecting odorant collections by SPME fiber exposure to headspace environments reflecting contrasting, pristine versus crushed states. Aromagrams displayed in mirror-image format.

The apparent simplicity of the benzyl mercaptan 'burnt match' odor at the frontal boundary progressed to a distinctly different odor, of much greater complexity, upon closer inspection relative to the source. In the case of the MDGC-MS-Olfactometry based odorant prioritization effort, the near-source, closer-inspection was the wide-mouth opening of the 1 L glass headspace vessel containing the crushed whole plants. In contrast to the simple 'burnt match' benzyl mercaptan odor at the odor frontal boundary, the near-source odor character, at or near the vessel opening, was perceived as 'grassy' / 'herbaceous'; dominated by the 'grassy' odor of hexanal, in combination with the 'earthy' / 'mushroom' odor of 1-octene-3-one and 'herbaceous' odor of 3-hexene-1-ol. As proposed by the first author, the overall impact-priority subset for the crushed virginia pepperweed source, was projected as: (**1**) benzyl mercaptan (**character-defining at the odor frontal boundary**); (**2**) hexanal 'grassy'; (**3**) 1-octene-3-one 'earthy'; (**4**) 3-hexene-1-ol 'herbaceous'; (**5**) unknown 'ketone' @13.59 min RT; (**6**) benzaldehyde 'cherry'; (**7**) unknown 'burnt' @24.95 min RT; (**8**) methyl mercaptan 'fecal' and (**9**) hydrogen sulfide 'sewer'.

Implications of the Rolling Unmasking Effect and Odorant Prioritization for Environmental Odor Mitigation and Monitoring Strategy Development

As a model for larger scale environmental odor sources, the virginia pepperweed, ***pt*** porcupine and prairie verbena results are believed to illustrate an important consideration: … '*with respect to focusing a community environmental odor issue, it is possible to look too closely at the source*'… In effect, looking too closely at the source often expands the study to include background noise; an unnecessary expenditure of effort if the goal is, in fact, the reduction of environmental odor impact upon human receptors. With respect to odor neutralization or mitigation, it is important to focus initial attention on the smallest subset of odorous chemicals which represent significant impact and reach, downwind

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relative to the source. In the illustrative cases presented herein these subsets are, respectively, (**1**) the benzyl mercaptan driven 'burnt match' odor of virginia pepperweed; (**2**) the two unknown 'onion' odorant driven 'grilled onion' odor of the *pt* porcupine and (**3**) the *p*-cresol driven 'barnyard' odor of prairie verbena. These subsets are the single, character-defining odorants, first recognizable at the odor frontal boundaries. Success in reducing or eliminating the highest impact odorants will often result in pushing the odor frontal boundary back toward the source; reducing its outward reach. Such a focused approach, outward to inward with respect to the source often represents the most efficient approach to development of effective strategies for remediation, monitoring and mitigation. Impact significance due to the remaining complex odorous 'noise', near the source, is often eliminated through the natural dilution process in dispersive migration outward. On a small scale the odor change upon distance separation from these small-scale sources are believed to constitute excellent natural representations of the RUE which has been previously described for larger environmental odor sources; both natural and man-made.

Unfortunately, while it is often relatively simple to identify the character-defining odorant(s) at the odor frontal boundary, it may be more challenging to accurately predict, in advance, the actual reduction in outward reach which might be realized by selective elimination of only those few critical compounds. As was shown in **Figure 1** the reduction in outward odor reach will be determined by the distance separation between the outermost frontal boundary and the nearest secondary boundary in retreating upwind toward the plume source. With regard to such a 'hypothetical' selective elimination strategy, the best-case scenario would be a considerable distance separation between the frontal boundary and the closest secondary boundary. This may or may not be what actually exists. An obvious corollary to such a selective elimination strategy / reach-reduction consideration is the corresponding impact on odor- character / odor-quality.

One unintended consequence of the selective elimination of only the character-defining odorant responsible for the odor at the frontal boundary might be the unmasking to an odor character which is even more offensive than the original; driven by the next-in-line character-defining odorant(s) responsible for the secondary boundary odor. An excellent example of this consideration is reflected in the ***pt*** porcupine odor profile wherein selective elimination of the 'grilled onion' character-defining odorants would elevate the rest of the impact-priority odorant subset. It is reasonable to predict that the emerging secondary odor boundary would be more 'barnyard' in character (Wright, et.al., 2006); owing to the secondary priority of *p*-cresol and the overall odor profile similarity between ***pt*** porcupine and swine barn summarized in **Table 2**.

Simply stated, the 'hypothetical' selective elimination of only those odorant(s) reflecting the greatest downwind reach could result in a relatively minor reduction in reach while uncovering a more offensive odor! Therefore, in practice, a more realistic strategy is to focus initial attention on the smallest character-impact subset of odorants responsible for frontal boundary + near-source combined odor character. Obviously this smallest target subset MUST include the character-defining odorant(s) shown to be responsible for the odor at the frontal boundary. As example, in the case of the ***pt*** porcupine, that smallest

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combined subset consists of the 5 to 7 odorants leading the comparative listing in **Table 2**.

Counter-intuitive odor masking… With regard to the RUE, many of the near-source versus frontal boundary impact-priority rankings described above run counter-intuitive. For example: (**1**) with respect to the Mexican Free-tail bat colonies, the near-source odor masking dominance of ammonia (a relatively weak odorant) over 2-amino acetophenone (the highly potent 'bat-cave' / 'taco shell' odorant ascending to 'un-masked' dominance at the odor frontal boundary) and (**2**) with respect to the virginia pepperweed clusters, the near-source odor masking effect of hexanal and 3-hexene-1-ol (comparatively weaker 'grassy' / 'herbaceous' odorants) over benzyl mercaptan (the highly potent 'burnt' / 'burnt-match' odorant ascending to 'unmasked' dominance at the odor frontal boundary).

The Odor Activity Value (i.e., OAV) concept has historically been applied as one way to gauge the difference in odor potency between different odorous compounds emitting from a source. OAV is defined as the simple ratio between the concentration of an odorous compound in the headspace above a source and the odor threshold concentration of that compound. The OAV concept fails to adequately explain the apparent 'flip' in odor dominance such as observed relative to the Mexican Free-tail bat colony since the OAVs for both ammonia and 2-amino-acetophenone are assumed to reflect relatively constant values spanning the short time and distance extremes reflected downwind from the high-density colonies. An alternate approach for representing this observed 'counter-intuitive' unmasking effect is proposed in the overlay odor threshold response curves in **Figure 16** below.

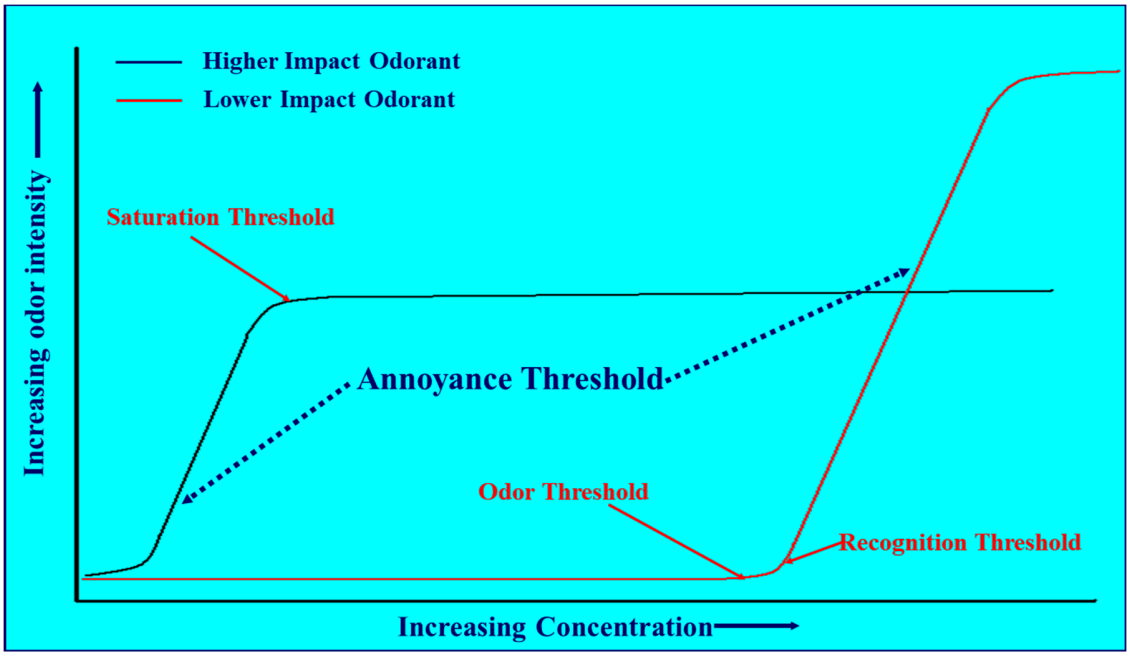

Figure 16: Representative Odor Threshold Curves; Higher impact (greater reach) odorant versus Lower impact (shorter reach but masking) odorant (e.g. 2-amino-acetophenone versus ammonia relative to Bracken Cave Mexican Free-tail bat colony).

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This is a general graphical representation of the odor response curves for two competing odorants; one reflecting relatively high odor potency, the other reflecting comparatively lower odor potency (e.g., 2-aminoacetophenone versus ammonia). As shown, the odor responses are reflected as overlay sigmoid curves; plotting concentration against odor intensity and delineated by: (**1**) **odor threshold value** - the minimal concentration that can be detected by a human receptor as a perceptible odor change; (**2**) **odor recognition threshold value** - the minimal concentration that can be detected and recognized by the human receptor as to odor character / odor source and (**3**) **odor saturation threshold value** - the concentration level at which all related olfactory receptors are activated and above which any additional concentration increase will fail to induce a corresponding increase in response intensity. While ammonia (i.e., the lower-impact and lower odor potency of the two) is pictured as requiring a much higher concentration to exceed the odor and recognition thresholds, once exceeded it rises to a response level which overtakes 2-amino acetophenone (i.e., the higher-impact and greater odor potency of the two). While OAV values account for the dominance of a higher-impact odorant up to the point of being masked, it fails to account for the apparent reversal in dominance above that juncture.

A number of mechanisms have been proposed for this observed non-linearity of the OVA values at a higher concentration; including: ***(1) synergistic effects; (2) receptor blocking effects; (3) receptor competition effects*** and possibly others. Addressing the challenge of defining the olfactory physiological mechanisms which are responsible for this observed counter-intuitive effect is beyond the scope of the work reported herein. Rather, the goal of these authors is qualitative illustration of these effects utilizing small-scale natural odor sources.

An interesting aside relative to the issue of odor threshold versus maximum odor intensity is believed reflected relative to 2,4,6-trichloroanisole (i.e., TCA) and tribromoanisole (i.e., TBA). These two 'ugly cousins' have been prioritized relative to many 'musty' consumer product odor issues; most notably, perhaps, the 'cork taint' issues from the wine industry. It is believed noteworthy that, concerning odor, the 20th Edition Merck Index describes TCA as '***faint odor similar to acetophenone***.' This reference to the odor of TCA as '***faint***' is believed noteworthy, considering its published odor threshold value of 10 parts per trillion. (Park, N., et.al., 2007) Consistent with this description, from the first author's personal experience , is the observation that the odor response to either TCA or TBA (i.e., reported odor threshold of ~30 ppq by Mallert, L., et.al., 2002) contamination can be initially masked by many other common odors co-emitting from the source; regardless of relative odor potency. In contrast, however, TCA and TBA will almost always be the 'last odors remaining' after all others have weathered away to levels below their respective odor 'masking' effect. It is believed that these observations are, at least partially, representative of the disconnect which can exist between odor threshold and odor intensity, as reflected in **Figure 16** above.

Implications of the RUE for the discourse regarding community environmental odor issues. As shown relative to the three natural environmental odor models, the complexity of odor composition is often greatly reduced upon distance separation from the source.

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An important by-product of this simplification is the potential for improving communication between critical stakeholders relative to a community environmental odor issue. In particular, the stake-holder standing to gain the most from improving communication is the downwind citizenry; the group most directly impacted by the issue. Historically, this is the stakeholder group which has been least effectively represented in community discussions regarding odor assessment, chemical prioritization, odor monitoring and mitigation strategy development.

The authors feel that the challenges which have led to this under-representation can be illustrated, at least partly, by drawing parallels from one of the other human senses; the sense of visual color perception. For example, if the cube, pictured in **Figure 17** below, was presented to a human 'sensory' panel and the panel asked to describe the color, a very high percentage of panelists are likely to describe the color as RED. If then asked to expand on this assessment, various descriptor modifiers might be added regarding the three discrete faces, such as 'tomato', 'blood' and 'fire engine'. However, these proposed modifiers would likely reflect a considerably lower level of consensus since they are cultural and/or personal experience based. Fortunately, concerning the sense of vision, physical color-wheels can be used to effectively neutralize these biases and reconcile the modifying descriptors to a closer approach back to consensus. In contrast, however, concerning the sense of smell, we are limited, solely, to such 'hazy' descriptor modifiers for reconciling communication regarding environmental odors of common interest (e.g., sewer-like, barnyard-like, skunky, musty etc.). Sensory professionals, representing various industries, have developed odor/aroma/flavor wheels which attempt to emulate the color-wheel (Torrice, M., et.al., 2017; Harbison, M., et.al., 2013). While these sensory wheels can be very effective tools in reconciling discussions between trained sensory professionals, these authors feel they are too cumbersome for practical use by the lay panel (e.g., such as populated by downwind citizenry). The practicality challenge for such odor wheels is the fact that they, too, rely on relatively imprecise descriptors such as 'musty', 'barnyard' etc.

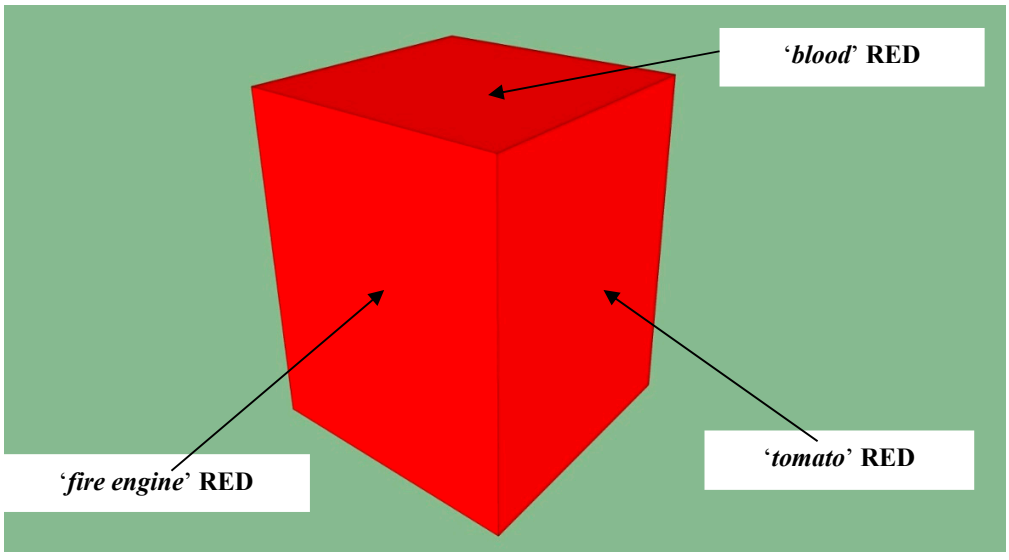

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Figure #17 Visual color perception challenge and its parallel to communication relative to odor;

The simplification of odor profiles, induced by the RUE, opens up the possibility of introducing a reconciling tool for odor which is more closely aligned with the simplicity of the color wheel for color. This tool is the use of chemical odor-matching (Wright et al., 2006). Taking as example the prairie verbena, virginia pepperweed and ***pt*** porcupine odor sources, reconciling the communication regarding odor-character at their respective odor frontal boundaries is reduced to its simplest form; confirming or rejecting their odor-match to 'suspect' high-purity odorants, *p*-cresol, benzyl mercaptan and unknown 'onion' odorant #1 + unknown 'onion' odorant #2, respectively.

In its simplest form, the odor-match query asked of a lay panelist relative to a targeted environmental odor is a simple YES or NO when presented with a trace amount of a 'suspect' character-defining odorant. This simplicity negates the requirement, on the part of the panelist, for extensive training, experience or memory acuity relative to odor recognition. The only requirement is the normal application of his / her sense of smell (i.e., assumed or demonstrated to be normally functioning). Such straight-forward odor-match surveys can be easily expanded to include query variations such as: (**1**) pick the best odor-match from a multi-unknown odorant line-up which includes the 'suspect' character-defining odorant and (**2**) apply a perceived odor-match quality grading to a perceived best odor-match selection. The odor-match validation process is the same whether the chemical reference is a single, character-defining odorant (e.g., dominant at the odor frontal boundary) or a multi-odorant formulation (e.g., synthetically replicating the combined frontal boundary + near-source odor character).

Although the odor-match approach to validation of a proposed odorant prioritization may be relatively simple and straight-forward, there are a number of practical challenges which can present. Unfortunately, even if the impact-priority odorants are isolated from the targeted sample utilizing MDGC-MS-Olfactometry, there is no guarantee that: (1) one will be able to achieve mass spectral identification of the impact-priority subset, so isolated; (2) even if one does achieve chemical identification, that the suspect odorous compound(s) will be found to be commercially available for synthetic odor-match blending or (3) even if identified and the suspect odorous compounds are found to be commercially available, that they will be available in sufficiently high purity (i.e. odor-purity).

It is noteworthy that, among the three natural sources discussed herein, the two plant sources proved to be straight-forward for odor-match validation while the ***pt*** porcupine yielded an excellent illustration of the potential challenges. Despite extraordinary efforts utilizing: (1) MDGC-MS-Olfactometry based target odorant purification / separation and (2) an 'onion' polysulfide targeted SPTD based pre-concentration enrichment protocol, the identities of the two character-defining 'onion' odor notes remain elusive (i.e., as of the time of this writing). As a result, the first author was forced to apply a novel 'work-around' which was recently developed relative to an unrelated investigation. The novel

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concept (Wright, D.; Provisional Patent Application; 2017) which is summarized below opens up the possibility of off-setting the challenges attendant with high-impact odorant 'unknowns' and 'unavailables'.

Double-heart cut isolation of high-impact odorants from crude source materials. In essence, the 'work-around' utilizes MDGC, in sample-prep mode, for on-the-fly purification / isolation / capture of the 'suspect', high-purity reference odorants from readily available crude source materials. Once refined and captured, the proposed priority odorants can be utilized off-line for presentation to the lay panelists for odor-match validation of impact-priority or character-defining status. In the case of the ***pt*** porcupine, 'dirty' urine (i.e., passive external collection; with minor entrained feces contamination) was collected from the male half of the primary Moody Gardens breeder pair and utilized as the crude source material.

For illustration purpose, **unknown 'onion' odorant #1** was the reference odorant targeted for initial odor-match validation; proceeding approximately as follows. It was experimentally determined that a timed heart-cut event; transferring the pre-column effluent to the analytical column between retention times 9.9 min and 11.20 min, effectively isolated the targeted **unknown 'onion' odorant #1** from the bulk of potential VOC interference peaks and odorants. This 78 s transfer window represented less than 6% of the total pre-column VOC separation profile of ~22 min. It was further determined experimentally, that a 12 s whole-air fraction collect when taken at the olfactory detector from this initial 78 s heart-cut separation band; further refined the targeted **unknown 'onion' odorant #1** fraction (i.e., essentially constituting a heart-cut collection from a heart-cut purification). Mechanically, an inert, low-odor, polyolefin gas-tight syringe was used to 'vacuum' aspirate this 12 sec fraction (i.e., **Figure #18**), between 13.93 min and 14.13 min; capturing the targeted **unknown 'onion' odorant #1** peak as it eluted to the olfactory detector nose-cone. This 2-stage, clean-up fraction represented less than 1.0% of the extremely complex 22 min pre-column VOC profile as collected from the headspace above the crude urine sample.

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Figure #18; Fraction Collect Process. Whole-air fraction collection; aspiration of olfactory detector effluent for deferred, off-line odor assessment.

Off-line composite odor assessment of the syringe vapor contents confirmed the odor purity of the isolated fraction. Upon off-line presentation to three collaborative associates, there was consensus agreement, with the first author, for the 'onion' / 'grilled onion' odor character descriptor. Likewise, upon off-line presentation to a collaborative associate from the Moody Gardens Rainforest Exhibit team, there was consensus agreement, with the first author, for the high-fidelity match to the characteristic odor of the ***pt*** porcupine exhibit (i.e., upon dilution of distance separation). When presented with the isolated **unknown 'onion' odorant #1** fraction, she agreed, enthusiastically, that it did reflect the odor character of the ***pt*** porcupine, upon dilution. However, it is also interesting to note, that she did not characterize the odor as 'onion' specifically; rather she volunteered that it had always reminded her of a favorite sauce that was frequently made by her grandmother. It is also noteworthy that a second member of the team, the Assistant Curator of the Rainforest exhibit, had volunteered, in the initial conversation with the first author in 2015, her impression that the dilute odor character of the ***pt*** porcupine was that of 'stale onion'. This observation is believed significant since, up to the time of that observation, the first author had not volunteered that he had already predicted that the odor would be traced to a specific compound from the extensive polysulfide 'onion' family of odorants. These contrasting odor character descriptors appear to reflect another manifestation of the need for reconciling the discussion relative to environmental odors; distinctly different contrasting descriptors, from multiple odor panelists, for the same refined chemical odorant. With the exception of the one 'stale onion' description, it is interesting to note that, driven by the RUE the balance of the assessments were assessed as relatively pleasant; in marked contrast to the assessment first encountered on the exhibit's instructional exhibit sign; '***what is that foul odor***?'.

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CONCLUSIONS

As scale-models for community environmental odor issues, the odorant prioritization results, presented herein, illustrate an important consideration. Regardless of the relative size and reach of an environmental odor source, a simplification of odor character and composition will typically develop in dispersive migration outward from that source. Extremes of odor simplification-upon-dilution were demonstrated for two Central Texas plant varieties, prairie verbena and virginia pepperweed. Their 'odor frontal boundaries' were shown to be dominated by single, character-defining odorants; prairie verbena presenting with a p-cresol dominated 'barnyard' odor and virginia pepperweed with a benzyl mercaptan dominated 'burnt match' odor. Similar odor simplification was also shown for the South American ***pt*** porcupine; it's downwind 'odor frontal boundary' dominated by two potent, character-defining odorants (i.e. as yet unidentified): (**1**) **'onion' / 'body odor' odorant #1** and (**2**) **'onion' / 'grilled' odorant #2**. In contrast with their boundary simplicities, each of these sources also presented, at the source, with odor compositions reflecting considerable complexity and corrresponding composite odor character differences.

Although simple odor dilution, as measured by odor concentration and intensity, certainly occurs during downwind dispersive migration from the source, the term ***dynamic dilution*** is limiting with respect to downwind environmental odor impact. The results presented herein suggest that the process of downwind environmental odorant prioritization can better be described as a ***rolling unmasking effect*** or RUE. The RUE is exhibited by the masking odors nearest the source sequentially 'falling away' with distance from the source, revealing a succession of increasingly simplified odor characteristic and composition, such as reflected in the three natural model sources.

As a result of scaling factors and meteorological unpredictability, the logistics involved in carrying-out odorant prioritization studies can be very challenging when targeting large-scale odor sources. However, for these author's illustrative purposes, these challenges were reduced significantly by selecting natural, 'scale-model' odor-sources which represented significant reductions in the primary scaling factors; especially, reductions in the size of the odor sources and the distance of their downwind reach.

Significant parallels for community odor issues can be drawn from odorant prioritization and the RUE driven simplification-upon-dilution, as demonstrated in these scale-model studies. Most notably are: (**1**) the potential for focusing of odor monitoring strategy development to the most technologically appropriate for the impact-priority subset of odorants; (**2**) the focusing of odor mitigation strategy development; enabling, potentially, a more focused resolution of the environmental odor issue and (**3**) making possible the integration of odor-matching as a reconciling tool for improving communication, among stakeholders, regarding community odor issues. The odor-matching strategy is suggested as more closely aligning with the simplicity of the color wheel as applied to communication regarding visual color perception. The authors also presented a novel MDGC-MS-O based technique for off-setting challenges attendant with blending of high-impact odorant 'unknowns' and 'unavailables'. The novel technique utilizes MDGC, in

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sample-prep mode, for on-the-fly purification / isolation / capture of 'suspect', high-purity reference odorants from readily available crude source materials.

REFERENCES

Abu-Lafi, S., J.W. Dembicki, P. Goldshlag, L. O. Hanuš, V.M. Dembitsky. 2004. The use of the 'Cryogenic' GC/MS and on-column injection for study of organosulfur compounds of the *Allium sativum*. *Journal of Food Composition and Analysis*, 17, 235–245. DOI: 10.1016/j.jfca.2003.09.002

Akdeniz, N., L.D. Jacobson, B.P. Hetchler, S.D. Bereznicki, A.J. Heber, J.A. Koziel, L. Cai, S. Zhang, D.B. Parker. 2012a. Odor and odorous chemical emissions from animal buildings: Part 2 – odor emissions. *Transactions of ASABE*, 55(6), 2335-2345.

Akdeniz, N., L.D. Jacobson, B.P. Hetchler, S.D. Bereznicki, A.J. Heber, J.A. Koziel, L. Cai, S. Zhang, D.B. Parker. 2012b. Odor and odorous chemical emissions from animal buildings: Part 4 – correlations between sensory and chemical concentrations. *Transactions of ASABE*, 55(6), 2347-2356.

Bleiler, R.; Iwasinska, A.; Kuhrt, F.; Wright, D. 2014. *Formulations: A stable chemical / sensory equivalent to natural products for permeation / package testing*. Proceedings of the Society of Sensory Professionals Meeting. Tucson, Arizona; Sept 17-19.

Block, E., D. Putman, S.-H. Zhao. 1992. Allium Chemistry: GC-MS Analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, chive, and Chinese chive. *J. Agric. Food Chem*. 40, 2431-2436.

Boelens, M.H. and van Gemert, L.J. 1993. *Volatile character-impact sulfur compounds and their sensory properties*; Perf. Flav.; 18(3); 29.

Bulliner E.A., J.A. Koziel, L. Cai, D. Wright. 2006. Characterization of livestock odors using steel plates, solid phase microextraction, and multidimensional - gas chromatography-mass spectrometry- olfactometry. *Journal of the Air & Waste Management Association*, 56, 1391-1403.

Caraway, E.A., D. Parker, M.Ruby, G. Green, J. Spears, M. Olsen, M. Rhodes, Z. Buser. 2007. Identification of malodorous compounds from a fishmeal plant. Proceedings of the International Symposium on Air Quality and Waste Management for Agriculture; Broomfield, Colorado.

Chung, I.-M. N. Praveen, S.H. Kim, A. Ahmad. 2012. Composition of the essential oil, neutral volatile oil and petroleum ether extract from *Allium sativum* of different regions in Korea and antioxidant activity. *Asian Journal of Chemistry*; 24(2), 827-831.

Harbison, M.; (adapted from Dredge, M.). 2013. *Daily infographics; beer edition*: *The beer flavor and aroma wheel;* Popular Science (web edition); Jan 10.

Jacobson, L.D., B.P. Hetchler, D.R. Schmidt, R.E. Nicolai, A.J. Heber, J. Ni, S.J., Hoff, J.A. Koziel, D.B. Parker, Y. Zhang, D.B. Beasley. 2008. Quality Assured Measurements of Animal Building Emissions: Odor Concentrations: *Journal of the Air & Waste Management Association*, 58, 806-811.

Koziel, J.A., J.P. Spinhirne, J. Lloyd, D. Parker, D. Wright, F. Kuhrt. 2005. Evaluation of sample recoveries of malodorous gases for odor bags, SPME, air sampling canisters, and sorbent tubes. *Journal of the Air & Waste Management Association*, 55, 1147-1157.

Koziel, J.A., L. Cai, D. Wright, S. Hoff. 2006. Solid phase microextraction as a novel air sampling technology for improved, GC-Olfactometry-based, assessment of livestock odors. *J Chromatogr. Sci.*, 44(7), 451-457.

Laor, J., J.A**.** Koziel, L. Cai, U. Ravid. 2008. Chemical-sensory characterization of dairy manure odor by headspace solid phase microextraction and multidimensional gas chromatography–mass spectrometry-olfactometry. *Journal of the Air & Waste Management Association*, 58, 1187-1197

Lo, Y.C., J.A. Koziel, L. Cai, S.J. Hoff, W.S. Jenks, H. Xin. 2008. Simultaneous chemical and sensory characterization of VOCs and semi-VOCs emitted from swine manure using SPME and multidimensional gas chromatography-mass spectrometry-olfactometry system. *Journal of Environmental Quality*, 37(2), 521-534.

Luo, J., A. vanOostrom. 1997. Biofilters for controlling animal rendering odour; a pilot plant study. Pure & Applied Chem., 69(11), 2403-2410.

Maurer, D., J.A. Koziel, J.D. Harmon, S.J. Hoff, A.M. Rieck-Hinz, D.S Andersen. 2016. Summary of performance data for technologies to control gaseous, odor, and particulate emissions from livestock operations: Air Management Practices Assessment Tool (AMPAT). *Data in Brief*, 7, 1413-1429. doi: 10.1016/j.dib.2016.03.070.

Mallert, L., A. Bruchet. 2002. A taste and odour episode caused by 2,4,6-tribromoanisole. Journal of American Water Works Association, 94(7), 84-95.

May-Chien Kuo; Mingjien Chien and Chi-Tang Ho. 1990. *Novel polysulfides identified in the volatile components from Welsh onions and scallions*. J. Agri. Food Chem.; 38, 1378-1381.

McGorrin, R.J. 2007. Chapt 9. *Character-impact flavor compounds*. In Sensory Directed Flavor Analysis. Marsili, R. ed.. Taylor & Francis Group; Boca Raton, Fl.

Nielsen, L.T., Eaton, D.K., Wright, D.W. and Schmidt-French, B. 2006 Characteristic odors of *Tadarida braziliensis Mexicana* chiroptera: molossidae. *J. of Cave and Karst Studies* 68, No. 1, p. 27-31.

Park, N., Y. Lee, S. Lee, J. Cho. 2007. Removal of taste and odor model compound (2,4,6-trichloroanisole) by tight ultrafiltration membranes. Desalination, 212(1-3), 28-36.

Parker, D.B, M.B. Rhoades, G.L. Schuster, J.A. Koziel, Z. Perschbacher. 2005. Odor characterization at open-lot cattle feedyards using triangular forced-choice olfactometry. *Transactions of the ASABE*, 48(4):1527-1535.

Parker, D.B., J.A. Koziel, L. Cai, L. Jacobson, N. Akdeniz, S. Bereznicki, T.T. Lim, E. Caraway, S. Zhang, S.J. Hoff, A.J. Heber, K. Heathcote, B. Hetchler. 2012. Odor and odorous chemical emissions from animal buildings: Part 6 – odor activity value. *Transactions of ASABE*, 55(6), 2357-2368.

Rice, S., J.A. Koziel. 2015a. Characterizing the smell of marijuana by odor impact of volatile compounds: An application of simultaneous chemical and sensory analysis. *PLoS ONE*, 10(12): e0144160. doi: 10.1371/journal.pone.0144160.

Rice, S., J.A. Koziel. 2015b. Odor impact of volatiles emitted from marijuana, cocaine, heroin and their surrogate scents. *Data in Brief*, 5, 653-706.

Rice, S., J.A. Koziel. 2015c. The relationship between chemical concentration and odor activity value explains the inconsistency in making a comprehensive surrogate scent training tool representative of illicit drugs. *Forensic Science International*, 257, 257-270.

Roze, U., K.T. Leung, E. Nix, G. Burton, D.M. Chapman. 2010. Microanatomy and bacterial flora of the perineal glands of the North American porcupine. *Can. J. Zool.* 88, 59-68.

Schiffman, S.S.; Bennett, J.L. and Raymer, J.H. 2001. *Quantification of odors and odorants from swine operations in North Carolina*; Agricult. Forest. Meteorol; 108; 213-240.

Soso, S.B., J.A. Koziel, A. Johnson, Y.J. Lee, W.S. Fairbanks. 2014. Analytical methods for chemical and sensory characterization of scent-markings in large wild mammals: a review. *Sensors*, 14(3), 4428-4465. doi:10.3390/s140304428.

Soso, S.B., J.A. Koziel. 2016. Analysis of odorants in marking fluid of Siberian tiger (*Panthera tigris altaica*) using simultaneous sensory and chemical analysis with headspace solid-phase microextraction and multidimensional gas chromatography-mass spectrometry-olfactometry. *Molecules*, 21(7), 834; doi: 10.3390/molecules21070834.

Soso, S.B., J.A. Koziel. 2017. Characterizing the scent and chemical composition of *Panthera leo* marking fluid using solid-phase microextraction and multidimensional gas chromatography-mass spectrometry-olfactometry. *Scientific Reports,* 7(1):5137. doi: 10.1038/s41598-017-04973-2.

Torrice, M.; (adapted from Suffet, M.). 2017. *The scientists who sniff water*; C&E News; July 3; pp. 16-19.

Wright, D.W.; Nielsen, L.; Eaton, D.; Kuhrt, F.; Koziel, J.A.; Spinhirne, J.P.; Parker, D.B. 2005. *Multidimensional GC-MS-olfactometry for identification and*

prioritization of malodors from confined animal feeding operations. *J. Agric. Food Chem. 53*: 8663-8672.

Wright, D.W., Eaton, D.K., Nielsen, L.T., Kuhrt, F.W. Koziel, J.A.; and Parker, D.B. 2005. *Improved, GC-olfactometry based, malodor assessment of swine CAFOs utilizing novel air sampling technologies*. Proceedings of the Air & Waste Management Association Symposium; Minneapolis, MN; June 21-24**.**

Wright, D.W.; Eaton, D.K.; Nielsen, L.T.; Kuhrt, F.W.; Koziel, J.A.; Lingshuang, Cai.; Yin-Cheung Lo.; Parker, D.B. and Buser Z.. 2006. *Synthetic CAFO odor formulation; an effective technique for validation of odorant prioritizations*. Proceedings of the Ecological Society of America Conference.; Washington, DC.; June 5-8.

Wright, D.W.; Nielsen, L.; Eaton, D.; Kuhrt, F.; Koziel, J.A.; Cai, L.; Parker, D.B. 2006. *Animal Odor Assessment – Chickens, Pigs, Bats or Cats; it is Still Analytical Chemistry*. Proceedings of the National Poultry Waste Management Symposium. Springdale, Arkansas; Oct 23-25.

Wright, D.; Wright, H.; Iwasinska, A.; Kuhrt, F.; Koziel, J. and Tippett-Mosby, L. 2008; *Carthage bottoms area odor study; A Missouri test case for odorant prioritization as a prelude to instrument based downwind odor monitoring protocol development*. Proceedings of the ASABE National Conference. Providence, R.I.; June 29- July 02.

Wright, D.W. 2017. *MDGC-MS-Olfactometry based device and strategy for preparation of odor-match blends from crude odor source materials*. Provisional Patent Application with USPTO.; Application number 62/605,374; filing date July 18.

Yabuki, Y., Mukaida, Y., Saito, Y., Oshima, K., Takahashi, T., Muroi, E., Hashimoto, K., Uda, Y. 2010. Characterisation of volatile sulphur-containing compounds generated in crushed leaves of Chinese chive (*Allium tuberosum* Rottler). *Food Chemistry*, 120, 343-348.

Yu, T.-H., L.-Y. Lin, C-T. Ho. 1994a. Volatile compounds of blanched, fried blanched, and baked blanched garlic slices. *J. Agric. Food Chem*. 42, 1342-1347.

Yu, T.-H., C.-M. Wu, R.T. Rosen, T.G. Hartman, C-T. Ho. 1994b. Volatile compounds generated from thermal degradation of alliin and deoxyalliin in an aqueous solution. *J. Agric. Food Chem*. 42, 146-153.

Zhang, S., L. Cai, J.A. Koziel, S. Hoff, D. Schmidt, C. Clanton, L. Jacobson, D. Parker, A. Heber. 2010. Field air sampling and simultaneous chemical and sensory analysis of livestock odorants with sorbent tube GC-MS/Olfactometry. *Sensors and Actuators: B. Chemical*, 146, 427-432.

Zhang, S., J.A. Koziel, L. Cai, S.J. Hoff, K. Heathcote, L. Chen, L. Jacobson, N. Akdeniz, B. Hetchler, D.B. Parker, E. Caraway, A.J. Heber, S. Bereznicki. 2015. Odor and

odorous chemical emissions from animal buildings: Part 5 – correlations between odor intensities and chemical concentrations (GC-MS/O). *Transactions of ASABE*, 58(5) 1349-1359. doi.10.13031/2013.32645.

Zhu, W., J.A. Koziel, L. Cai, D. Wright, F. Kuhrt. 2015. Testing odorants recovery from a novel metalized fluorinated ethylene propylene gas sampling bag. *Journal of the Air & Waste Association*, 65(12), 1434-1445.

Zarra, T., V. Naddeo, V. Belgiorno, M.Reiser, M. Kranert. 2008. Odour monitoring of small wastewater treatment plant located in a sensitive environment. *Water Science & Technology*, 58.1: 89-94.

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SUPPLEMENTARY MATERIAL

Figure S1. Prairie Verbena cluster; p-cresol 'barnyard' odor source;

Figure S2. Prairie Verbena field; p-cresol 'barnyard' odor source;

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Figure S3. Virginia Pepperweed; benzyl mercaptan 'burnt match' odor source;

Figure S4; Cora; female porcupine at Moody Gardens, Galveston, Texas.

Figure S5. Sampling *point at a* porcupine exhibit chamber at Moody Garden. SPME fibers protected by affixing to hanging fixture.

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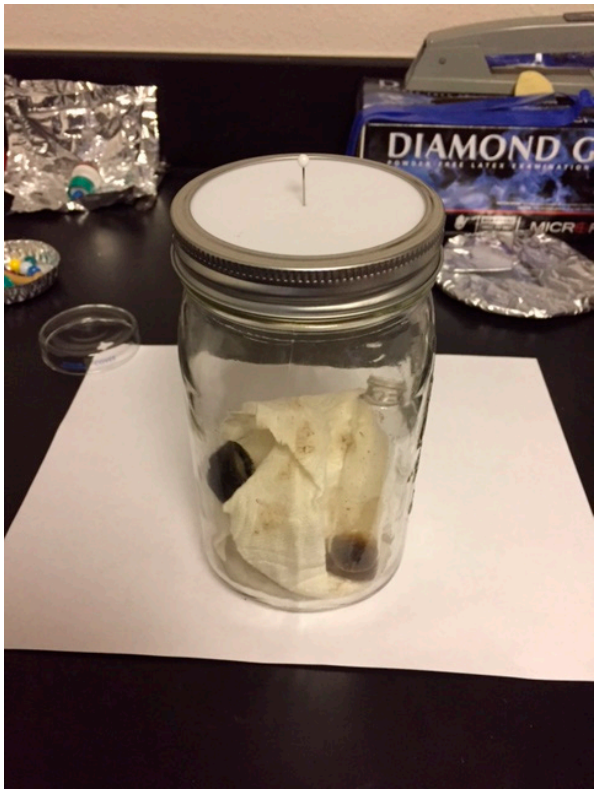

Figure S6. One-quart glass sampling jar, equilibrating between SPME fiber insertions. Combination urine deposited on low-odor paper towel + excess neat urine sample in open vial.