OPTIONS FOR THE EVALUATION OF NOVEL SOLVENTS

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1. **Introduction**

As amine-based solvent vendors seek to balance the efficiency of CO_2 capture against the dangers posed by nitrosamine/nitramine formation from NO_x reactions, a variety of novel amine-based solvents are likely to be proposed for use in CO_2 capture. Regulators and operators of CO_2 capture plants must develop a strategy for evaluating the risks to human health and safety posed by these novel solvents. Here we focus on human health endpoints, because it is likely that these endpoints would necessitate lower emission limits than environmental endpoints. This report proposes a strategy based upon a combination of chemical and toxicological assays to evaluate novel amine-based solvents. Solvents not based upon amines are not addressed in this strategy. Chemical assays would address dangers posed by specific chemical families known to pose health hazards to humans at low concentrations. Here, we consider nitrosamines and nitramines. Toxicological assays would target human health-related endpoints more broadly. General chemical and toxicological analytical approaches and their rationale are described first. Afterwards, a strategy for their application is proposed.

2. Chemical Analytical Approaches

2.1. Prediction of specific N-nitrosamine/N-nitramine byproducts and their analyses:

When the chemical constitution of the solvent is provided by the vendor, it is possible to predict important, specific nitrosamine/nitramine byproducts that may form. The prediction would be based on the formation pathways for these byproducts outlined in our literature summary. For example, although we are not examining MDEA as a solvent in this project, we can apply our knowledge of general nitrosamine/nitramine formation pathways to predict the structures in Figure 1 to be potentially important nitrosamine/nitramine products of MDEA-NO_x reactions. Formation pathways indicate that tertiary amines lose an alkyl group. When the methyl group is removed, diethanolamine is one product. Accordingly, nitrosamines/nitramines relevant to diethanolamine may form, including N-nitromonoethanolamine, N-nitrosodiethanolamine, N-nitrodiethanolamine, N-nitrosodimethylamine, N-nitrodimethylamine, N-nitrosomorpholine, and N-nitromorpholine. Alternatively, an ethanol functional group may be removed, leaving methylethanolamine. N-nitromethylethanolamine and N-nitrosomethylethanolamine are potential byproducts.

These byproducts could be targeted for specific chemical analysis. Note that this would require synthesis of relevant chemical standards. We anticipate that the analytical methods being developed by this project could be modified to these target compounds. For example, our EPA Method 521 analysis should capture nitrosamines and nitramines that do not contain hydrophilic alcohol groups. In the case of MDEA, N-nitrodimethylamine, N-nitrosodimethylamine, N-nitromorpholine, and N-nitrosomorpholine would be captured, and chemical analyses for these compounds are being developed as part of this project. More polar products containing alcohol groups would be captured by the method we are developing for N-nitromonoethanolamine, N-nitrosodiethanolamine, and N-nitrodiethanolamine. In the case of MDEA, this method could be modified to include N-nitromethylethanolamine and N-nitrosomethylethanolamine.

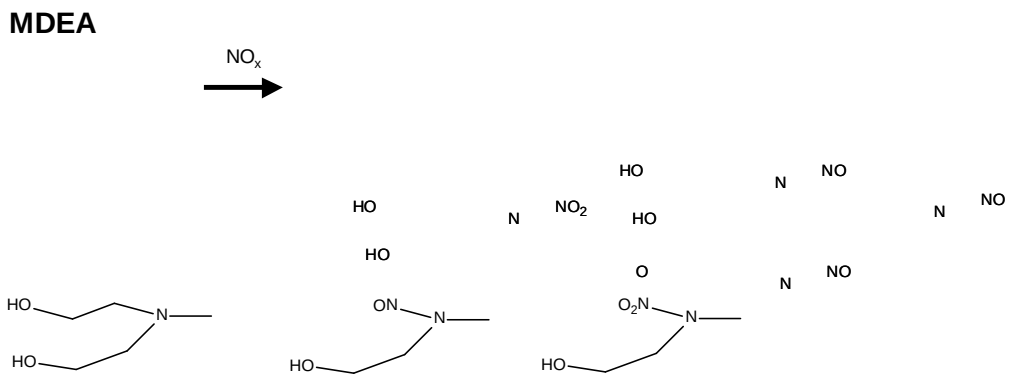

Figure 1 Predicted byproducts of MDEA exposure to NO_x.

2.2. Bulk nitrosamine/nitramine analyses: Our literature review on general nitrosamine/nitramine formation pathways provides some insights into specific, likely byproducts, and hopefully enables prediction of the most important byproducts. However much of the reviewed literature was obtained from other research fields under conditions far from those observed in CO_2 capture units (e.g., biological literature on nitrosation reactions in vivo). The unique conditions of CO_2 capture units, including temperatures up to 120 °C (desorber unit) and high gas-liquid transfer potentials, are likely to foster a plethora of unanticipated reactions and associated byproducts. For example, we have detected concentrations of N-nitrosopiperidine comparable to those of N-nitrosomorpholine and N-nitrosodimethylamine in washwaters downstream of a CO_2 capture absorber unit employing a monoethanolamine-based solvent. While our literature review indicated that N-nitrosomorpholine and N-nitrosodimethylamine were expected products, we could find no pathways relevant to the formation of N-nitrosopiperidine.

Given that it is unlikely that all specific nitrosamine/nitramine byproducts will be predicted by the current formation literature, bulk nitrosamine/nitramine analyses that quantify the total molar concentration of nitrosamines and nitramines in a sample would be useful. Combined with the specific chemical analyses, bulk analyses would indicate what fraction of the total nitrosamine/nitramine pool is constituted by the specific nitrosamines/nitramines analyzed. Toxicological information available on the U.S. EPA's Integrated Risk Information System (IRIS) database indicate that cancer potencies for nitrosamines exhibiting a range of physical properties (i.e., polar N-nitrosodiethanolamine to hydrophobic N-nitrosodibutylamine) range over only 2 orders of magnitude, with no specific correlation with hydrophobicity. As a first approximation, we could consider cancer potencies of nitrosamines lacking toxicological data as comparable to those on the U.S. EPA IRIS database. Accordingly, if the bulk nitrosamine/nitramine analyses indicate that the specific nitrosamines/nitramines anticipated for a solvent constitute a minor fraction of the total nitrosamine/nitramine pool, there would be a significant motivation to characterize the additional nitrosamines/nitramines. Alternatively, if the bulk nitrosamine/nitramine analyses indicate that the specific nitrosamines/nitramines

anticipated for a solvent constitute a significant fraction of the total nitrosamine/nitramine pool, there would be less motivation to characterize the additional nitrosamines/nitramines, because the specific nitrosamines/nitramines would account for the majority of the nitrosamine/nitramine-related carcinogenicity of the sample. A bulk nitrosamine analysis is being developed as part of this project. Although a bulk nitramine analysis is not being developed for this project, there are modifications to the method that could be pursued, if nitramines are determined to be important.

2.3. General nitrosamine/nitramine analyses: A general nitrosamine/nitramine analysis is defined as an analysis that identifies a specific compound as either a nitrosamine or nitramine, without further characterization of the specific compound. For example, such methods include those that separate compounds chromatographically and employ a nitrosamine or nitramine specific detector, but do not characterize the rest of the compound. An example is a thermal energy analyzer (TEA), devices that were widely available in the 1970s, but are currently difficult to find. These techniques could be employed when the combination of specific nitrosamine/nitramine and bulk nitrosamine/nitramine analyses indicate that the specific predicted nitrosamines/nitramines constitute only a minor fraction of the total nitrosamine/nitramine pool. The general nitrosamine/nitramine analyses would help in the identification of the missing nitrosamines/nitramines. Nitrosamine or nitramine-specific detectors are anticipated to provide signals proportional to the concentration of the nitrosamines or nitramines. Accordingly, the most important products (e.g., those with the largest peaks) could be further characterized by combining this analysis with mass spectral analysis.

3. Toxicological Analytical Approaches

3.1. Background: Regulatory action for nitrosamines/nitramines has focused on a human cancer endpoint, because much lower permissible concentrations are associated with this endpoint than with non-cancer endpoints for humans or the environment. Carcinogenic modes of action fall into two primary categories. The largest class of carcinogens consists of genotoxins that induce cancer through direct DNA damage or through altering the proper functioning of the genome. Modes of damage include genomic damage (genomic instability), gene mutation, chromosomal aberration and aneuploidy (an abnormal number of chromosomes). The other class of carcinogens acts indirectly. They are not directly genotoxic, but can induce cancer by aberrant epigenetic processes altering gene expression, without induced damage to the genomic DNA. Of particular importance in the second category are compounds that induce alterations in the expression of genes involved with controlling the cell cycle. Improperly-controlled cell cycle progression results in tumor formation. Such non-genotoxic modes of action include cytotoxicity and associated regenerative proliferation, and xenobiotics acting as receptor agonists to induce either undesirable hormonal responses, or to trigger peroxisome proliferator-activated receptors. Peroxisome induction triggers reactive oxygen species (ROS) that can then directly damage genomic DNA and act as genotoxins. During the past decade the number of non-genotoxic rodent carcinogens has increased; yet the fact remains that >90% of the known human carcinogens are detected in conventional short-term tests for genotoxicity. Accordingly, many of the biological assays described below focus on delineating this sort of damage to predict carcinogenic activity. No short-term biological assay can adequately predict human carcinogenic risk of a chemical or chemical mixture. However, using the weight of evidence from a battery of assays one can rank order agents as to their increasing potential for carcinogenicity. We selected biological assays to detect a broad range of genomic insults while conforming to the characteristics of amine-

based byproducts, sample size and the generation of complex mixtures. The battery of assays that we propose is listed below.

3.2. Mutagenicity in the bacterial point mutation assay, *Salmonella typhimurium*: This assay is being further developed and applied to carbon capture solvents and washwaters as part of the current project [1-3]. The assay, originally developed by Ames and Hartman, measures point mutation in strain YG7108 with and without metabolic activation was optimized for to evaluate the mutagenicity of nitrosamine/nitramines. The *S. typhimurium* mutation assay is a reliable predictor of carcinogenic potential of genotoxic carcinogens.

3.3. Mammalian cell chronic cytotoxicity: This assay is being further developed and applied to carbon capture solvents and washwaters as part of the current project [5]. The assay measures chronic cytotoxicity in Chinese hamster ovary cells. The use of mammalian cells is a major advantage. The assay provides a general measure of adverse biological impacts in cells after chemical exposure. This assay measures global toxic stress of individual chemicals and mixtures.

3.4. Mammalian cell acute genotoxicity: This assay (single cell gel electrophoresis) is being further developed and applied to carbon capture solvents and washwaters as part of the current project [4, 5]. The assay measures genomic DNA damage in Chinese hamster ovary cells induced by chemicals or complex mixtures with and without metabolic activation. The use of mammalian cells is a major advantage. Like mutagenicity, genomic DNA damage is associated with genotoxic carcinogens.

3.5. Quantitative high throughput screening: Assays for qHTS in human and mammalian cells are being further developed and applied to carbon capture solvents and washwaters as part of the current project [6]. The use of mammalian and human cells is a major advantage. The assays screen for specific cellular responses upon chemical exposure, and so help indicate mechanisms behind toxicity. Accordingly, these assays build upon the chronic cell cytotoxicity assay by determining if the solvent or washwater induces the cytotoxic response by poisoning general cellular systems or specifically acting as a mitochondrial poison. The assay addresses cellular responses associated with both genotoxic and non-genotoxic carcinogens. These qHTS assays identify pathways of toxicity including mitochondrial poisoning as well as employing reporter genes for biological pathways involved in the development of neoplasia.

All further assays described below are not being developed as part of the current project.

3.6. Quantitative flow cytometry to measure ROS activity: Samples inducing cytotoxicity or genomic DNA damage in CHO cells can be further evaluated to understand the mechanism underlying the genotoxicity. One potential mechanism includes the induction of oxidative stress toxicity by reactive oxygen species. ROS can be induced by non-genotoxic carcinogens. ROS can directly damage genomic DNA or oxidize other cellular constituents (i.e., induce a more general cytotoxic response). Quantitative flow cytometry employing either CHO or human FHs cells can be employed to analyze for ROS toxicity. Although there are several methods for measuring free radical production in cells, we propose to use conversion of 2′-7′-dichlorodihydrofluorescein diacetate to 2′,7′-dichlorofluorescein via ROS to directly measure the redox state of treated cells [7].

3.7. Quantitative Real Time PCR Arrays: Should we find a positive ROS response we can directly analyze the impact of the samples on human ROS toxic response genes by toxicogenomic techniques using human cell qRT-PCR gene arrays (Table 1). Data indicating whether the ROS are damaging genomic DNA can be obtained in nontransformed human cells (FHs cells) using a DNA damage signaling pathway array (Table 2). Dr. Plewa's laboratory has published research on both DNA damage and ROS damage using qRT-PCR toxicogenomic analyses [8-10].

Table 1. List of Genes for Human Toxic Response to ROS (Array PAHS-065A, SABiosciences).

Antioxidants:
Glutathione Peroxidases (GPx): GPX1, GPX2, GPX3, GPX4, GPX5, GPX6, GPX7, GSTZ1.
Peroxiredoxins (TPx)**:** PRDX1, PRDX2, PRDX3, PRDX4, PRDX5, PRDX6.
Other Peroxidases: CAT, CSDE1, CYGB, DUOX1, DUOX2, EPX, GPR156, IPCEF1, LPO, MGST3, MPO, PTGS1, PTGS2, PXDN, PXDNL, TPO, TTN.
Other Antioxidants: ALB, APOE, GSR, MT3, SELS, SOD1, SOD3, SRXN1, TXNDC2, TXNRD1, TXNRD2.

Genes Involved in Reactive Oxygen Species (ROS) Metabolism:
Superoxide Dismutases (SOD): SOD1, SOD2, SOD3.
Other Genes Involved in Superoxide Metabolism: ALOX12, CCS, CYBA, DUOX1, DUOX2, GTF2I, MT3, NCF1, NCF2, NOS2, NOX5, PREX1, PRG3.
Other Genes Involved in ROS Metabolism: AOX1, BNIP3, EPHX2, MPV17, SFTPD.
Oxidative Stress Responsive Genes: ANGPTL7, APOE, ATOX1, CAT, CCL5, CSDE1, CYGB, DGKK, DHCR24, DUOX1, DUOX2, DUSP1, EPX, FOXM1, GLRX2, GPR156, GPX1, GPX2, GPX3, GPX4, GPX5, GPX6, GPX7, GSS, IPCEF1, KRT1, LPO, MBL2, MPO, MSRA, MTL5, NME5, NUDT1, OXR1, OXSR1, PDLIM1, PNKP, PRDX2, PRDX5, PRDX6, PRNP, RNF7, SCARA3, SELS, SEPP1, SGK2, SIRT2, SOD1, SOD2, SRXN1, STK25, TPO, TTN, TXNRD2.

Table 2. List of Genes for Human DNA Damage Signaling Pathway (Array PAHS-029, SABiosciences).

Apoptosis: ABL1, BRCA1, CIDEA, GADD45A, GADD45G, GML, IHPK3, PCBP4, AIFM1 (PDCD8), PPP1R15A, RAD21, TP53, TP73.

Cell Cycle:

Cell Cycle Arrest: CHEK1, CHEK2, DDIT3 (CHOP), GADD45A, GML, GTSE1, HUS1, MAP2K6, MAPK12, PCBP4, PPP1R15A, RAD17, RAD9A, SESN1, ZAK.

Cell Cycle Checkpoint: ATR, BRCA1, FANCG, NBN (NBS1), RAD1, RBBP8, SMC1A (SMC1L1), TP53.

DNA Repair:

Damaged DNA Binding: ANKRD17, BRCA1, DDB1, DMC1, ERCC1, FANCG, FEN1, MPG, MSH2, MSH3, N4BP2, NBN (NBS1), OGG1, PMS2L3 (PMS2L9), PNKP, RAD1, RAD18, RAD51, RAD51L1, REV1 (REV1L), SEMA4A, XPA, XPC, XRCC1, XRCC2, XRCC3.

Base-excision Repair: APEX1, MBD4, MPG, MUTYH, NTHL1, OGG1, UNG.

Double-strand Break Repair: CIB1, FEN1, XRCC6 (G22P1), XRCC6BP1 (KUB3), MRE11A, NBN (NBS1), PRKDC, RAD21, RAD50.

Mismatch Repair: ABL1, ANKRD17, EXO1, MLH1, MLH3, MSH2, MSH3, MUTYH, N4BP2, PMS1, PMS2, PMS2L3 (PMS2L9), TP73, TREX1.

3.8. Quantitative flow cytometry for induction of micronuclei: This assay measures the induction of chromosomal aberrations, chromosomal nondisjunction and aberrant cell divi-

sion by the induction of micronuclei and can be applied to mammalian or human cells [11]. Micronuclei are abnormal nuclei that are resolved during cell division. Accordingly, their formation can indicate genetic alterations in the daughter cells, as well as chromosome fragments that are associated with cancer. The micronucleus assay is predictive of cancer potency of physical and chemical agents as well as complex mixtures.

3.9. qRT-PCR arrays of transcriptome biomarker genes to distinguish modes of carcinogenicity: This assay represents the most detailed characterization of the mechanism of carcinogenicity for the samples. As summarized by Waters et al., [12], predictive toxicogenomics have shed insight on the potential of transcriptome profiles to classify both genotoxic and nongenotoxic carcinogens and to predict the carcinogenicity of chemicals. Transcriptome profiles indicate activation of genes by measuring the level of transcribed messenger RNA. Toxicogenomics has demonstrated that altered expression of genes involved in DNA damage/response indicates direct DNA damage whereas increased expression of genes involved in cell cycle progression is more characteristic of the indirect-acting agents (e.g., oxidative stress). Metabolism genes are prominently represented among gene expression profiles associated with nongenotoxic modes of action (e.g., cytotoxicity and regenerative proliferation, xenobiotic receptor agonists, peroxisome proliferator-activated receptors, or hormonal-mediated processes). The evidence suggests that gene expression profiles reflect underlying modes or mechanisms of action, which may be exploited in the prediction of chemical carcinogenicity, especially in conjunction with conventional short-term tests for gene mutation, chromosomal aberration and aneuploidy. In this proposed assay, qRT-PCR arrays of 15-30 transcriptome biomarker genes [13, 14] would be fabricated that can be used to rank the carcinogenic potency of samples, and to distinguish their modes of action. This assay would have to be fabricated within our laboratory or outsourced. The cancer biomarker gene array provides a more focused use of specific alteration in gene expression that is statistically associated with the induction of carcinogenicity. However, it would be limited and other types of toxic response may not be resolved. The previous qRT-PCR arrays give broader molecular bases for toxicity, mutagenicity, altered DNA repair, and toxic responses to oxidative stress and neoplasia. This highly focused gene array would focus on a set of genes derived from the literature that have a higher association with cancer induction or cancer progression.

4. Application of Analytical Approaches to Novel Solvent Evaluation

4.1. Strategy: The strategy described below will compare novel solvents to 30% monoethanolamine, one of the most common solvents for amine-based carbon sequestration. This solvent can be used as a benchmark against which to compare other solvents for both chemical and toxicological assays. Such a benchmark is needed because the absolute numbers obtained from chemical and toxicological assays are not directly understandable in terms of risk to the public. For example, how does a concentration of nitrosamines in a solvent translate into an actual exposure to a receptor population? How should an absolute measure of genomic DNA damage in a genotoxicity assay be interpreted in terms of risk to the public? Comparison of responses measured for a novel solvent against that measured for monoethanolamine under comparable conditions would indicate whether the novel solvent enhances or reduces the risks associated with using the novel solvent for carbon capture. Comparing the battery of toxic responses of a novel solvent to monoethanolamine will generate one metric of relative toxicity. The benefit to the company would be to evaluate all novel solvents and rank order each solvent as to its toxicity.

We propose a hierarchical approach in three phases in which solvents that are acceptable at one level proceed for more detailed analyses to the next level. The company could choose to employ all three phases for all solvents or a strategy could be to choose only the least toxic (but effective in CO_2 capture) solvents to evaluate at the next phase in the battery. Figures 2 and 3 present flowcharts summarizing the toxicological and chemical assays to be applied for each phase.

4.1. Phase I: Phase I assays represent a battery of relatively rapid screening assays to rule out solvents that do not significantly reduce nitrosamine/nitramine risk compared to the 30% monoethanoamine standard.

4.1.1. ***Initial Solvent Screening:*** Before expending significant effort on pilot-testing a novel solvent, an initial screening of the raw solvent should be conducted. The raw solvent should be analyzed for total nitrosamines/nitramines. Even if the constitution of the solvent is not disclosed by the vendor due to proprietary reasons, this type of assay would indicate whether there is gross nitrosamine/nitramine contamination of the solvent, and does not require the synthesis of specific nitrosamines/nitramines relevant to specific solvent constituents. Additionally, the raw solvent should be subjected to the following toxicological assays.

1. Mutagenicity in the bacterial point mutation assay, *Salmonella typhimurium*
2. Mammalian cell chronic cytotoxicity
3. Mammalian cell acute genotoxicity

For each novel solvent, the bulk nitrosamine/nitramine and toxicological profiles should be compared to that of 30% monoethanolamine. For these assays or the raw solvent, sample volume should not be a limiting factor.

4.1.2. ***Pilot-testing:*** After the initial solvent screening, novel solvents would be compared against 30% monoethanolamine within a pilot test unit. The pilot test unit should contain the complete array of relevant process units (e.g., adsorber and desorber units) to ensure the results are relevant. After exposure to a representative flue gas exhaust, samples of solvent, washwater and exhaust gas would be collected. To ensure that comparisons of solvents are valid, operational conditions must be kept constant. In practice, because the conditions responsible for nitrosamine/nitramine formation are not clearly delineated, this likely requires that all novel solvents be evaluated within the same pilot plant unit. This requirement ensures that differences in seemingly minor details do not skew the results. Such details include the gas and solvent loading rates, average residence time of washwater, nature of packing materials, amongst others. Although exhaust gas samples are not being subjected to the analyses being developed as part of the current project, we anticipate that the analytical methods could be modified to accommodate such samples. For example, solid phase resins used to collect exhaust gases could be eluted into solvents relevant to each analytical method.

For Phase I testing, we recommend that samples of solvent, washwater and exhaust gas collected from the pilot plant be analyzed for bulk nitrosamines/nitramines. Methods for specific nitrosamines/nitramines are likely to reach method detection limits on the order of 1-10 ng/L for washwaters, while those for bulk nitrosamines/nitramines should reach 100 pM (comparable to 10-100 ng/L for most nitrosamines/nitramines). For solvents, method detection limits are likely to be an order of magnitude higher, due to the need to dilute the samples to reduce the viscosity in order to conduct the extractions; however, solvents are anticipated to have

higher concentrations of target contaminants. The lower method detection limits for the specific nitrosamine/nitramine methods represents an advantage in terms of verifying that solvents exhibit low concentrations of target contaminants. However, particularly where vendors do not disclose the specific amine constituents of solvents, the bulk nitrosamine/nitramine analyses would suffice for Phase I testing, and do not require synthesis of specific nitrosamine/nitramine standards. For toxicological assays, we recommend the following:

1. Mutagenicity in the bacterial point mutation assay, *Salmonella typhimurium*
2. Mammalian cell chronic cytotoxicity
3. Mammalian cell acute genotoxicity
4. Quantitative high throughput screening.

Within Phase I, we estimate that this battery of assays would require minimum sample volumes of 4 L of solvent and 10 L of washwater. We cannot estimate volumes of gas that would be required for exhaust gas analyses as we have not conducted gas phase analyses as part of this project. Solvents would be rank-ordered based on their total nitrosamines/nitramines and toxicity measurements.

4.2. Phase II: Depending upon the requirements of the company all solvents or selected solvents would pass to Phase II evaluation. The battery of Phase II assays would further evaluate the toxic risk of novel solvents. Phase II assays would include the evaluation of specific nitrosamines/nitramines anticipated based upon the amine constituents in the solvent. These assays would feature lower detection limits than the total nitrosamine/nitramine assays, but would require synthesis of chemical standards, a process that can be time-consuming and costly. When combined with the total nitrosamine/nitramine results, these assays would enable an understanding of to what extent the hypothesized specific nitrosamines/nitramines constitute the entire nitrosamine/nitramine pool. Alternatively, how prone are the amine constituents to fragment into unanticipated amine products? Solvents for which only a low percentage of nitrosamines/nitramines are accounted for by those hypothesized from known reaction pathways should be avoided. Their tendency to form a wider array of products would hinder exposure characterization.

Solvent vendors may seek to avoid disclosing the chemical constitution of the amine solvents. Our opinion is that the ability of solvent vendors to avoid disclosure of amine constituents should be curtailed. Knowing the types of nitrosamines/nitramines that form is important because it would aid in determining the fate and transport of nitrosamines/nitramines that may be emitted from the exhaust stack. However, if disclosure is not possible, larger volumes of samples could be extracted and concentrated to enable lower detection limits for the total nitrosamine method. Additionally, the relative concentrations of total nitrosamines/nitramines in washwater and exhaust gas compared to the 30% monoethanolamine base case could be employed to estimate the physical properties of the uncharacterized nitrosamines/nitramines. For example, if the total nitrosamine concentration in washwater is higher for the novel solvent than for 30% monoethanolamine, but the reverse is true in exhaust gas, the novel solvent is likely to generate more polar nitrosamine products.

We recommend the following battery of toxicological assays for Phase II to initiate an understanding of the modes of action of the potential carcinogens:

1. Quantitative flow cytometry to understand ROS genotoxicity

2. Quantitative PCR Arrays
3. Quantitative flow cytometry for induction of micronuclei

We estimate that to adequately complete Phase II assays to evaluate CSS washwaters we would require minimum sample volumes of 8 L of solvent and 30 L of washwater.

4.3. Phase III: For solvents demonstrating promise as a result of Phase II assays, Phase III would further characterize their tendency to form nitrosamines/nitramines. For solvents for which >50% of the total nitrosamine/nitramine pool is uncharacterized, we recommend that further evaluation of the solvent be avoided, as the inability to characterize products would hinder fate and transport studies. If >95% of the total nitrosamine pool is accounted for by specific hypothesized nitrosamines/nitramines, then further chemical characterization to identify the remaining 5% is likely to be unimportant. However, if only 50-95% of the total nitrosamine/nitramine pool is characterized, the general nitrosamine/nitramine analyses could be applied to identify a significant fraction of the uncharacterized compounds.

We recommend that the most promising novel solvents be subjected to qRT-PCR arrays of transcriptome biomarker genes to distinguish modes of carcinogenicity. We estimate that to complete the Phase III assays would require 8 L of solvent and 30 L of washwater.

5. Task 2 Personnel

5.1. Yale University

Dr. William A. Mitch
Dr. Amisha D. Shah
Ms. Ning Dai

5.2. University of Illinois at Urbana-Champaign

Dr. Michael J. Plewa
Dr. Elizabeth D. Wagner
Ms. Jennifer Osiol
Ms. Azra Dad

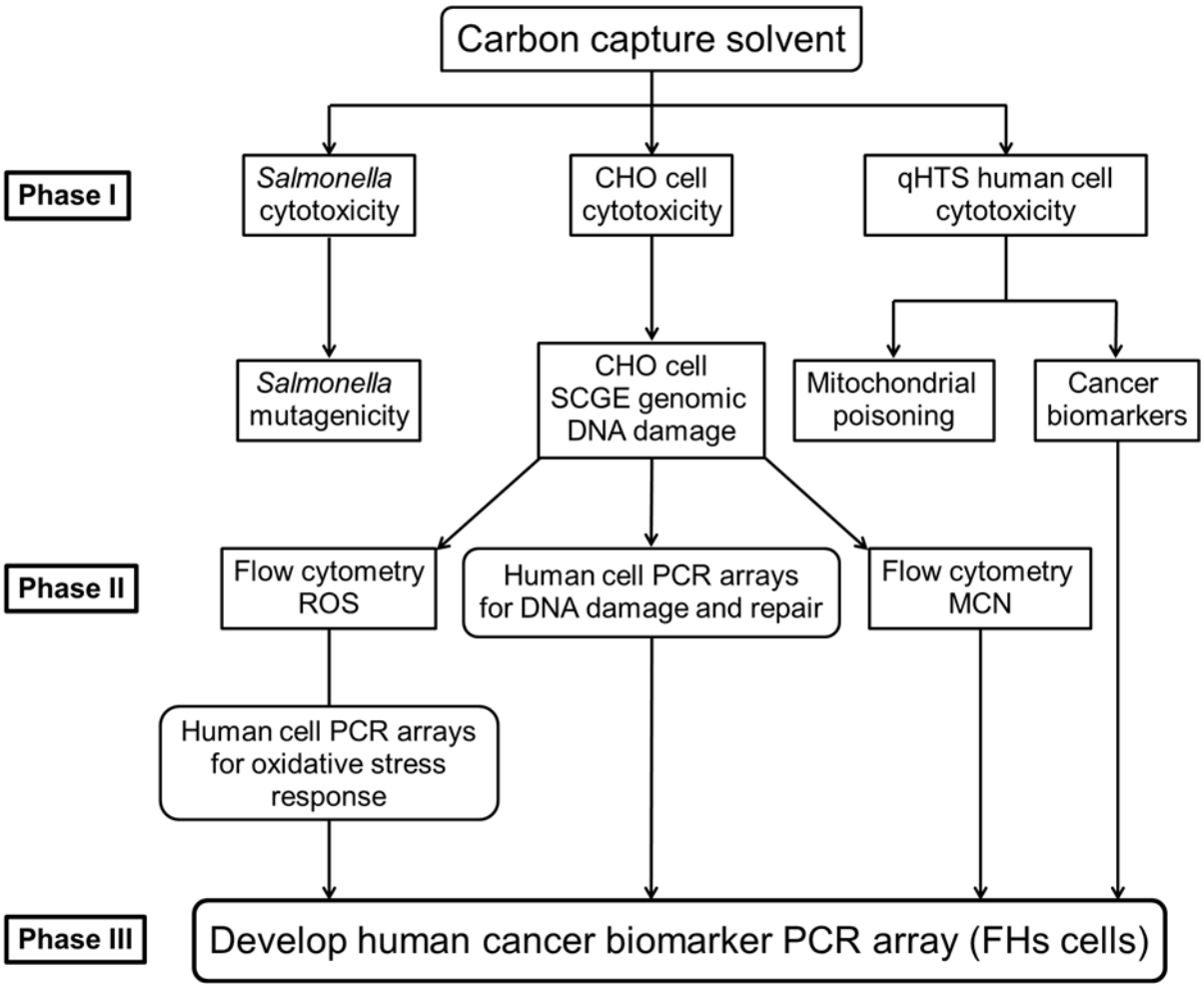

Figure 2 A three-phase hierarchical battery of *in vitro* biological and molecular assays to evaluate carbon sequestration solvents and their resulting CSS emissions for a comparison of their relative toxicological characteristics.

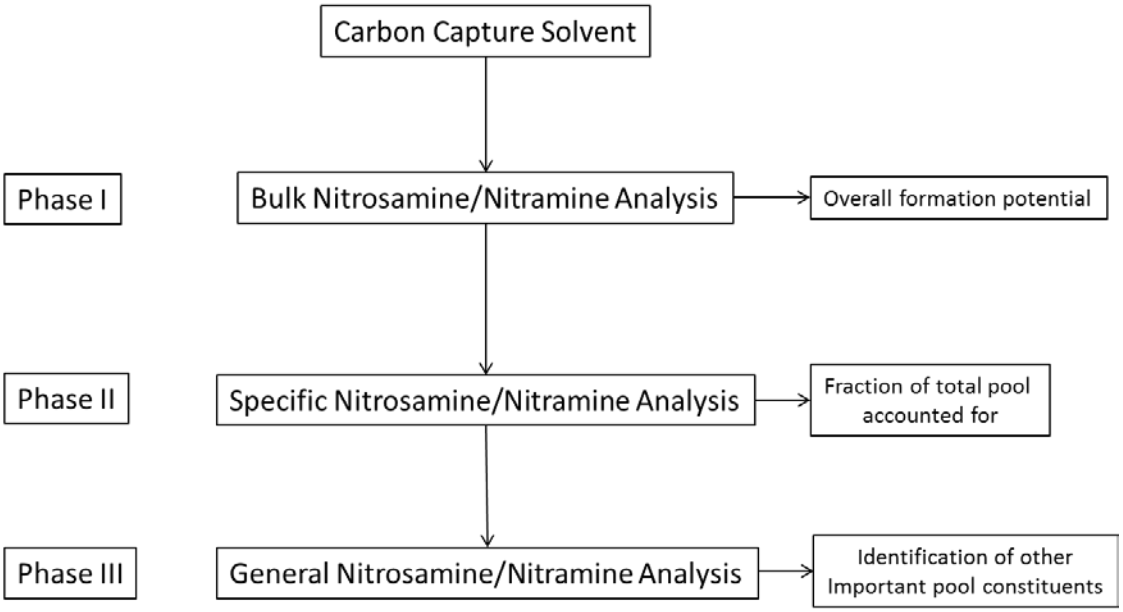

Figure 3 A three-phase hierarchical battery of chemical assays to evaluate carbon sequestration solvents and their resulting CSS emissions for nitrosamine and nitramine emissions.

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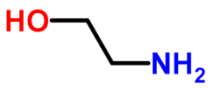
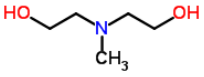
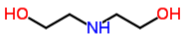
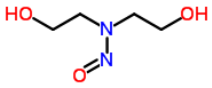
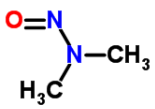
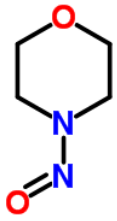
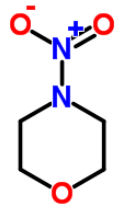
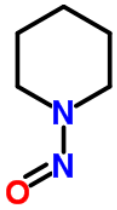
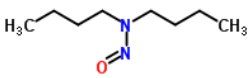
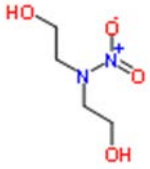
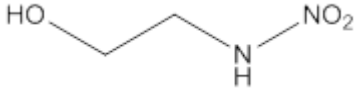
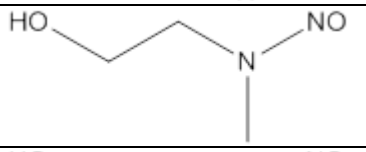
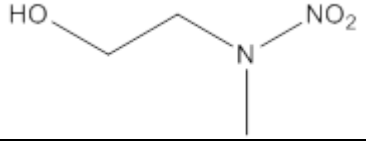
Appendix 1: List of chemicals, abbreviations, formulas, CAS Numbers and structures of agents discussed in this report.

Name	Abbreviation	Formula	CAS	Structure
Monoethanolamine	MEA	C_2H_7NO	141-43-5	
Methyldiethanolamine	MDEA	$C_5H_{13}NO_2$	105-59-9	
Diethanolamine	DEA	$C_4H_{11}NO_2$	11-42-2	
N-nitrosodiethanolamine	NDELA	$C_4H_{10}N_2O_3$	1116-54-7	
N-nitrodimethylamine	NO_2-DMA	$C_2H_6N_2O_2$	4164-28-7	
N-nitrosodimethylamine	NDMA	$C_2H_6N_2O$	571-61-9	

N-nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	59-89-2	
N-nitromorpholine	NO_2-MOR	$C_4H_8N_2O_3$	4164-32-3	
N-nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	100-75-4	
N-nitrosodibutylamine	NDBA	$C_8H_{18}N_2O$	924-16-3	
N-nitrodiethanolamine	NO_2-DELA	$C_4H_{10}N_2O_4$	Not available	
N-nitromono-ethanolamine	NO_2-MEA	$C_2H_6N_2O_3$	Not available	
N-nitrosomethyl-ethanolamine	NO-MELA	$C_3H_8N_2O_2$	Not available	
N-nitromethyl-ethanolamine	NO_2-MELA	$C_3H_8N_2O_3$	Not available	

Appendix 2: List of Terms	
carcinogen	A carcinogen is a substance, radionuclide, or radiation that is directly involved in inducing neoplasia resulting in cancer
CHO	Chinese hamster ovary cells
chromosomal aberrations	A structural abnormality in one or more chromosomes of a cell that may include, a deletion, insertion, inversion, break, translocation or ring formation of chromatin. Chromosome aberrations can lead to birth defects or to cancer.
chromosomal nondisjunction	A failure of chromosome pairs to separate properly during cell division which can lead to fetal death, birth defects or to cancer.
FHs cells	
genotoxin	A genotoxin is a substance, radionuclide, or radiation that induces damage to the genome or alters the proper functioning of the genome.
IRIS	Integrated Risk Information System, a U.S. Environmental Protection Agency database of human health effects from exposure to environmental substances.
µM	micromolar, Molar $\times 10^{-6}$.
micronucleus	A small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division.
nM	nanomolar, Molar $\times 10^{-9}$
neoplasia	An abnormal mass of tissue as a result of the abnormal proliferation of cells. The growth of neoplastic cells causes tumor that may be benign, premalignant or malignant.
nitroamine	Nitroamines are organic compounds that contain the nitroamino functional group, $R_2N\text{-}NO_2$
nitrosamine	Nitrosamines are compounds of the chemical structure $R_1N(\text{-}R_2)\text{-}N{=}O$
pM	picomolar, Molar $\times 10^{-12}$
qHTS	Quantitative high throughput screening.
qRT-PCR	Quantitative real time polymerase chain reaction which is used to amplify and simultaneously quantify a targeted DNA molecule.
ROS	Reactive oxygen species that is chemically-reactive molecules containing oxygen that include oxygen ions and peroxides. Reactive oxygen species are highly reactive due to the presence of unpaired valence shell electrons. ROS can lead to oxidative stress. ROS are also generated by exogenous sources such chemical toxins or as ionizing radiation.
TEA	Thermal energy analyzer
toxicogenomics	The science that deals with the collection, interpretation, and storage of information about gene and protein activity within cells or tissues in response to toxic substances. Toxicogenomics combines toxicology with genomics or other high throughput molecular profiling technologies.
transcriptome profiles	The result of high-throughput sequencing technologies to sequence cDNA in order to get information about a cell's RNA content in which to determine the level of gene expression.
xenobiotic	A chemical which is found in an organism but which is not normally produced or expected to be present in it; a toxic substance or poison.
YG7108	A strain of *S. typhimurium* with enhanced sensitivity for nitrosamines.

Appendix 3. Published Papers on the Bioassays Presented in this Report	
Bioassays	Methology and or Review Papers
S. typhimurium mutation assay	Maron, D. M.; Ames, B. N., Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* **1983,** *113*, (3-4), 173-215.
CHO cell chronic cytotoxicity assay	Plewa, M. J.; Wagner, E. D., *Mammalian cell cytotoxicity and genotoxicity of disinfection by-products*. Water Research Foundation: Denver, CO, 2009; p 134.
CHO SCGE genotoxicity assay	Wagner, E. D.; Plewa, M. J., Microplate-based comet assay. In *The Comet Assay in Toxicology*, Dhawan, A.; Anderson, D., Eds. Royal Society of Chemistry: London, 2009; pp 79-97.
qHTS	Inglese, J.; Auld, D. S.; Jadhav, A.; Johnson, R. L.; Simeonov, A.; Yasgar, A.; Zheng, W.; Austin, C. P., Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc. Natl. Acad. Sci. U. S. A.* **2006,** *103*, (31), 11473-11478.
ROS flow cytometry	Eruslanov, E.; Kusmartsev, S., Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods Mol. Biol.* **2010,** *594*, 57-72.
qRT-PCR Toxicogenomics	Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J., Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts. *Envion. Sci. Technol.* **2010,** *44*, (19), 7206-7212.
Micronucleus flow cytometry	Bryce, S. M.; Shi, J.; Nicolette, J.; Diehl, M.; Sonders, P.; Avlasevich, S.; Raja, S.; Bemis, J. C.; Dertinger, S. D., High content flow cytometric micronucleus scoring method is applicable to attachment cell lines. *Environ. Mol. Mutagen.* **2010,** *51*, (3), 260-266.
Human Cancer Biomarker Analyses	Waters, M. D.; Jackson, M.; Lea, I., Characterizing and predicting carcinogenicity and mode of action using conventional and toxicogenomics methods. *Mutat. Res.* **2010,** *in press*.