Step 1 of 9: Background/Introduction

The HHEAR Application is for investigators who wish to apply for HHEAR laboratory and data analysis services to add or broaden analyses of environmental exposures in their studies of human health.

Before submitting an Initial Application, please review the policies and procedures for accessing HHEAR services.

Contact HHEARHelp@westat.com with any questions about this Application.

Instructions

• Step 1: Review background/introduction.
• Step 2: Provide information about yourself, a project contact person, and any co-investigators.
  o Indicate your agreement to the Privacy Acknowledgement
  o If you do not have the authority to commit to the transfer of biological and/or environmental specimens and data, list contact information for those who do.
  o Upload biographical sketches for yourself and co-investigators. Use the standard NIH biographical sketch format PHS 398.
• Step 3: Complete the section on investigator eligibility. If you have questions about your eligibility, contact HHEARHelp@westat.com.
• Step 4: Review HHEAR policies and indicate your agreement.
• Step 5: Specify which HHEAR services you are requesting.
• Step 6: Provide information on your parent study.
  o Provide succinct responses to each item. Note word/character limits.
• Step 7: Provide information about your proposed HHEAR project.
  o Provide succinct responses to each item. Note word/character limits.
  o Upload a list of citations of key references that provide scientific premise for the proposed project including the rationale for each requested service.
• Step 8: Preview application.
• Step 9: Complete application.
Step 2 of 9: Applicant/Investigator Information

Project number (will be filled in automatically upon submission of this form): 2020-00500

Principal Investigator: Please provide contact information and biosketch for the Principal Investigator (PI). Indicate whether the PI has the authority to commit for the transfer of samples and/or data.

Principal Investigator:
- Name: xxxxxxxxxx xxxxxx xxxxxxxx
- Institution: xxxxxxxxxxx xx xxxxxxxxx xxxxxxxxxx
- Phone: xxx-xxx-xxxx
- Email: xxxxxxxx@xxx.edu
- Biosketch: [UPLOAD HERE]

Privacy acknowledgement. Please check the box below to indicate that you understand and agree with the following privacy acknowledgment.

I acknowledge that all information in the application and documents submitted with the application will be shared with members of the HHEAR consortium and the HHEAR Scientific Expert Panel for the purpose of reviewing the application.

If you do not agree to the privacy acknowledgement, your application will not be reviewed, and the application and all documents submitted with the application will be deleted from the myHHEAR system.

[X] Yes, I understand and agree with the privacy acknowledgement.

Authority to commit to transfer

- Biological Samples: [X] Yes ☐ No ☐ N/A
- Environmental Samples: ☐ Yes ☐ No [X] N/A
- Data: [X] Yes ☐ No
Other Investigator: Complete this item for the investigator with the authority to commit to transfer of biological/environmental samples and/or data, if it is not the PI. If you propose to use samples and/or data from more than one cohort, click the “Add Other Investigator(s)” button below to provide this information for all biological/environmental samples and data sources.

Other Investigator:

Name: __________
Institution: __________
Phone: __________
Email: __________
Role: ☐ Co-Investigator
☐ Other (specify): __________

Biosketch: [UPLOAD HERE]

Authority to commit to transfer

Biological Samples: ☐ Yes ☐ No ☐ N/A
Environmental Samples: ☐ Yes ☐ No ☐ N/A
Data: ☐ Yes ☐ No

[ADD OTHER INVESTIGATOR(S)]

Project Contact Person (if different from Principal Investigator):

Name: __________
Institution: __________
Phone: __________
Email: __________
Step 3 of 9: Investigator Eligibility

Please provide responses to each item below to indicate your eligibility for HHEAR services.

You have an ongoing or completed epidemiological or clinical study (parent study) with human biological and/or environmental samples linked to health outcome data.

[X] Yes  ☐ No

You want to add environmental exposure data to your parent study or need more extensive analysis of exposures to support your scientific hypothesis related to health outcomes.

[X] Yes  ☐ No

Although your parent study may be ongoing, you have collected all the data and biological and environmental samples that you will provide to HHEAR for the proposed project prior to submitting your final application.

[X] Yes  ☐ No

You meet at least one of the following funding criteria (mark all that apply).

- Your ongoing or completed parent study is/was funded at least in part by NIEHS extramural funds. In addition, NIEHS will consider support for studies with significant NIEHS engagement that are administered by other NIH Institutes such as the Environmental Health Disparities Centers (administered by NIMHD), the GEOHealth Centers (administered by FIC), and the ABCD study (administered by NIDA) as well as others. Applicants are encouraged to inquire about potential eligibility before submitting an application. Eligible studies supported by NIEHS may request all HHEAR services including targeted and untargeted analysis of biological and environmental samples.

  ☐ Yes  [X] No

- Your parent study is/was funded by the NIEHS Superfund Research Program. Studies funded by the NIEHS Superfund Research Program are eligible for targeted and untargeted analysis of biological and environmental samples.

  ☐ Yes  [X] No

- Your parent study is currently funded at least in part by NHLBI extramural funds. Studies funded by NHLBI extramural funds are eligible for targeted and untargeted analysis of only biological samples.

  ☐ Yes  [X] No
Human Health Exposure Analysis Resource (HHEAR)
Initial Application for HHEAR Services

Step 3 of 9: Investigator Eligibility

- Your parent study is currently funded by NCI extramural funds and has more than one year of funding remaining at the time you submit the HHEAR Initial Application. Studies funded by NCI extramural funds are eligible for only targeted analysis of biological samples.
  [X] Yes  [ ] No

- Your study is an ECHO-wide cohort analysis proposal that has been approved through the ECHO Publications Program, or an ECHO Opportunities and Infrastructure Fund (OIF) proposal approved through the ECHO OIF Program. Studies funded by ECHO are eligible for targeted and untargeted analysis of only biological samples.
  [ ] Yes  [X] No
  - ECHO-wide and OIF supported projects are managed through the ECHO program and don’t require an additional application for HHEAR services. ECHO cohorts may be eligible for cohort specific analyses through NIEHS, NHLBI, or NCI support through a HHEAR application.
  - Email echocc-publications@dm.duke.edu for more information on the ECHO Publications Program. Email echocc-oif@duke.edu for more information on the ECHO OIF Program.

You are eligible to apply for an NIH grant at your home institution, and you have the authority to commit to documentation such as the Material Transfer Agreement, Data Submission Agreement, and Data Sharing Plan.

[ ] Yes  [X] No

You agree to share your experimental design details and supporting data, including phenotypic data at the individual level, needed to achieve the aim(s) of your proposal.

[ ] Yes  [X] No

If your study will use data or samples collected or owned by one or more institutions other than your own, obtain the informed consent form used by each institution. Confirm that the consent language is consistent with the use of the data, biological and environmental (if applicable) samples for future unspecified research; this includes the public sharing of de-identified data.
Step 4 of 9: Agreement to HHEAR Policies

Please indicate that you have read and will comply with the Policies for Access to HHEAR Services by adding your eSignature in the space provided.

I have read and will comply with the HHEAR policies for accessing services.

[X] Yes   [ ] No

If no, please provide an explanation:

(100 words remaining)

Are you subject to any other data sharing policies (e.g., a consortium agreement that your data must adhere to)?

[ ] Yes  [X] No

If yes, please provide an explanation:

(100 word limit)
Step 5 of 9: Request HHEAR Services

Please indicate the HHEAR services you are requesting (select all that apply):

- [X] Laboratory analysis of biological samples
- [ ] Laboratory analysis of environmental samples
- [X] Statistical analysis
Step 6 of 9: Parent Study Information

Please complete each item below to provide the key information that can be used by reviewers to understand the parent study for the proposed project. If there is more than one parent study providing data and biological and/or environmental samples, provide the information for each parent study. Do not leave any items blank.

1. **Parent study project title**: Understanding the Determinants of Racial/Ethnic Disparities in Liver Cancer and Chronic Liver Disease in Understudied and High-Risk Populations

2. **Parent study cohort name and website link (if available)**: Multiethnic Cohort Study (MEC)
   
   [https://www.uhcancercenter.org/mec](https://www.uhcancercenter.org/mec)

3. **Parent study funding source(s), including grant number(s)**: NCI, R01CA228589

4. **Parent study Principal Investigator and institution**:

5. **Parent study key publications (limit to 3; provide as PMIDs)**:
   
   PMID: 31553803
   PMID: 27301913
   PMID: 30859154

6. **Primary hypothesis of the parent study**:

   We hypothesize that lifestyle, genetic, social, and contextual factors explain racial/ethnic differences in liver cancer and NAFLD risks.

   *(18 words/20 word limit)*

7. **Summary of main published findings for parent study**:

   We revealed ethnic differences in HCC incidence and NAFLD being the most common cause of liver disease and cirrhosis. We showed that diabetes is a risk factor for HCC that eliminating diabetes could potentially reduce HCC incidence in all ethnic groups with the largest potential benefit among Latinos. We found coffee consumption and better diet quality may reduce HCC incidence and that diets low in meat and cholesterol and high in fiber may reduce the risk for NAFLD and cirrhosis.

   *(80 words/100 word limit)*
8. Parent study design: (Check all that apply)

- [ ] Cross-sectional
- [ ] Hospital-based
- [ ] Ambispective cohort
- [ ] Case-control
- [X] Prospective cohort
- [ ] Intervention study
- [ ] Population-based
- [ ] Retrospective cohort
- [ ] Clinical trial
- [ ] Other: ____________________________

9. Parent study population description:

MEC is a large prospective cohort study designed to investigate dietary, lifestyle and genetic factors in relation to cancer and other chronic conditions. The cohort includes over 215,000 men and women, aged 45 - 75 years at cohort enrollment during 1993 - 1996. It consists of participants primarily from five different racial and ethnic groups (African Americans, Japanese Americans, Latinos, Native Hawaiians and Whites) living in Hawaii and California. Blood samples were collected from about 70K participants.

Sample size for parent study*: (e.g., # cases and controls if case-control study, number of cohort members if cohort study): 215,000 at baseline; 70,000 with blood samples

*If study is longitudinal, please indicate the sample size at the first time point as well as the last time point.

HHEAR encourages proposals from parent studies of a wide range of sample sizes.

Age range(s) of parent study population (at study entry): 45-75 at cohort entry

Has the parent study population been included in a previous CHEAR/HHEAR project or in a previous ECHO project?

- [ ] Yes, specify
- [X] No

Specify: _____________________________________________________________________________________________

Geographic location(s) of the parent study population: Los Angeles County and Hawaii

If the parent study was conducted outside of the United States, please provide the geographic location of the foreign study participants, if all participants were located in the United States, please write N/A. For each location outside of the United States, please provide the City (specific location or, if it is a regional location, list the capital city), Region (if applicable), and Country: N/A

N/A
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Step 6 of 9: Parent Study Information

f. Years in which the parent study was conducted: Baseline 1993-1996 and follow up until present

g. Method of data collection (e.g., survey, in-person visits, medical records) for the parent study: Baseline and follow up questionnaires; SEER linkage, Medicare linkage, state death files, National Death Index

h. Number of data collection time points and interval between data collection time points for the parent study: Baseline between 1993 and 1996; follow up every 5 years until present. Blood collection in early 2000.

10. Main exposures (environmental and/or non-environmental) investigated for the parent study: Demographic, lifestyle (smoking, alcohol drinking, physical activity, etc.), diet, comorbid conditions, medication use, family history of cancer, germline genetic variations.

11. Type of biological and/or environmental samples collected (i.e., whole blood, plasma, urine) for the parent study: blood; plasma

a. Years in which biological samples were collected for the parent study: Early 2000

b. Number of samples collected per study participant/interval between sample collection for the parent study: One time collection

c. Years in which environmental samples were collected for the parent study: N/A
Step 7 of 9: Proposed HHEAR Project

Please complete each item below to provide the key information that can be used by reviewers to evaluate your proposed project.

Proposed Project Title: The role of PFAS exposures in nonalcoholic fatty liver disease and hepatocellular carcinoma in the Multiethnic Cohort

1. **Abstract:** Please provide a summary (hypotheses, study design, methods and statistical analysis) of your proposed HHEAR project in the context of the parent study.

   HCC and NAFLD rates have continued to increase over the past three decades. The health impact of the increasing incidence of HCC is compounded by its dismal survival rate. NAFLD is now recognized as a major contributor to cirrhosis and HCC development. Emerging evidence indicates that PFAS exposure disrupts lipid homeostasis in the liver and has an influence on the initiation and progression of a cascade of pathological conditions associated with NAFLD. Epidemiological evidence is scarce and there are no studies on the impact of PFAS exposure on NAFLD, cirrhosis and HCC. Our objective is to examine the associations between pre-diagnostic plasma PFAS concentrations and NAFLD, cirrhosis and HCC (n~1450) and matched controls (n~1450) in the MEC. This study is novel and cost efficient (leveraging existing data and samples from MEC), has the potential to advance our understanding of hepatotoxic effects of environmental pollutants.

   (144 words/150 word limit)

2. **Specific aim(s) for proposed HHEAR project:**
   
a. **Specific aim 1:** To examine the association between plasma PFAS concentrations and risk of NAFLD, NAFLD-related cirrhosis and HCC in the MEC.

b. **Specific aim 2 (if applicable):** To evaluate whether NAFLD, cirrhosis and HCC risk associated with PFAS exposures differs by genetic risk score (GRS) and diet profile

c. **Specific aim 3 (if applicable):**

3. **Exposures to be investigated for proposed project:** PFAS, genetic risk score, diet
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Step 7 of 9: Proposed HHEAR Project

4. Significance:

a. Describe the scientific premise for the proposed HHEAR project, including the rationale for each requested laboratory analysis (targeted and untargeted for biological and/or environmental samples). Please provide citations when applicable and indicate which are “key” references for the rationale:

While incidence and mortality rates have declined for most cancers, HCC rates have continued to increase over the past 35 years (1). NAFLD can progress to nonalcoholic steatohepatitis, fibrosis, cirrhosis and HCC (2). NAFLD is now a major contributor to HCC development (3). Because of the increasing incidence of NAFLD-related HCC, there is a pressing need to identify the factors responsible for HCC development in the setting of NAFLD. Emerging evidence indicates that exposure to environmental pollutants, including PFAS disrupts lipid homeostasis in the liver and has an influence on the initiation and progression of a cascade of pathological conditions associated with NAFLD. PFAS are a group of synthetic chemicals widely used in industrial applications and consumer products such as protective coatings for cookware, food packaging and furniture (4,5). PFAS are extremely resistant to degradation, they bioaccumulate in food chains and drinking water and have long half-lives in humans. Animal studies show that PFAS exposures cause liver enlargement, hepatic steatosis, and hepatocellular hypertrophy (6-13). Data in human are scarce and there are no studies on the impact of PFAS exposure on NAFLD and HCC. Cross-sectional studies in adults showed that elevated serum concentrations of PFOA were associated with increased levels of alanine aminotransferase (ALT), a surrogate marker for NAFLD (14,15), and cytokeratin 18, a marker for liver apoptosis (16). These findings suggest that PFAS may be an important toxicant contributing to NAFLD and HCC.

List of citations: [Uploaded]
b. Explain how the proposed project will improve scientific knowledge of the comprehensive effects of environmental exposures on human health noting advancements over previous research on this topic or how the proposed project will address gaps in scientific knowledge. Include any information related to life stage (e.g., infants, adolescents, adults, seniors) that the project may focus on:

With the increasing prevalence of NAFLD and NAFLD-related cirrhosis and HCC in the US, more efforts are needed to understand their etiology in order to improve prevention and early detection. Given the high prevalence of PFAS exposures in population and the link between PFAS and hepatotoxicity and liver dysfunction, it is important to evaluate whether these compounds are involved in NAFLD, cirrhosis and HCC etiology. The potential association between PFAS concentrations and NAFLD, cirrhosis and HCC has not been studied in humans. The MEC provides a unique opportunity to investigate this relationship with its prospective design, long follow-up, and diverse population. This study has the potential to advance our understanding of hepatotoxic effects of environmental pollutants, and may open new avenues for NAFLD, cirrhosis and HCC prevention.

(127 words/200 word limit)

c. Describe how the requested HHEAR analyses will enhance the findings from the parent study:

The project will provide key additional data which allow us to investigate the role of environmental pollutants in NAFLD and HCC etiology – which may help explain racial differences in risk. Furthermore, we will be able to examine genetic variants and gene-PFAS interactions in NAFLD and liver cancer risk. We hypothesize that known variants associated with HCC and NAFLD interact with PFAS exposures to further increase disease risks. This project will greatly expand the scientific scope of the parent study and will make this the first and biggest population-based study of PFAS and NAFLD and liver cancer.

(96 words/100 word limit)
5. **Study design of proposed HHEAR project:**

   **The objectives and analysis plan should be clearly stated so that non-experts in the field can easily follow the project plan.**

   We will utilize nested case-control design in the MEC. Three case groups: NAFLD (n=1194), NAFLD-related cirrhosis (n=154), and NAFLD-related HCC (n=101) and matched controls (n~1450). Incident HCC cases (C22.0, ICD-O-3 histology codes 8170-8175) are identified after cohort entry through the latest tumor linkage. To determine NAFLD and cirrhosis and underlying etiology of liver disease, we utilized Medicare claims data. Only cases with pre-diagnostic blood samples will be included in this study (i.e. blood collected prior to cancer diagnosis or NAFLD/cirrhosis identification). The median time between blood collection and diagnosis of NAFLD, cirrhosis or HCC is ~8 years. Eligible controls will be individually matched to each case by birth year, sex, race/ethnicity and study area (Los Angeles/Hawaii).

   *(116 words/250 word limit)*

**HHEAR encourages proposal of a wide range of sample sizes. Successful applications will justify the proposed sample size and exposure analyses to achieve the specific aims (see Item 10b).**

   **a. Study sample size:** Three case groups: NAFLD (n=1194), NAFLD-related cirrhosis (n=154), and NAFLD-related HCC (n=101) and matched controls (n=1449).

   **b. Relationship between participants (if applicable) (e.g., mother–child, siblings, family-based trios):**

      Unrelated

      *(1 word/50 word limit)*

   **c. Provide, in a narrative, a breakdown of the total number of participants with biological and/or environmental samples available for analysis by visit and/or age:**

      Three case groups: NAFLD (n=1194), NAFLD-related cirrhosis (n=154), and NAFLD-related HCC (n=101) and matched controls (n=1449).

      *(16 words/100 word limit)*
6. Define your proposed HHEAR project according to the following criteria (please check all that apply):

- [X] Hypothesis testing (for example a new hypothesis or replication of published studies)

- [X] Hypothesis generation
a. Provide an explanation to demonstrate how your HHEAR project meets the criteria for hypothesis testing, hypothesis generation, or both:

This study is novel and has the potential to advance our understanding of hepatotoxic effects of environmental pollutants. The potential association between PFAS concentrations and NAFLD, cirrhosis, and HCC has not been studied in humans. The MEC provides a unique opportunity to investigate this relationship with diverse subjects.

(48 words/100 word limit)
Schedule a pre-submission consultation with HHEAR Lab Hub scientists if you have questions about which exposure analyses are appropriate or about the suitability of your samples for proposed exposure analyses (HHEARHelp@westat.com)

7. Proposed Project Biological Sample Characteristics and Analyses: Complete Table 1 to provide information on characteristics of participants, the associated biological samples that will be provided, the requested laboratory analyses, and the scientific rationale for the laboratory analysis.

- Complete a separate row for each unique combination of characteristics. Provide the biological sample information in as much detail as possible. For example, if you are providing serial serum and urine samples collected from men and women at Time 1 and Time 2, you would complete 8 rows of the Table.

- **Below each selected analysis, please provide a brief scientific rationale for that analysis.** The rationale should describe how the analysis can be used to assess the relationship between the exposure and health outcomes in your HHEAR project.

- If you have a sample matrix that does not match a specified option, select “O-Other” and specify the matrix in the Other Comments section.

- If you need to add rows to the table, contact HHEARHelp@westat.com.

### Table 1: Proposed Project Biological Sample Characteristics and Analyses

<table>
<thead>
<tr>
<th>Priority Order</th>
<th>Laboratory Analyses (exposure measures)</th>
<th>Participant Type</th>
<th>Age/Stage at Collection (e.g., ages 0-2, first trimester)</th>
<th>Time in Study (T1, T2, T3)</th>
<th>Sample Matrix (select from drop down)</th>
<th># Participants</th>
<th># Samples per Participant</th>
<th># Total Samples</th>
<th>Available Volume Per Sample for this Analysis (with units)</th>
<th>Collection Method (e.g., morning void, fasting, passive drool)</th>
<th>Storage Temp (with units)</th>
<th># of Freeze-Thaws</th>
<th>Sample Collection Status (All or Some)</th>
<th>Other Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PFAS</td>
<td>Adults (18+ years)</td>
<td>&gt;45</td>
<td>T1</td>
<td>PE – Plasma EDTA</td>
<td>2898</td>
<td>1</td>
<td>2898</td>
<td>205 ul</td>
<td>fasting</td>
<td>-80 C</td>
<td>1</td>
<td>All or Some</td>
<td></td>
</tr>
</tbody>
</table>

Provide a scientific rationale for the analysis requested above: Emerging evidence indicates that PFAS disrupt lipid homeostasis in the liver and have an influence on the initiation and progression of a cascade of pathological conditions associated with NAFLD. The potential association between PFAS concentrations and NAFLD, cirrhosis, and HCC has not yet been studied in humans – though there is evidence of PFAS exposure having negative hepatic impacts in animal models. This analysis provides an opportunity to advance our understanding of hepatotoxic effects of environmental pollutants on humans by assessing the association between plasma PFAS concentrations and risk of NAFLD, NAFLD-related cirrhosis and HCC.

**Scroll to the right to ensure you complete all columns.**

For information on which samples are suitable for each HHEAR exposure analysis and minimum volume requirements, see the HHEAR Available Analyses by Sample Matrix.
8. Provide information for each type of biological sample to be analyzed for your proposed project. Describe the type of specimen collected (e.g., venous -whole blood, -plasma, -serum, urine, saliva, etc.), the collection method (e.g., spot urine, passive drool saliva, etc.) and any processing (e.g. centrifugation, and/or aliquoting into secondary containers, etc.). Include a description of any additives (e.g. type of anticoagulant, type of preservative, etc.) that were included with or added to the primary or secondary container during collection and/or processing. Describe the storage containers, storage temperature, length of time in storage, and number of freeze thaws. Please note that if your proposed HHEAR project progresses to the Feasibility Assessment consultation, you will be required to provide information on sample collection, processing, and storage.

We collected blood in lavender top CaEDTA tubes. Samples were collected at fasting at (one time point). Samples have been stored at -80°C since they were collected in early 2000. Samples have undergone one freeze-thaw.

(83 words/250 word limit)
Investigators requesting analysis of environmental samples are strongly encouraged to consult HHEAR Lab Hub scientists to discuss proposed analyses and sample collection, processing and storing requirements before submitting an application. Contact the HHEAR coordinating center (HHEARHelp@westat.com) to schedule a pre-submission consultation.

9. Environmental Samples and Analyses: Complete Table 2 to provide information on the environmental samples that will be provided to HHEAR and requested lab analyses.
   • **Below each selected analysis, please provide a brief scientific rationale for that analysis.** The rationale should describe how the analysis can be used to assess the relationship between the exposure and health outcomes in your HHEAR project.
   • **Complete a separate row for each unique combination of characteristics.** Provide the environmental sample information in as much detail as possible.
   • **If you have a sample matrix that does not match a specified option, select “O-Other” and specify the matrix in the Other Comments section.**
   • **If you need to add rows to the table, contact HHEARHelp@westat.com.**

   **Table 2: Proposed Project Environmental Sample Characteristics and Analyses**

<table>
<thead>
<tr>
<th>Priority Order for Analyses</th>
<th>Laboratory Analyses (select from drop down)</th>
<th>Time in Study (T1, T2, T3)</th>
<th>Sample Matrix (select from drop down)</th>
<th># Samples</th>
<th>Available Total Volume/Quantity (with units)</th>
<th>Storage Temp (with units)</th>
<th># of Freeze-Thaws</th>
<th>Other Comments</th>
<th>Briefly provide the rationale for the analysis requested above:</th>
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<tr>
<td>SELECT ONE:</td>
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<td>Briefly provide the rationale for the analysis requested above:</td>
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</table>

10. Provide information on the collection method employed, including the tools and materials used to collect the environmental sample, and any sample processing that was conducted (e.g., sieving) and the containers used to store the samples. Please also include the location where the samples were collected (e.g., if dust, where in the house was the dust collected?):

N/A

(1 word/100 word limit)
Data and Statistical Analysis

11. In the items below, provide information about the variables that will be submitted to HHEAR for data analysis:

a. **Outcome(s):** (Check all that apply)

- [ ] Asthma
- [X] Cancer
- [ ] Autism
- [ ] Cardiovascular disease/risk
- [ ] Diabetes
- [ ] Infectious disease
- [X] Liver disease
- [ ] Obesity/growth
- [ ] Pregnancy outcomes
- [ ] Respiratory health
- [ ] Other: ____________________________

b. How was/were the outcome(s) assessed? For each outcome, please indicate the clinical definitions, symptoms checklists or standardized questionnaires used to obtain outcome measures. For example: asthma diagnosis is based on spirometry lung function- FEV1/FVC; recurrent wheezing/asthma is determined by validated ISAAC questionnaire; incident cancer cases (C22.0, ICD-XX histology codes XX-XX) are identified via SEER tumor registry linkage; Type 2 Diabetes is defined as fasting serum glucose ≥126mg/dl and/or currently taking anti-diabetic medication.

Incident HCC cases (C22.0, ICD-O-3 histology codes 8170-8175) are identified via SEER tumor registry linkage. NAFLD and cirrhosis cases are identified using ICD-9 and ICD-10 codes via Medicare claims linkage. Algorithms for case ascertainment have been previously published.

(38 words/100 word limit)

c. List key covariates. Along with the covariate, please provide the name of the standardized/validated questionnaires (symptom checklists, scales, etc.), if any, used to ascertain the variable.

Age, race/ethnicity, sex, study area, body mass index, diabetes, smoking status, alcohol intake, selected dietary factors and genetic risk score

(20 words/100 word limit)
**Human Health Exposure Analysis Resource (HHEAR)**

**Initial Application for HHEAR Services**

**Step 7 of 9: Proposed HHEAR Project**

d. **Provide frequency tables of key covariates and outcomes for the Proposed HHEAR study, including missing (by time point if applicable) as an attachment. This should be similar to a usual table 1 of a study population (Example Populations Characteristics Table). If not currently possible, provide a description of expected missingness on all key covariates and outcomes. If you don’t currently have access to this information, please explain why.** [Uploaded]

12. **Statistical analysis plan**

a. **Provide a summary description of the analysis strategy and statistical approaches proposed to address each aim. In your explanation, address the following points, as applicable (e.g., confounding, non-linearity, mixtures, combined effect of multiple exposures, potential interactions), and indicate how the proposed strategy will be evaluated to ensure validity, generalizability, and interpretability:**

The association between plasma PFAS concentrations and NAFLD, NAFLD-related cirrhosis and HCC in the matched case-control set will be assessed using conditional logistic regression with age, sex, race and study area as matching factors, adjusting for additional potential confounders, including BMI and other obesity related comorbidities. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) will be calculated for each case group compared to the reference group. We will test the association for the corresponding effect via a LRT. We will primarily focus on a composite PFAS variable, but will also investigate individual PFAS independently and we will use Bayesian kernel machine regression (BKMR) to further understand any non-linear relationships that may exist and to clarify which PFAS might be driving the association, if any. This later inference is possible via the posterior inclusion probabilities in the BKMR model. For aim 2, we will include selected diet variables and genetic risk score (GRS) generated with all of the known NAFLD/HCC SNPs genotyped in the parent R01. We assume that each SNP is independently associated with risk according to an additive genetic model. We will calculate GRS by summing the number of risk alleles at each locus and assume that each SNP contributed equally to the risk of NAFLD/HCC. The genetic risk score distribution among the controls with complete genotype data will be used to generate risk percentile categories.

(228 words/500 word limit)
b. Provide power calculations (e.g., measurable effect size, sample size calculations) for each aim or explain the rationale for why the anticipated sample size is sufficient:

We assume a significance level of $\alpha=0.05$ for our primary composite PFAS exposure variable from a two-sided test. We have 80% power to detect OR of 1.5 with 101 cases (the smallest group, HCC) and 101 matched controls for a one standard deviation increase in an exposure variable. For the largest group (NAFLD: 1194 cases/1194 controls), we can detect OR of 1.15 or 1.20, respectively.

(65 words/100 word limit)

13. Challenges and biases that might be encountered in conducting the proposed study analysis:

PFAS concentrations will be measured once, however, PFAS has long term half-lives in humans and are not lipophilic, thus a single measurement provides good estimate of long-term exposure.

(28 words/50 word limit)