## Re: Form 9-3090 - EcoHealth Alliance

Meicher, Lisa K <lmeicher@usgs.gov>
Tue 5/29/2018 1:47 PM
**To:** Richgels, Katherine L <krichgels@usgs.gov>

Wow! That was quick. I will get the remaining signatures too.

Lisa K. Meicher
Budget Analyst
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
608-270-2410
fax 608-270-2415
lmeicher@usgs.gov

On Tue, May 29, 2018 at 1:42 PM, Richgels, Katherine <krichgels@usgs.gov> wrote:

> Ok, great. We need this form to then go to Jonathan and on to Leon (I believe). Can you usher it through the process for me?
>
> Thanks,
> Katie
>
> On Tue, May 29, 2018 at 1:40 PM, Ethics Office, GS-O <ethicsoffice@usgs.gov> wrote:
>
> > Hello,
> >
> > Please find the signed form attached.
> >
> > Please let me know if you need anything else.
> >
> > Thanks,
> > Liz
> >
> > USGS Ethics Office
> >
> > ~~~~~~~~~~~~
> > U.S. Geological Survey

12210 Sunrise Valley Drive, MS 603
Reston, VA 20192
EthicsOffice@usgs.gov
USGS Ethics Office website: www2.usgs.gov/quality_integrity/ethics

On Tue, May 29, 2018 at 2:20 PM, Meicher, Lisa <lmeicher@usgs.gov> wrote:

> Please review and sign the attached Form 9-3090.
>
> Thanks!
> Lisa
>
> Lisa K. Meicher
> Budget Analyst
> USGS National Wildlife Health Center
> 6006 Schroeder Rd
> Madison, WI 53711
> 608-270-2410
> fax 608-270-2415
> lmeicher@usgs.gov

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Katherine L. D. Richgels, Ph.D.
Branch Chief, Applied Wildlife Health Research
Responsible Official, Federal Select Agent Program
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
(608) 270 - 2450 (office)
(608) 381 - 2492 (cell)
(608) 270 - 2415 (fax)
krichgels@usgs.gov
www.nwhc.usgs.gov

Form 9-3090
(June 2016 - Supersedes all previous versions)
**INTERIM FORM FOR USE WHILE REVISING SHAREPOINT VERSION**

**NOTE – This form along with SM 500.18 and SM 205.13 are in the review process before becoming final policy adjustments. There is a need to utilize this form now in order to obtain the information necessary to proceed in approving Grant applications. This form will be updated per the comments received from the afore mentioned review. Upon completion of the review process the 9-3090 will be developed into a Sharepoint form which will reduce its length and offer ease in its utilization.**

6. *Complete this section only if USGS is applying to RFP as a Subawardee, else skip to next section*

Original Source of Funding Being Awarded to the Grant Source

| | |
|---|---|
| Legal Name of Original Source of Funds being provided to Grant Source | Department of Defense (DoD), Defense Advanced Research Projects Agency (DARPA), Preventing Emerging Pathogenic Threats (PREEMPT) |
| Short Name or Acronym | PREEMPT |
| Address | 675 N. Randolph St |
| Phone Number | DARPA-SN-18-18@darpa.mil |
| Contact Name | Dr. Jim Gimlett, Program Manager |
| Website of Original Source | https://www.darpa.mil/ |
| Website of RFP | https://www.fbo.gov/index?s=opportunity&mode=form&id=4e14aa2d9a172c92a41d8fc181128435&tab=core&_cview=0 |

Make a Selection: ☐ University ☐ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit ☒ Federal Governmental Unit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

| | |
|---|---|
| State of Incorporation (if applicable) | |

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

| | |
|---|---|
| Identify the Parent Company | |
| Website of Parent Company | |

---

7. USGS Office Submitting Grant Information

| | |
|---|---|
| Principal Investigator (PI) Name | Tonie Rocke |
| PI Title | Epizootiologist |
| PI Phone Number | 608-270-2451 |
| PI Email | trocke@usgs.gov |

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## Request to Apply for a Grant Funds-In Award

**(See Appendix A for Instructions for completion of Form 9-3090)**

1. Type of Award: ☐ Prime Award ☒ Subaward

2. Provide documentation confirming USGS can accept the funds as either a prime or sub-awardee.
   *We can accept funds for this RFP using the authority in 31 U.S.C. 1535A Economy Act.*

3. Grant Application Deadline

| |
|---|
| 3/27/2018 |

4. Projected Grant Award Date

| |
|---|
| 12/1/2018 |

5. Grant Source Information (Entity from whom USGS is directly receiving awarded funds)

| | |
|---|---|
| Name | EcoHealth Alliance |
| Address | 460 W. 34th St, 17th Floor |
| Phone Number | 212-380-4474 |
| Contact Name | Dr. Peter Daszak |
| Website of Grant Source | |
| Web Link to RFP | |

Make a Selection: ☐ University ☒ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

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If Yes,

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| | |
|---|---|
| Name of Science Center | National Wildlife Health Center |
| List Other USGS Science Team Members Include Name, Phone, Email, Center | N/A |

8. Science Center/Cost Center Director (Manager)

| | |
|---|---|
| Name of Director | Jonathan Sleeman |
| Title | Center Director |
| Phone Number | 608-270-2401 |
| Email | jsleeman@usgs.gov |
| Name of Science Center | National Wildlife Health Center |
| Organization Code of Center | GGEMNC0000 |
| Administrative Officer (AO) | Thomas Hankins, thankins@usgs.gov |

9. Estimated Dollar Value of Requested Grant (funds coming into USGS)

| |
|---|
| 847,292.29 |

10. Briefly Describe the Proposed Project or attach an SOW if applicable at this time

| |
|---|
| See attached proposal. |

11. Explain how this grant will support a USGS mission or project

| |
|---|
| The application and validation of the technique outlined in this project will also be applied to on-going development of a white-nose syndrome vaccine for bats. Managing white-nose syndrome in bat populations is a priority for the NWHC, Ecosystems Mission Area, and the U.S. Fish and Wildlife Service. |

12. Does Grant solicitation/award limit Center from charging its full overhead? ☐ Yes ☒ No
If Yes,

| | |
|---|---|
| Explain the Limitation | |
| **Contact Center AO to discuss whether the limitation meets Bureau established accounting practices.** | |

13. Do you intend to provide an out-going sub-award of grant funds received through either a contract or financial assistance (cooperative agreement)? ☐ Yes ☒ No

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If Yes,

| | |
|---|---|
| Briefly Explain the Out-Going Sub-Award Plan | |
| **Contact Center AO for review of sub-award plans and appropriateness of sub-award in consultation with OAG as necessary.** | |

14. Is there a Grant Agreement template associated with the RFP that the providing entity is requesting be utilized in the award of funds? ☐ Yes ☒ No

| | |
|---|---|
| If No, | **Start agreement negotiations utilizing the Technical Assistance Agreement template found on the Office of Policy and Analysis site at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx** |
| If Yes, | **Contact Center AO for review of Grant award template and appropriateness of agreement language.** |

15. Does the grant require project reporting and/or special invoicing? ☐ Yes ☐ No ☒ Unknown

If Yes,

| | |
|---|---|
| Describe any concerns the submitting office/PI has regarding the project reporting or invoicing | |

16. Does the grant have special auditing requirements? ☐ Yes ☐ No ☒ Unknown

If Yes,

| | |
|---|---|
| Describe the audit requirements | |

17. Does the Grant contain Intellectual Property (IP) conditions and provisions?
☐ Yes ☐ No ☒ Unknown

If Yes,

| | |
|---|---|
| Does the Grant require a license to new IP? | |
| Does the Grant require assignment of IP? | |
| Is there background IP of either party used to accomplish the SOW? | |
| Is a party identified who will take the lead when new IP is made? | |

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| |
|---|
| **If yes, on any of the above, please contact Center AO for review of compliance with Bureau IP policy. Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html** |

18. Does the Grant RFP contain restrictions on data use rights or access to research results?
☐ Yes ☒ No ☐ Unknown
If Yes,

| | |
|---|---|
| Are there confidentiality terms for protection of the data or research and development information? If yes, explain. | |
| Are there data ownership terms? If yes, explain. | |
| Are there access terms and conditions? If yes, explain. | |
| Is a data management or distribution plan required? If yes, explain. | |
| **If yes on any of the above, please contact Center AO for review of compliance with Bureau policy and consultation with the Office of Policy and Analysis.** | |

19. Does the Grant RFP contain restrictions on preparation or submission of publications or reports?
☐ Yes ☒ No ☐ Unknown
If Yes,

| | |
|---|---|
| Is a review by the Grantor required prior to publication? If yes, explain. | |
| Is there a request for naming entity as support or as designated author? If yes, explain. | |
| **If yes on any of the above, please contact Center AO for review of compliance with OSQI policy related to the Fundamental Science Practices and the Office of** | |

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**Communications for guidance related publications and copyrights.**

20. Business, Contract or Litigation Relationships

| **A. USGS Center** | **B. Principal Investigator (PI)** |
|---|---|
| (1) Is there a Purchase Contract with the Grant Source?<br>☐ Yes ☒ No | (1) Are you involved with any type of project involving the Grant Source?<br>☐ Yes ☒ No |
| (2) Is there an existing CRADA with the Grant Source?<br>☐ Yes ☒ No | (2) Are you involved in development of a prospective CRADA with Grant Source?<br>☐ Yes ☒ No |
| (3) Is there a Cooperative/State/Local Agreement with the Grant Source?<br>☐ Yes ☒ No | (3) Do you have any type of financial interest in the Grant Source?<br>☐ Yes ☒ No |
| (4) Are you aware of any litigation pending or anticipated with the Grant Source? ☐ Yes ☒ No | |
| (5) Is the Grant Source listed in the current Financial Guide for USGS Employees Interests?<br>http://www.usgs.gov/quality_integrity/ethics<br>☐ Yes ☒ No | |

If the answer is 'Yes' to any of the questions in the above section please provide details below: (Attach additional sheets if necessary)

Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

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Question: ☐ (Input Question you are answering, i.e. 1(a), 1(b), etc.)

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Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

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## SIGNATURE/CERTIFICATION SECTION

### I. CERTIFICATION AND CONFLICT OF INTEREST
**This section to be completed by USGS employee applying for competitive grant**

I have completed the above information and certify they are accurate to the best of my knowledge and belief. The activities proposed by the competitive grant will not conflict with my responsibilities to USGS, and my responsibility to report government inventions. I further certify that to the best of my knowledge neither I, nor my spouse, child, parent, sibling nor any organization in which I/we serve as officer, director, trustee or employee:

i) holds financial interest in the above entity;
ii) has or will receive a gift or gratuity from the above entity or any entity that has a substantial interest in the preparation, negotiation or approval of my competitive grant.

I understand that if the facts change during the term of any grant that I may receive, I have an obligation to advise my Supervisor and the Ethics Office at ethicsoffice@usgs.gov in writing.

Principal Investigator Tonie Rocke

Signature: Tonie Rocke Date: 4/18/2018

### II. ADMINISTRATIVE CERTIFICATION

I have reviewed the RFP associated with this Grant. ☐ Yes ☐ No

Answers provided above are determined to be accurate and made to the best of individual ability based on the information available at this time.

Administrative Officer: T Thomas Hankins Jr

Signature: T Thomas Hankins Date: 18 APR 2018

### III. ETHICS OFFICE DETERMINATION
**This section to be completed by USGS Ethics Office**

☒ Based upon the information presented in this form, no prohibited source or conflict of interest issues have been identified.

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☐ Based upon the information presented in this form, prohibited source or conflict of interest issues have been identified.

Comments *(optional)*

Signature: Elizabeth D. Bacca Associate Ethics Counselor Date: 05.29.2018

**IV. CENTER DIRECTOR DETERMINATION**
**This section to be completed by Center Director/Manager**

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: T Sleeman Date: 6/6/18

**V. ASSOCIATE/REGIONAL DIRECTOR DETERMINATION**
**When applicable given thresholds in the SM 500.18 and SM 205.13**
***SM Drafts out for Comments due 7/15/16***

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: _____ Date: _____

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**INSTRUCTIONS for Completing a Request to Apply for a Grant Funds-In Award, Form 9-3090**

**Purpose:** The purpose of the form is to determine if it is appropriate for USGS to apply to Requests for Proposals (RFP) publicly announced and open to Federal entities, and whether USGS policies and procedures allow the project to proceed as outlined/envisioned.

Prior to completing Form 9-3090, a determination of whether the funding it is applicable as an incoming Grant is needed. Guidance on applicability can be found in Survey Manual 500.18 (current draft is out for review) and the Policy and Grant Handbook (under construction).

While SM 500.18 is in Draft form, the following definitions are provided for guidance:
See Definition of a Grant in section 1 below. In addition, the funding is not considered a grant when the following situations are present:

1) When Federal entities invite only other Federal entities to apply for a grant.
2) When there is an internal USGS competition for funds.
3) If an entity requests the USGS submit a proposal for a science effort, but it is not in a competitive situation (even if the providing entity calls the document a grant).
4) When USGS enters into a reimbursable agreement with an entity that has been awarded a grant where the USGS was not specifically involved or identified in the proposal and/or competition.

Blocks on the Form:

1. Definitions for Prime Award and Subaward can be found in Survey Manual 500.18. For additional information and discussion, see the Grant Handbook.

   While SM 500.18 is in Draft form, the following definitions are provided for guidance:

   *Grant.* A grant is a program in which the grant-making entity makes funds or other resources available to eligible participants through a competitive process. A grant, if awarded by a grant-making entity, may be awarded directly or indirectly to the USGS. For instructional guidance and forms specific to incoming grants, see Grant Application and Acceptance Handbook 500.18-H. Financial management procedures are outlined in the Financial Operating Procedures Handbook (FOP).

   (1) Direct Grant (commonly referred to as a "prime award") is one in which the USGS receives the grant funding as a prime recipient.
   (2) Indirect grant (commonly referred as a "subaward") is one in which the USGS has collaborated with an entity to submit a proposal as a co-Principal Investigator (co-PI).
2. Documentation used to confirm that USGS can accept the grant funds might be written language from the RFP itself, an email from the prime award entity indicating that USGS can be a sub-awardee or something from the Grants source.
3. Enter the date the Request For Proposal (RFP) closes.
4. Enter the date it is anticipated the Grant will be awarded.
5. Enter information specific to the Entity from whom USGS is directly receiving awarded funds.
6. Enter information specific to the Original source of funds if USGS is applying as a sub-awardee.
7. Enter Principal Investigator (PI) and Co-PI specific information.
8. Enter Center Director/Manager and Center information.
9. Enter dollar amount of USGS specific requested funds.
10. Provide a brief description of the proposed project or attach an SOW if one has been prepared.
11. Provide an explanation of how the grant will support a USGS mission or project.

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12. Identify whether the Grant has an overhead limitation. If there is a limitation, provide a narrative explanation. For information on overhead structure, management and flexibilities, see Financial Operating Procedures Handbook Chapters 5, 6 and 7.
13. Provide information on intentions for out-going contracts or financial assistance agreements. These will be handled by the Office of Acquisitions and Grants (OAG).
https://insight.usgs.gov/aei/offices/oa/oag/AOP/introduction.pdf
14. Determine if there is a pre-identified agreement template from the granting entity to be used. If so, guidance can be provided by the Office of Policy and Analysis after consultation with the Center's Administrative Officer. A USGS Grant Agreement template found at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx if we have the opportunity to initiate the agreement.
15. Describe any special requirements on project reporting and/or invoicing. Discuss these requirements with Administrative Officer to ensure they can be implemented.
16. Outline special auditing requirements identified in the RFP if applicable.
17. Outline any Intellectual Property (IP) provisions or expectations for this project. Additional information on IP can be accessed at Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html.
18. Outline any restrictions or expectations on data use rights and access to research results. Additional information on these topics can be obtained from the OPA at GS-AEI_opa@usgs.gov.
19. Outline restrictions on preparation or submission of publications or reports. Additional information on these topics can be obtained from OSQI related to the Fundamental Science Practices (FSP) and the Office of Communications for guidance related publications and copyrights. FSP guidelines can be found at https://www2.usgs.gov/fsp/.
20. Answer the questions from the perspective of both the local USGS Center and the PI. Answers will be considered in determining a conflict of interest or appearance thereof. Any "Yes" answers will require an explanation.

Signature/Certification Section:

I. The Principal Investigator signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Administrative Officer for review and signature.

II. The Administrative Officer signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Ethics Office for review and signature.

III. The Ethics Office signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Center Director/Manager for review and signature.

IV. The Center Director/Manager signs this section certifying per the statement provided in accordance with SM 500.18. This may be the final signature if it is under the thresholds outlined in the Delegations of Authority, SM 205.13. If additional approval is needed, upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Associate/Regional Director (AD/RD) for final review and signature.

V. The AD/RD signs this section certifying per the statement provided in accordance with SM 500.18. Upon signature the signed form will be returned to the Center Director/Manager filing. Upon award of the Grant this 9-3090 must be part of the Agreement packet that is submitted for OPA Review at GS-AEI_opa@usgs.gov.

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- Associate Directors and Regional Directors – grants from Federal, State, and local governments of $750,000 or more, and grants of $250,000 or more from sources other than Federal, State, and local governments.
- Science Center Directors and Cost Center Managers – grants from Federal, State, and local governments of less than $750,000, and grants less than $250,000 from sources other than Federal, State, and local governments.

## Form 9-3090 - EcoHealth Alliance

Meicher, Lisa K <lmeicher@usgs.gov>

Tue 5/29/2018 1:20 PM

**To:** Ethics Office, GS-O <ethicsoffice@usgs.gov>
**Cc:** Richgels, Katherine L <krichgels@usgs.gov>

Please review and sign the attached Form 9-3090.

Thanks!
Lisa

Lisa K. Meicher
Budget Analyst
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, WI 53711
608-270-2410
fax 608-270-2415
lmeicher@usgs.gov

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3. Grant Application Deadline

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| 3/27/2018 |

4. Projected Grant Award Date

| |
|---|
| 12/1/2018 |

5. Grant Source Information (Entity from whom USGS is directly receiving awarded funds)

| | |
|---|---|
| Name | EcoHealth Alliance |
| Address | 460 W. 34th St, 17th Floor |
| Phone Number | 212-380-4474 |
| Contact Name | Dr. Peter Daszak |
| Website of Grant Source | |
| Web Link to RFP | |

Make a Selection: ☐ University ☒ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

| | |
|---|---|
| State of Incorporation (if applicable) | |

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

| | |
|---|---|
| Identify the Parent Company | |
| Website of Parent Company | |

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6. *Complete this section only if USGS is applying to RFP as a Subawardee, else skip to next section*

Original Source of Funding Being Awarded to the Grant Source

| | |
|---|---|
| Legal Name of Original Source of Funds being provided to Grant Source | Department of Defense (DoD), Defense Advanced Research Projects Agency (DARPA), Preventing Emerging Pathogenic Threats (PREEMPT) |
| Short Name or Acronym | PREEMPT |
| Address | 675 N. Randolph St |
| Phone Number | DARPA-SN-18-18@darpa.mil |
| Contact Name | Dr. Jim Gimlett, Program Manager |
| Website of Original Source | https://www.darpa.mil/ |
| Website of RFP | https://www.fbo.gov/index?s=opportunity&mode=form&id=4e14aa2d9a172c92a41d8fc181128435&tab=core&_cview=0 |

Make a Selection: ☐ University ☐ Non-Profit ☐ State/Local Governmental Unit
☐ Individual ☐ For Profit ☒ Federal Governmental Unit

International Entity? ☐ Yes ☒ No

Private Corporation? ☐ Yes ☒ No

| | |
|---|---|
| State of Incorporation (if applicable) | |

If a corporation, is it a subsidiary of a larger entity? ☐ Yes ☐ No ☒ NA

If Yes,

| | |
|---|---|
| Identify the Parent Company | |
| Website of Parent Company | |

7. USGS Office Submitting Grant Information

| | |
|---|---|
| Principal Investigator (PI) Name | Tonie Rocke |
| PI Title | Epizootiologist |
| PI Phone Number | 608-270-2451 |
| PI Email | trocke@usgs.gov |

Form 9-3090
(June 2016 - Supersedes all previous versions)
**INTERIM FORM FOR USE WHILE REVISING SHAREPOINT VERSION**

**NOTE – This form along with SM 500.18 and SM 205.13 are in the review process before becoming final policy adjustments. There is a need to utilize this form now in order to obtain the information necessary to proceed in approving Grant applications. This form will be updated per the comments received from the afore mentioned review. Upon completion of the review process the 9-3090 will be developed into a Sharepoint form which will reduce its length and offer ease in its utilization.**

| | |
|---|---|
| Name of Science Center | National Wildlife Health Center |
| List Other USGS Science Team Members Include Name, Phone, Email, Center | N/A |

8. Science Center/Cost Center Director (Manager)

| | |
|---|---|
| Name of Director | Jonathan Sleeman |
| Title | Center Director |
| Phone Number | 608-270-2401 |
| Email | jsleeman@usgs.gov |
| Name of Science Center | National Wildlife Health Center |
| Organization Code of Center | GGEMNC0000 |
| Administrative Officer (AO) | Thomas Hankins, thankins@usgs.gov |

9. Estimated Dollar Value of Requested Grant (funds coming into USGS)

| |
|---|
| 847,292.29 |

10. Briefly Describe the Proposed Project or attach an SOW if applicable at this time

| |
|---|
| See attached proposal. |

11. Explain how this grant will support a USGS mission or project

| |
|---|
| The application and validation of the technique outlined in this project will also be applied to on-going development of a white-nose syndrome vaccine for bats. Managing white-nose syndrome in bat populations is a priority for the NWHC, Ecosystems Mission Area, and the U.S. Fish and Wildlife Service. |

12. Does Grant solicitation/award limit Center from charging its full overhead? ☐ Yes ☒ No
If Yes,

| | |
|---|---|
| Explain the Limitation | |
| **Contact Center AO to discuss whether the limitation meets Bureau established accounting practices.** | |

13. Do you intend to provide an out-going sub-award of grant funds received through either a contract or financial assistance (cooperative agreement)? ☐ Yes ☒ No

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If Yes,

| | |
|---|---|
| Briefly Explain the Out-Going Sub-Award Plan | |
| **Contact Center AO for review of sub-award plans and appropriateness of sub-award in consultation with OAG as necessary.** | |

14. Is there a Grant Agreement template associated with the RFP that the providing entity is requesting be utilized in the award of funds? ☐ Yes ☒ No

| | |
|---|---|
| If No, | **Start agreement negotiations utilizing the Technical Assistance Agreement template found on the Office of Policy and Analysis site at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx** |
| If Yes, | **Contact Center AO for review of Grant award template and appropriateness of agreement language.** |

15. Does the grant require project reporting and/or special invoicing? ☐ Yes ☐ No ☒ Unknown

If Yes,

| | |
|---|---|
| Describe any concerns the submitting office/PI has regarding the project reporting or invoicing | |

16. Does the grant have special auditing requirements? ☐ Yes ☐ No ☒ Unknown

If Yes,

| | |
|---|---|
| Describe the audit requirements | |

17. Does the Grant contain Intellectual Property (IP) conditions and provisions?
☐ Yes ☐ No ☒ Unknown

If Yes,

| | |
|---|---|
| Does the Grant require a license to new IP? | |
| Does the Grant require assignment of IP? | |
| Is there background IP of either party used to accomplish the SOW? | |
| Is a party identified who will take the lead when new IP is made? | |

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| |
|---|
| **If yes, on any of the above, please contact Center AO for review of compliance with Bureau IP policy. Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html** |

18. Does the Grant RFP contain restrictions on data use rights or access to research results?

☐ Yes ☒ No ☐ Unknown

If Yes,

| | |
|---|---|
| Are there confidentiality terms for protection of the data or research and development information? If yes, explain. | |
| Are there data ownership terms? If yes, explain. | |
| Are there access terms and conditions? If yes, explain. | |
| Is a data management or distribution plan required? If yes, explain. | |
| **If yes on any of the above, please contact Center AO for review of compliance with Bureau policy and consultation with the Office of Policy and Analysis.** | |

19. Does the Grant RFP contain restrictions on preparation or submission of publications or reports?

☐ Yes ☒ No ☐ Unknown

If Yes,

| | |
|---|---|
| Is a review by the Grantor required prior to publication? If yes, explain. | |
| Is there a request for naming entity as support or as designated author? If yes, explain. | |
| **If yes on any of the above, please contact Center AO for review of compliance with OSQI policy related to the Fundamental Science Practices and the Office of** | |

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**Communications for guidance related publications and copyrights.**

20. Business, Contract or Litigation Relationships

| **A. USGS Center** | **B. Principal Investigator (PI)** |
|---|---|
| (1) Is there a Purchase Contract with the Grant Source? ☐ Yes ☒ No | (1) Are you involved with any type of project involving the Grant Source? ☐ Yes ☒ No |
| (2) Is there an existing CRADA with the Grant Source? ☐ Yes ☒ No | (2) Are you involved in development of a prospective CRADA with Grant Source? ☐ Yes ☒ No |
| (3) Is there a Cooperative/State/Local Agreement with the Grant Source? ☐ Yes ☒ No | (3) Do you have any type of financial interest in the Grant Source? ☐ Yes ☒ No |
| (4) Are you aware of any litigation pending or anticipated with the Grant Source? ☐ Yes ☒ No | |
| (5) Is the Grant Source listed in the current Financial Guide for USGS Employees Interests? http://www.usgs.gov/quality_integrity/ethics ☐ Yes ☒ No | |

If the answer is 'Yes' to any of the questions in the above section please provide details below: (Attach additional sheets if necessary)

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

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Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

Question: [ ] (Input Question you are answering, i.e. 1(a), 1(b), etc.)

[ ]

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## SIGNATURE/CERTIFICATION SECTION

### I. CERTIFICATION AND CONFLICT OF INTEREST
This section to be completed by USGS employee applying for competitive grant

I have completed the above information and certify they are accurate to the best of my knowledge and belief. The activities proposed by the competitive grant will not conflict with my responsibilities to USGS, and my responsibility to report government inventions. I further certify that to the best of my knowledge neither I, nor my spouse, child, parent, sibling nor any organization in which I/we serve as officer, director, trustee or employee:

i) holds financial interest in the above entity;
ii) has or will receive a gift or gratuity from the above entity or any entity that has a substantial interest in the preparation, negotiation or approval of my competitive grant.

I understand that if the facts change during the term of any grant that I may receive, I have an obligation to advise my Supervisor and the Ethics Office at ethicsoffice@usgs.gov in writing.

Principal Investigator Tonie Rocke

Signature: Tonie E Rocke Date: 4/18/2018

### II. ADMINISTRATIVE CERTIFICATION

I have reviewed the RFP associated with this Grant. ☐ Yes ☐ No

Answers provided above are determined to be accurate and made to the best of individual ability based on the information available at this time.

Administrative Officer: T Morris Hankins Jr

Signature: T Morris Hankins Date: 18 APR 2018

### III. ETHICS OFFICE DETERMINATION
This section to be completed by USGS Ethics Office

☐ Based upon the information presented in this form, no prohibited source or conflict of interest issues have been identified.

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☐ Based upon the information presented in this form, prohibited source or conflict of interest issues have been identified.

Comments *(optional)*

Signature: _____ Date: _____

## IV. CENTER DIRECTOR DETERMINATION
**This section to be completed by Center Director/Manager**

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: _____ Date: _____

## V. ASSOCIATE/REGIONAL DIRECTOR DETERMINATION
**When applicable given thresholds in the SM 500.18 and SM 205.13**
***SM Drafts out for Comments due 7/15/16***

This application for grant meets USGS policy and procedures, as described in SM 500.18. Based on the information presented in this form, I have evaluated this grant and determined it is acceptable to proceed with the application for a grant.

☐ Yes ☐ No

Comments *(optional)*

Signature: _____ Date: _____

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## INSTRUCTIONS for Completing a Request to Apply for a Grant Funds-In Award, Form 9-3090

**Purpose**: The purpose of the form is to determine if it is appropriate for USGS to apply to Requests for Proposals (RFP) publicly announced and open to Federal entities, and whether USGS policies and procedures allow the project to proceed as outlined/envisioned.

Prior to completing Form 9-3090, a determination of whether the funding it is applicable as an incoming Grant is needed. Guidance on applicability can be found in Survey Manual 500.18 (current draft is out for review) and the Policy and Grant Handbook (under construction).

While SM 500.18 is in Draft form, the following definitions are provided for guidance:
See Definition of a Grant in section 1 below. In addition, the funding is not considered a grant when the following situations are present:

1) When Federal entities invite only other Federal entities to apply for a grant.
2) When there is an internal USGS competition for funds.
3) If an entity requests the USGS submit a proposal for a science effort, but it is not in a competitive situation (even if the providing entity calls the document a grant).
4) When USGS enters into a reimbursable agreement with an entity that has been awarded a grant where the USGS was not specifically involved or identified in the proposal and/or competition.

Blocks on the Form:

1. Definitions for Prime Award and Subaward can be found in Survey Manual 500.18. For additional information and discussion, see the Grant Handbook.

   While SM 500.18 is in Draft form, the following definitions are provided for guidance:

   *Grant.* A grant is a program in which the grant-making entity makes funds or other resources available to eligible participants through a competitive process. A grant, if awarded by a grant-making entity, may be awarded directly or indirectly to the USGS. For instructional guidance and forms specific to incoming grants, see Grant Application and Acceptance Handbook 500.18-H. Financial management procedures are outlined in the Financial Operating Procedures Handbook (FOP).

   (1) Direct Grant (commonly referred to as a "prime award") is one in which the USGS receives the grant funding as a prime recipient.
   (2) Indirect grant (commonly referred as a "subaward") is one in which the USGS has collaborated with an entity to submit a proposal as a co-Principal Investigator (co-PI).
2. Documentation used to confirm that USGS can accept the grant funds might be written language from the RFP itself, an email from the prime award entity indicating that USGS can be a sub-awardee or something from the Grants source.
3. Enter the date the Request For Proposal (RFP) closes.
4. Enter the date it is anticipated the Grant will be awarded.
5. Enter information specific to the Entity from whom USGS is directly receiving awarded funds.
6. Enter information specific to the Original source of funds if USGS is applying as a sub-awardee.
7. Enter Principal Investigator (PI) and Co-PI specific information.
8. Enter Center Director/Manager and Center information.
9. Enter dollar amount of USGS specific requested funds.
10. Provide a brief description of the proposed project or attach an SOW if one has been prepared.
11. Provide an explanation of how the grant will support a USGS mission or project.

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12. Identify whether the Grant has an overhead limitation. If there is a limitation, provide a narrative explanation. For information on overhead structure, management and flexibilities, see Financial Operating Procedures Handbook Chapters 5, 6 and 7.
13. Provide information on intentions for out-going contracts or financial assistance agreements. These will be handled by the Office of Acquisitions and Grants (OAG). https://insight.usgs.gov/aei/offices/oa/oag/AOP/introduction.pdf
14. Determine if there is a pre-identified agreement template from the granting entity to be used. If so, guidance can be provided by the Office of Policy and Analysis after consultation with the Center's Administrative Officer. A USGS Grant Agreement template found at https://insight.usgs.gov/aei/offices/oa/opa/SitePages/Home.aspx if we have the opportunity to initiate the agreement.
15. Describe any special requirements on project reporting and/or invoicing. Discuss these requirements with Administrative Officer to ensure they can be implemented.
16. Outline special auditing requirements identified in the RFP if applicable.
17. Outline any Intellectual Property (IP) provisions or expectations for this project. Additional information on IP can be accessed at Patents and Licensing: http://internal.usgs.gov/ops/opa/index.html.
18. Outline any restrictions or expectations on data use rights and access to research results. Additional information on these topics can be obtained from the OPA at GS-AEI_opa@usgs.gov.
19. Outline restrictions on preparation or submission of publications or reports. Additional information on these topics can be obtained from OSQI related to the Fundamental Science Practices (FSP) and the Office of Communications for guidance related publications and copyrights. FSP guidelines can be found at https://www2.usgs.gov/fsp/.
20. Answer the questions from the perspective of both the local USGS Center and the PI. Answers will be considered in determining a conflict of interest or appearance thereof. Any "Yes" answers will require an explanation.

Signature/Certification Section:

I. The Principal Investigator signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Administrative Officer for review and signature.

II. The Administrative Officer signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Ethics Office for review and signature.

III. The Ethics Office signs this section certifying per the statement provided. Upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Center Director/Manager for review and signature.

IV. The Center Director/Manager signs this section certifying per the statement provided in accordance with SM 500.18. This may be the final signature if it is under the thresholds outlined in the Delegations of Authority, SM 205.13. If additional approval is needed, upon signature submit the form along with the RFP and/or Draft Agreement if applicable to the Associate/Regional Director (AD/RD) for final review and signature.

V. The AD/RD signs this section certifying per the statement provided in accordance with SM 500.18. Upon signature the signed form will be returned to the Center Director/Manager filing. Upon award of the Grant this 9-3090 must be part of the Agreement packet that is submitted for OPA Review at GS-AEI_opa@usgs.gov.

While SM 500.18 is in Draft form, the following signature thresholds are provided for guidance:

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- Associate Directors and Regional Directors – grants from Federal, State, and local governments of $750,000 or more, and grants of $250,000 or more from sources other than Federal, State, and local governments.
- Science Center Directors and Cost Center Managers – grants from Federal, State, and local governments of less than $750,000, and grants less than $250,000 from sources other than Federal, State, and local governments.

## Re: [EXTERNAL] RE: DEFUSE documents as submitted

# Richgels, Katherine L <krichgels@usgs.gov>

Wed 3/28/2018 1:01 PM

**To:** Rocke, Tonie E <trocke@usgs.gov>

Thanks Tonie, I appreciate the support. I can't find a final proposal email from Luke, can you forward it when you get a chance?

Katie

Sent from my Verizon, Samsung Galaxy smartphone

-------- Original message --------
From: "Rocke, Tonie" <trocke@usgs.gov>
Date: 3/28/18 1:47 PM (GMT-05:00)
To: Katherine Richgels <krichgels@usgs.gov>
Subject: Re: [EXTERNAL] RE: DEFUSE documents as submitted

I believe Luke Hamel sent you all the documents including our budget. By the way, thanks for your letter; it was thoughtful of you, and rest assured, I am very supportive of you in this position (despite my initial hesitance which I now regret). I have had 3 branch chiefs during my term here, and you are by far the best! Keep up the good work. It will get easier. Best - Tonie

On Wed, Mar 28, 2018 at 12:30 PM, Katherine Richgels <krichgels@usgs.gov> wrote:

> Congrats! Can you send me a copy if the submitted proposal?
>
> Thanks,
> Katie
>
> Sent from my Verizon, Samsung Galaxy smartphone
>
> -------- Original message --------
> From: "Rocke, Tonie" <trocke@usgs.gov>
> Date: 3/28/18 1:18 PM (GMT-05:00)
> To: Peter Daszak <daszak@ecohealthalliance.org>
> Cc: Luke Hamel <hamel@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Rachel Abbott <rabbott@usgs.gov>, "Richgels, Katherine" <krichgels@usgs.gov>
> Subject: Re: [EXTERNAL] RE: DEFUSE documents as submitted
>
> My pleasure - your team did an amazing job getting all the information together in a very short time! Best -Tonie
>
> On Wed, Mar 28, 2018 at 12:07 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
>
> > ..also want to add my thanks for your help getting this together Tonie!

Cheers,

Peter

**Peter Daszak**

*President*

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4474

www.ecohealthalliance.org

@PeterDaszak

@EcoHealthNYC

*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

**From:** Luke Hamel [mailto:hamel@ecohealthalliance.org]
**Sent:** Wednesday, March 28, 2018 1:00 PM
**To:** Rocke, Tonie
**Cc:** Peter Daszak; Alison Andre; Rachel Abbott; Richgels, Katherine
**Subject:** DEFUSE documents as submitted

Hi Tonie,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

Attached files include:

- Technical and Management Proposal (Vol. I)
- Executive Summary Slide
- NWHC budget packet
- NWHC budget justification

Best,

**Luke Hamel**
*Program Assistant*

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

# Fwd: [EXTERNAL] DEFUSE documents as submitted

Richgels, Katherine L <krichgels@usgs.gov>
Wed 3/28/2018 1:10 PM
**To:** Sleeman, Jonathan M <jsleeman@usgs.gov>; Center Director, GS-MWA-NWHC <nwhc_director@usgs.gov>

4 attachments (7 MB)
PREEMPT Volume 1 no ESS HR001118S0017 EcoHealthAlliance DEFUSE.pdf; Executive Slide HR001118S0017 EcoHealthAlliance DEFUSE.pptx; NWHC budget packet HR001118S0017 EcoHealthAlliance DEFUSE.xlsx; NWHC budget Justification HR001118S0017 EcoHealthAlliance DEFUSE.pdf;

FYI Tonie has submitted the PREEMPT grant.

Katie

Sent from my Verizon, Samsung Galaxy smartphone

-------- Original message --------
From: "Rocke, Tonie" <trocke@usgs.gov>
Date: 3/28/18 2:07 PM (GMT-05:00)
To: "Richgels, Katherine" <krichgels@usgs.gov>
Subject: Fwd: [EXTERNAL] DEFUSE documents as submitted

---------- Forwarded message ----------
From: **Luke Hamel** <hamel@ecohealthalliance.org>
Date: Wed, Mar 28, 2018 at 11:59 AM
Subject: [EXTERNAL] DEFUSE documents as submitted
To: "Rocke, Tonie" <trocke@usgs.gov>
Cc: "Dr. Peter Daszak" <daszak@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>, Rachel Abbott <rabbott@usgs.gov>, "Richgels, Katherine" <krichgels@usgs.gov>

Hi Tonie,

Peter had asked me to send these files to you. They are the final versions of our DEFUSE proposal, as submitted yesterday.

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Best,

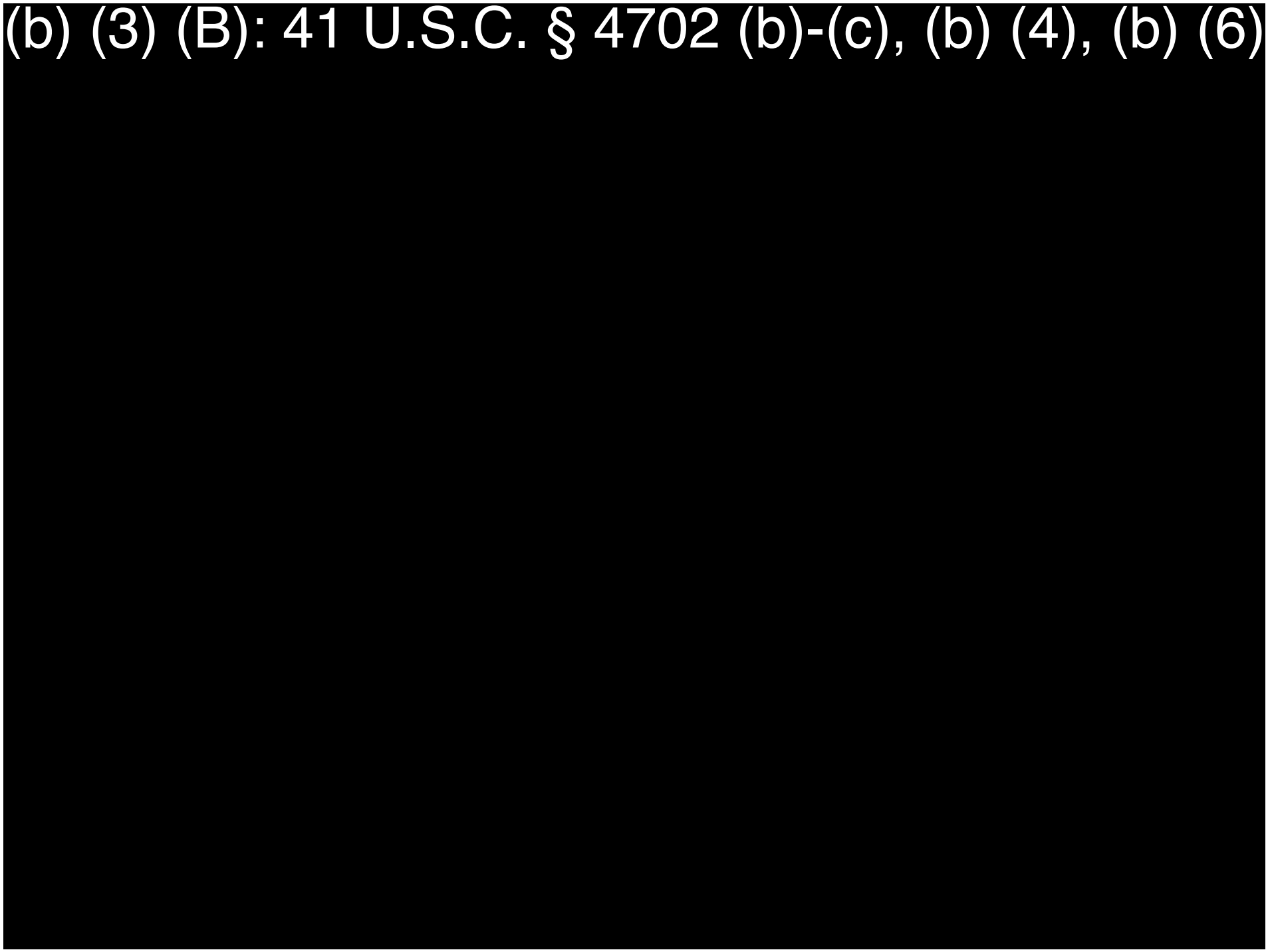
**Luke Hamel**
*Program Assistant*

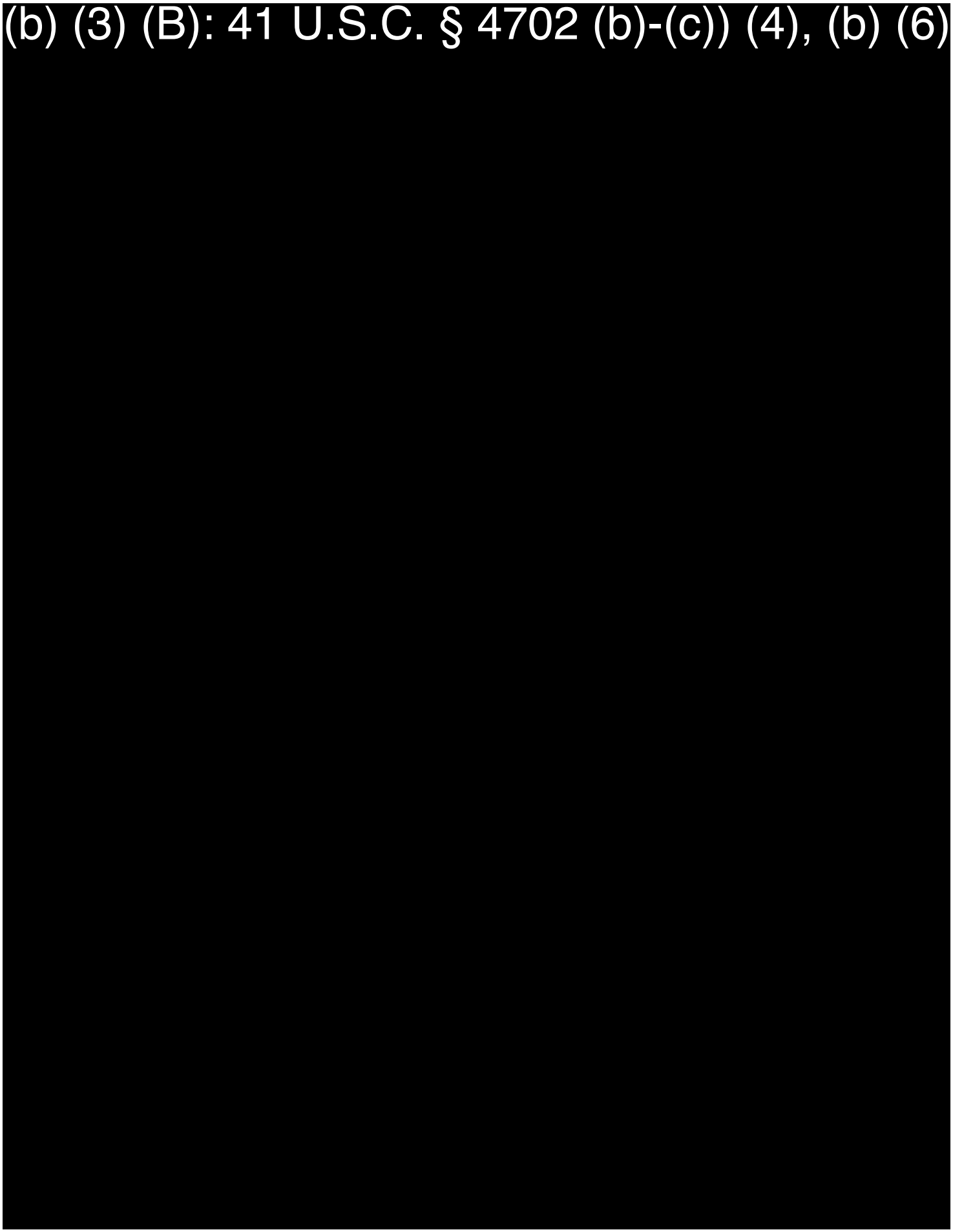
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

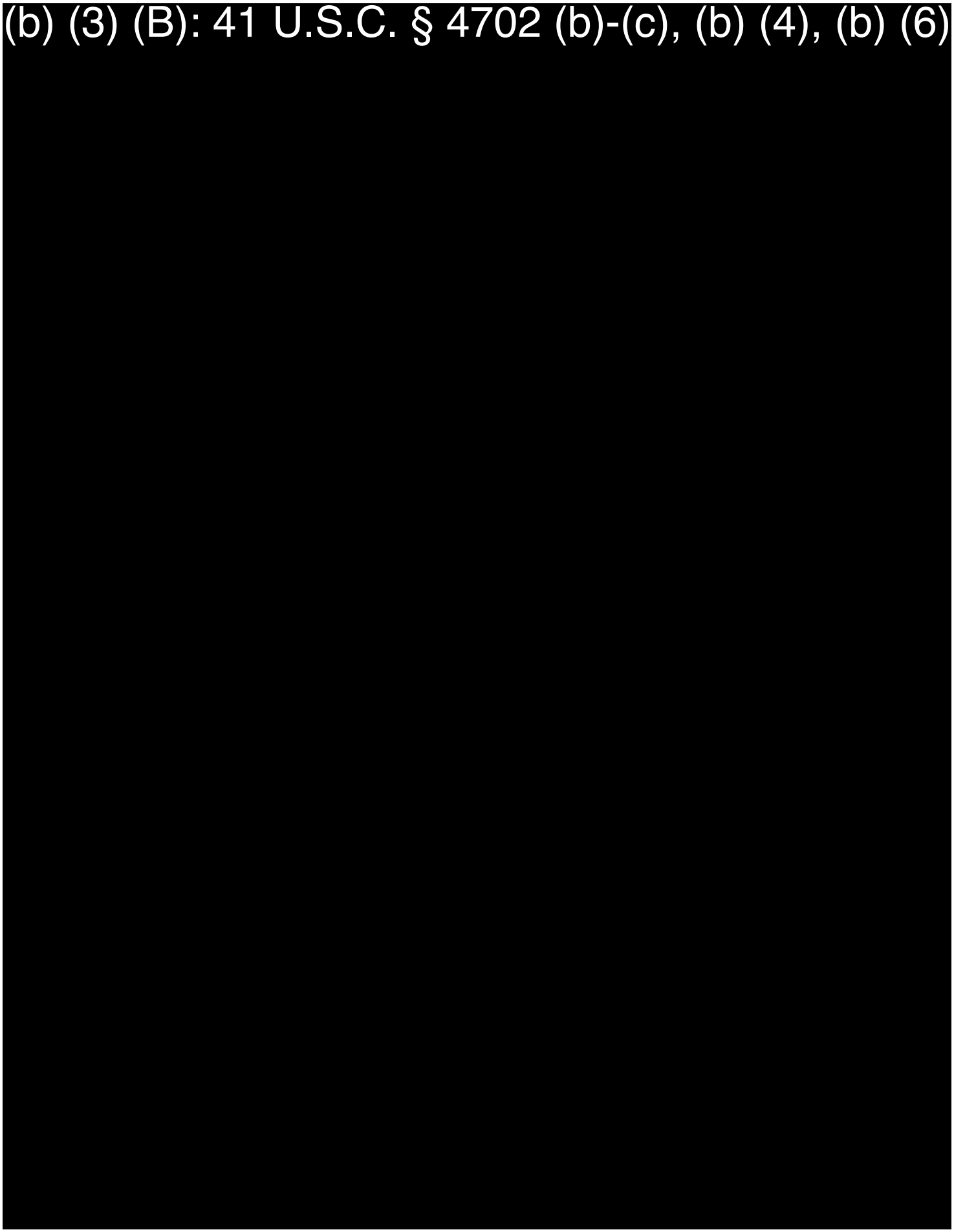
(b) (6) (direct)
(b) (6) (mobile)
www.ecohealthalliance.org

**EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.**

--
Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c)) (4), (b) (6)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)


(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4), (b) (6)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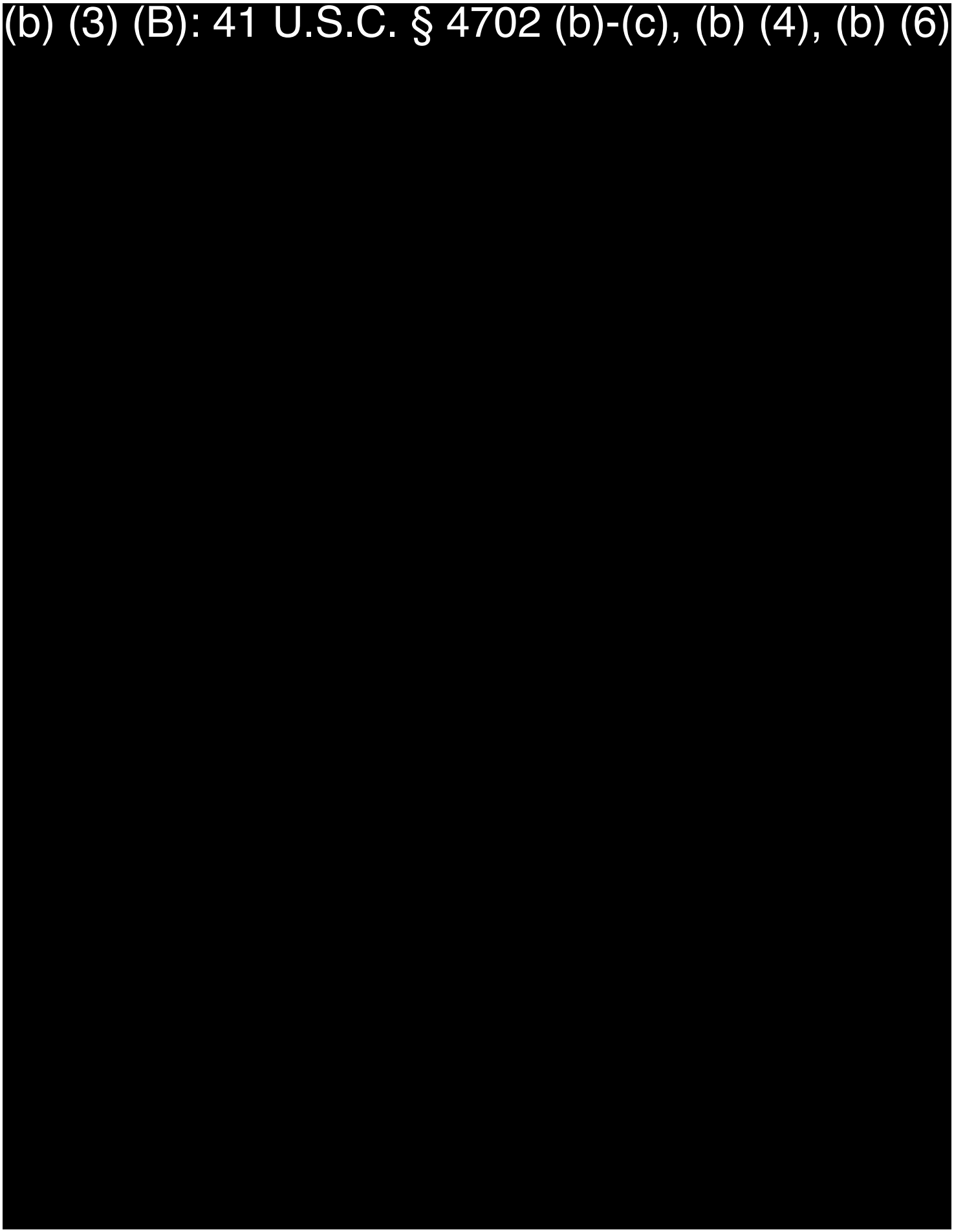
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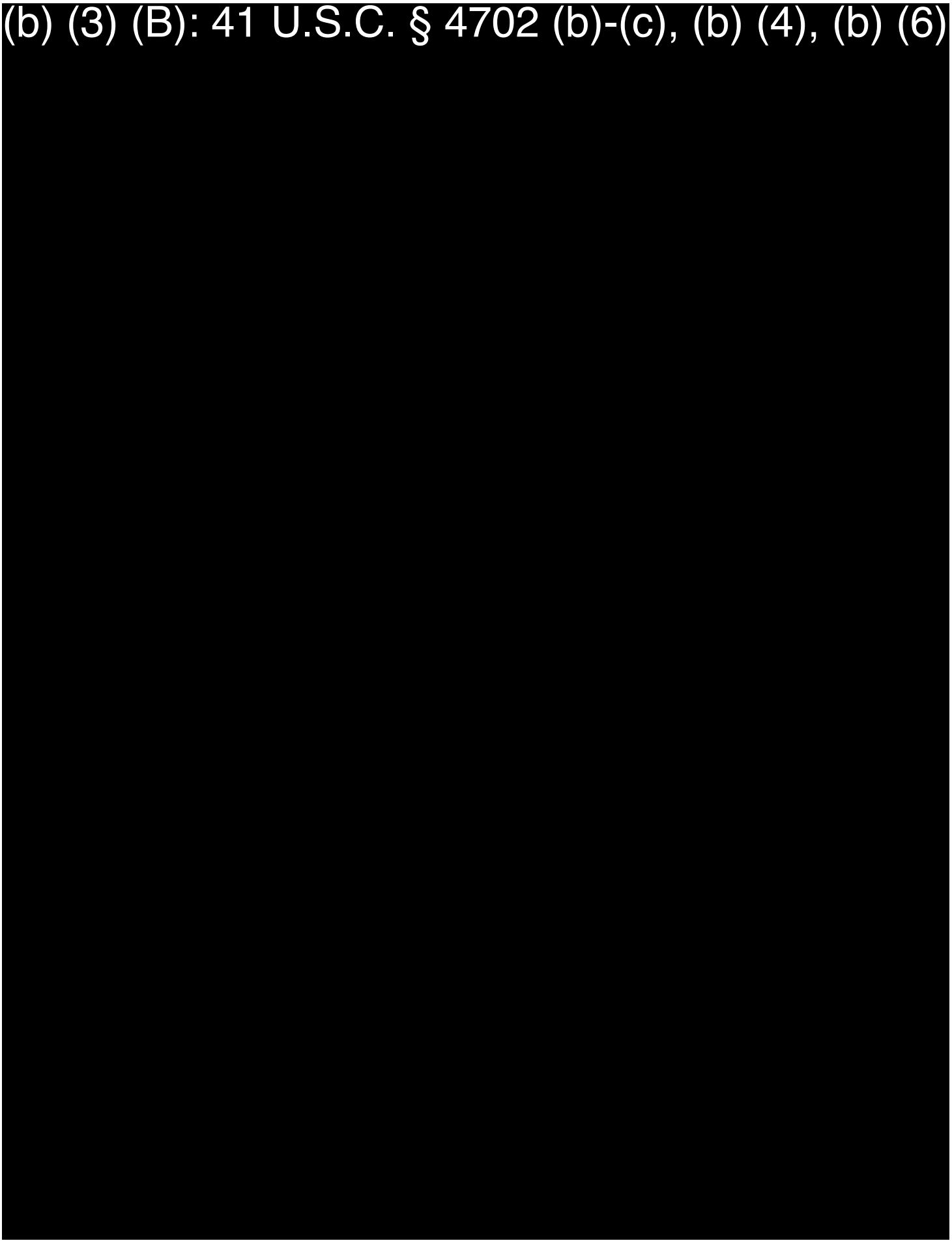
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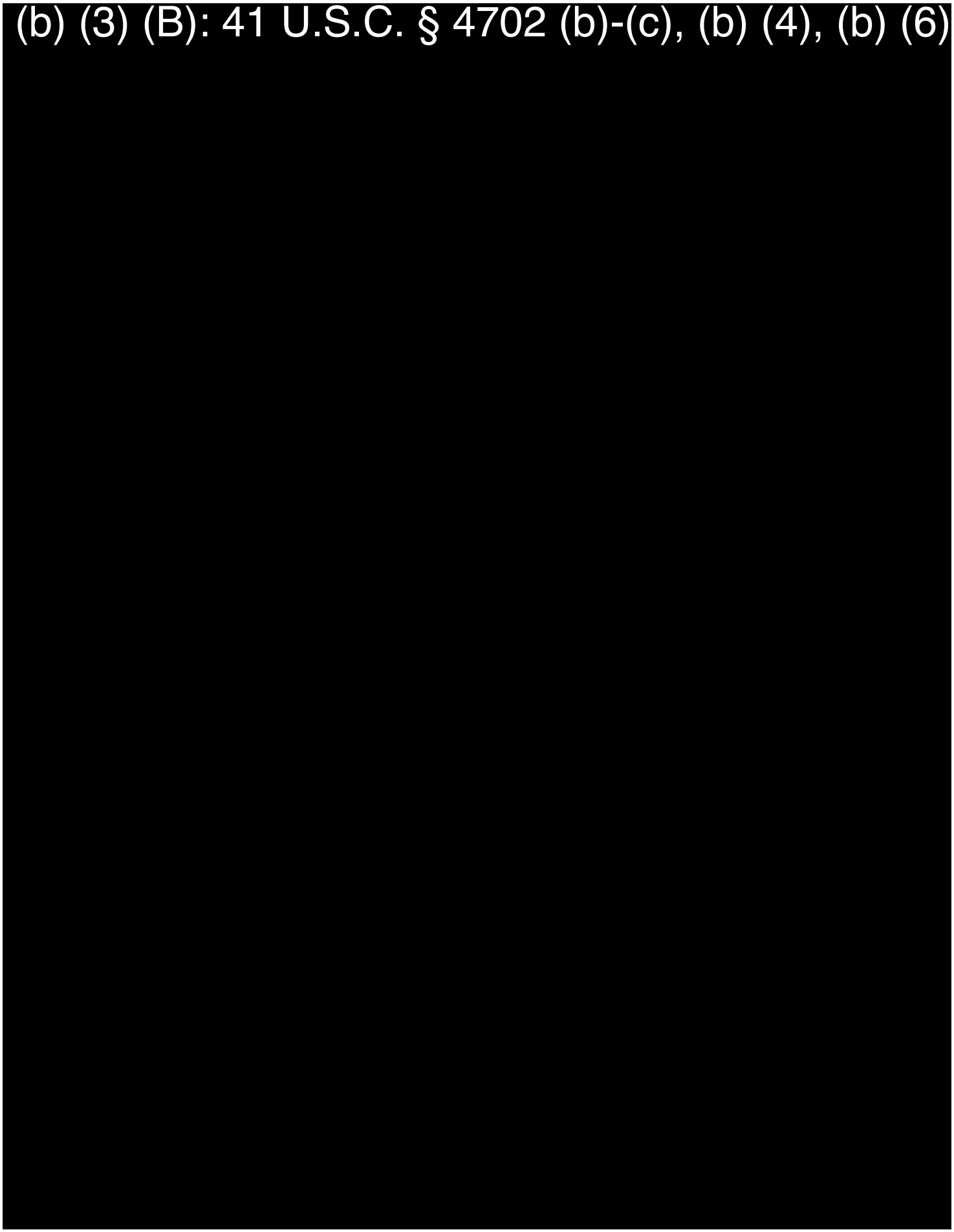
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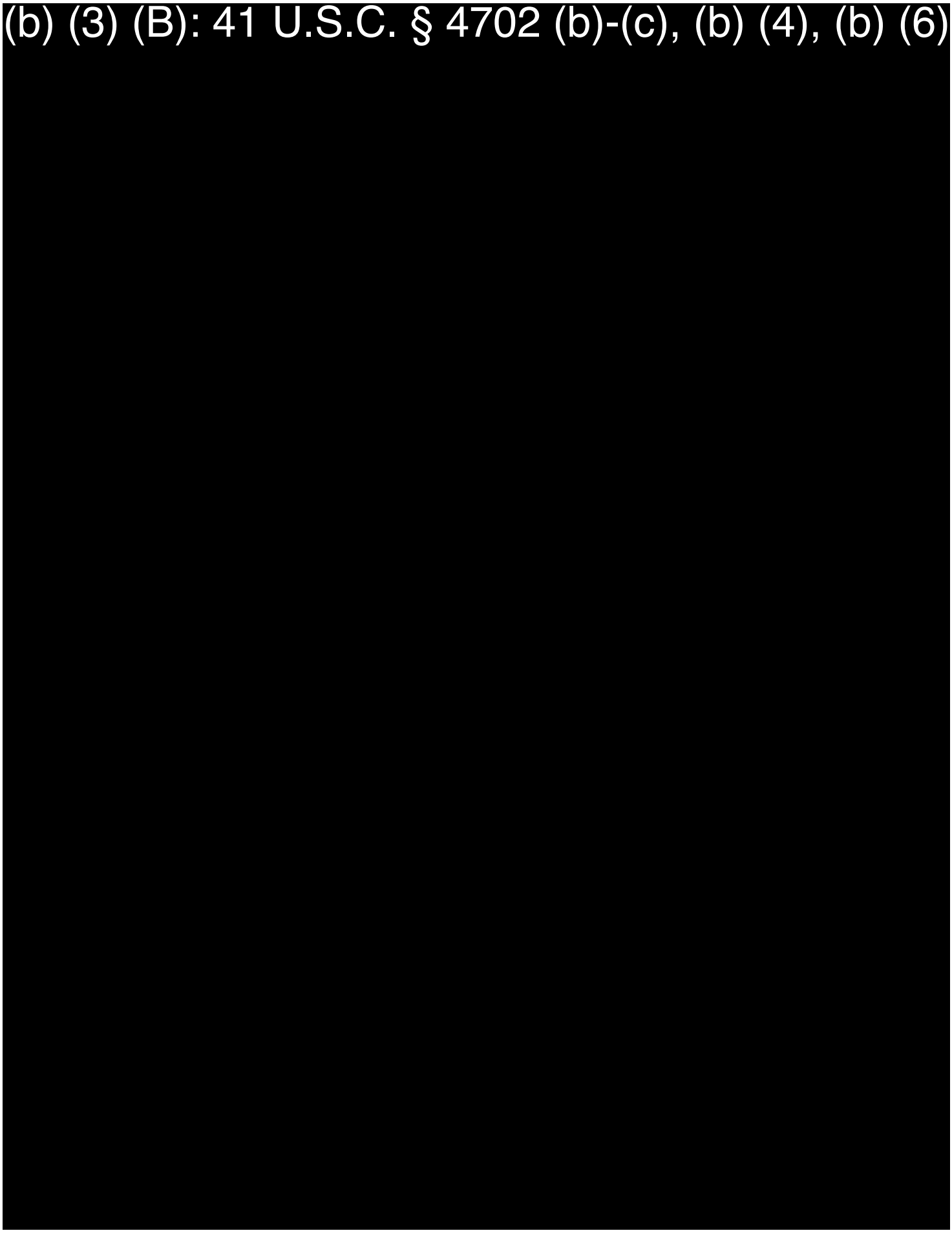
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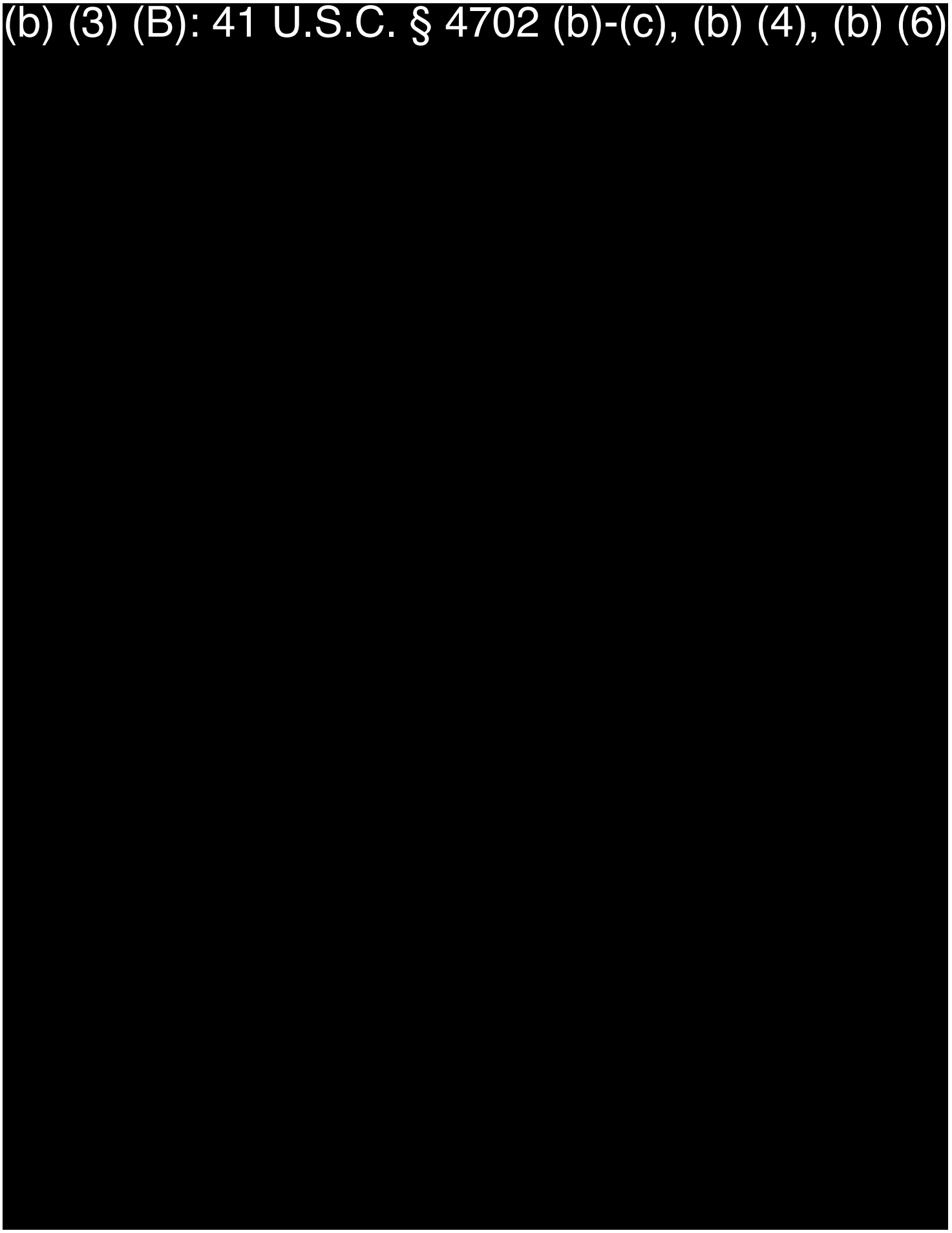
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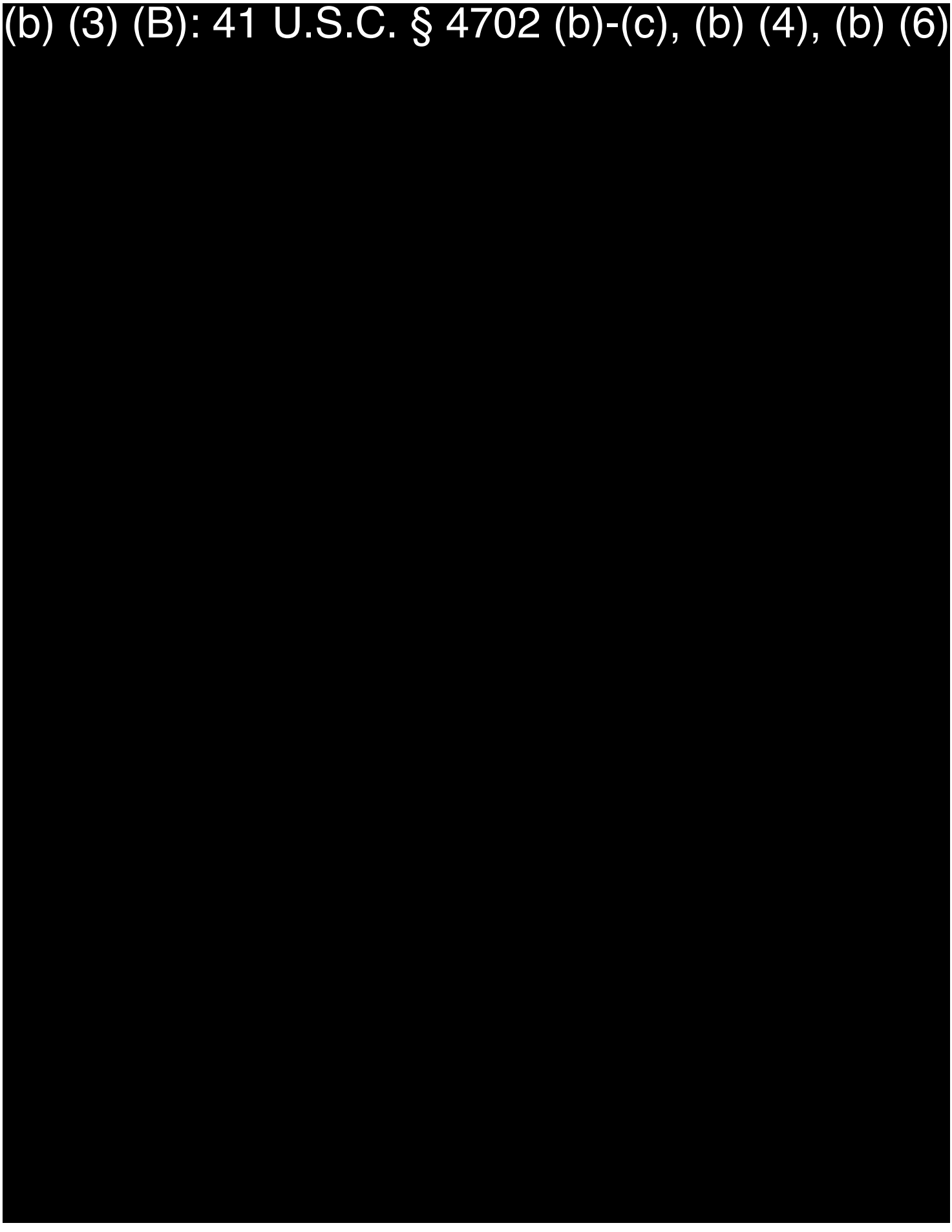
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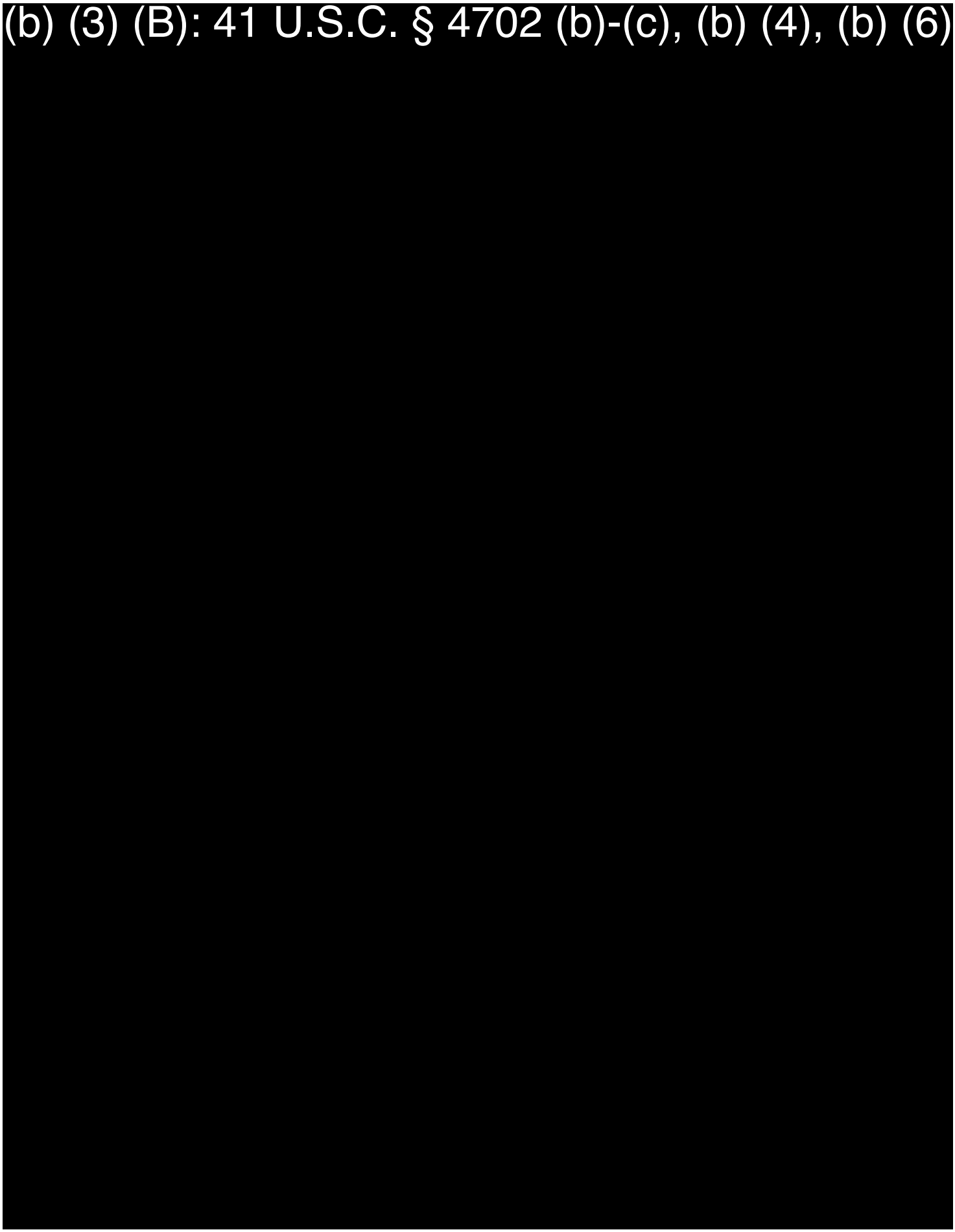
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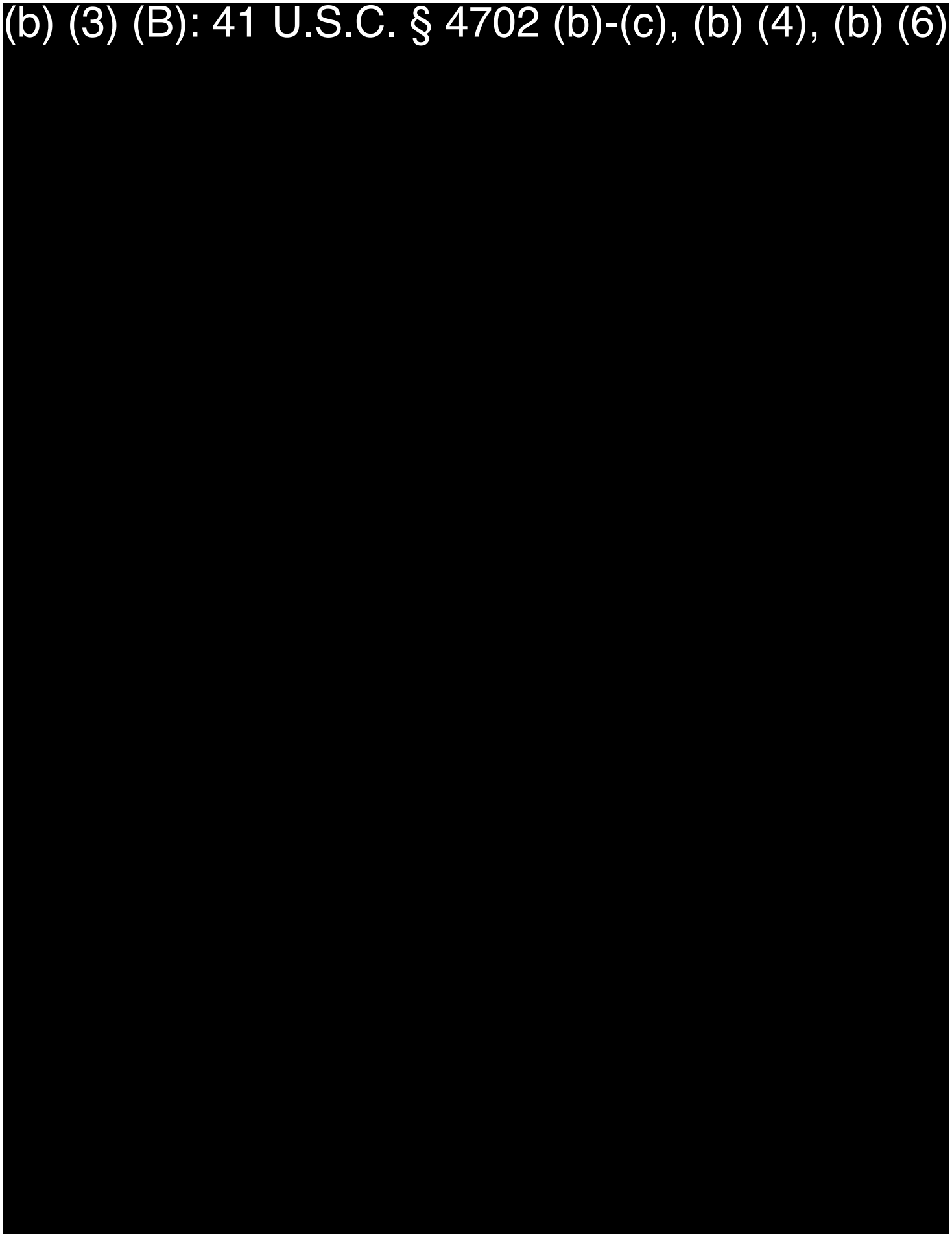
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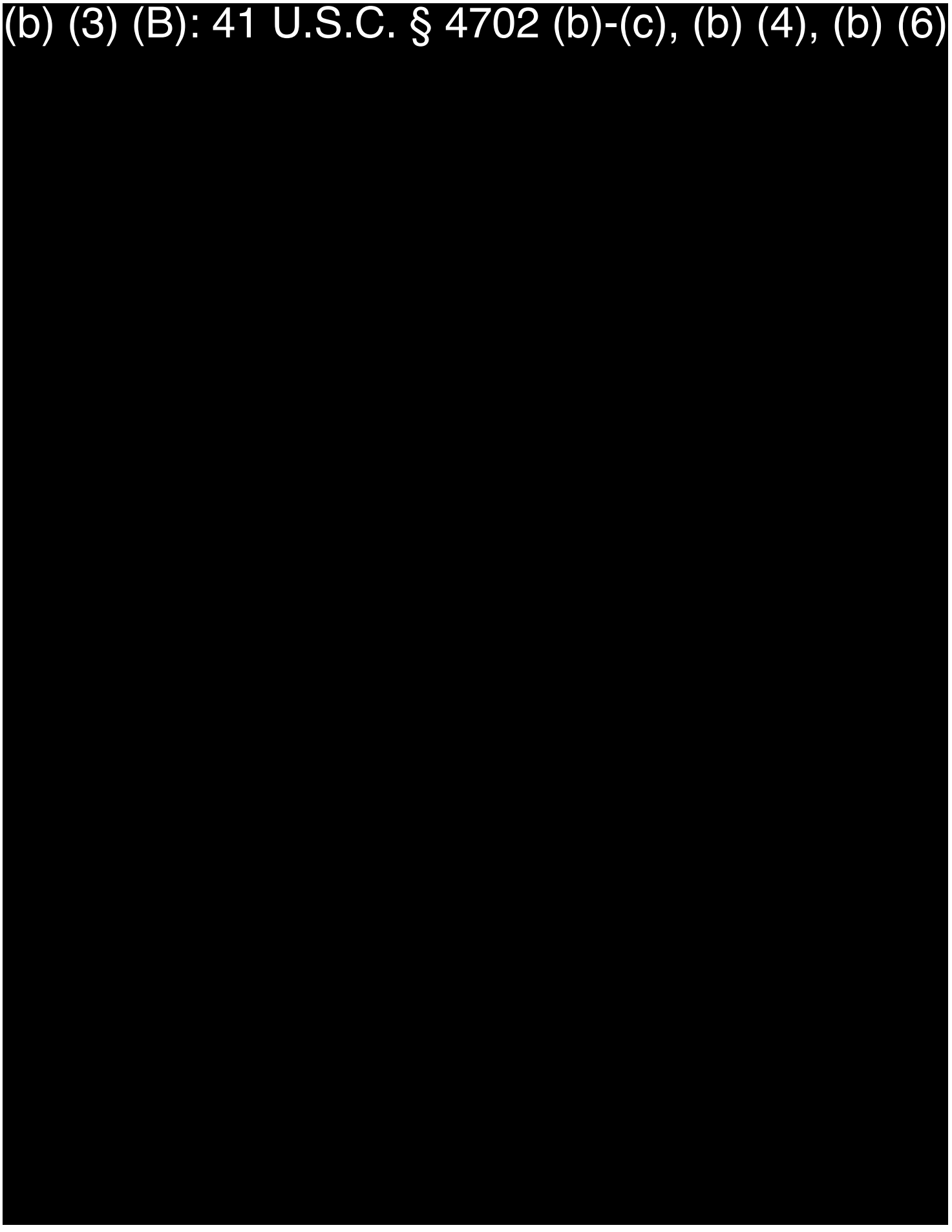
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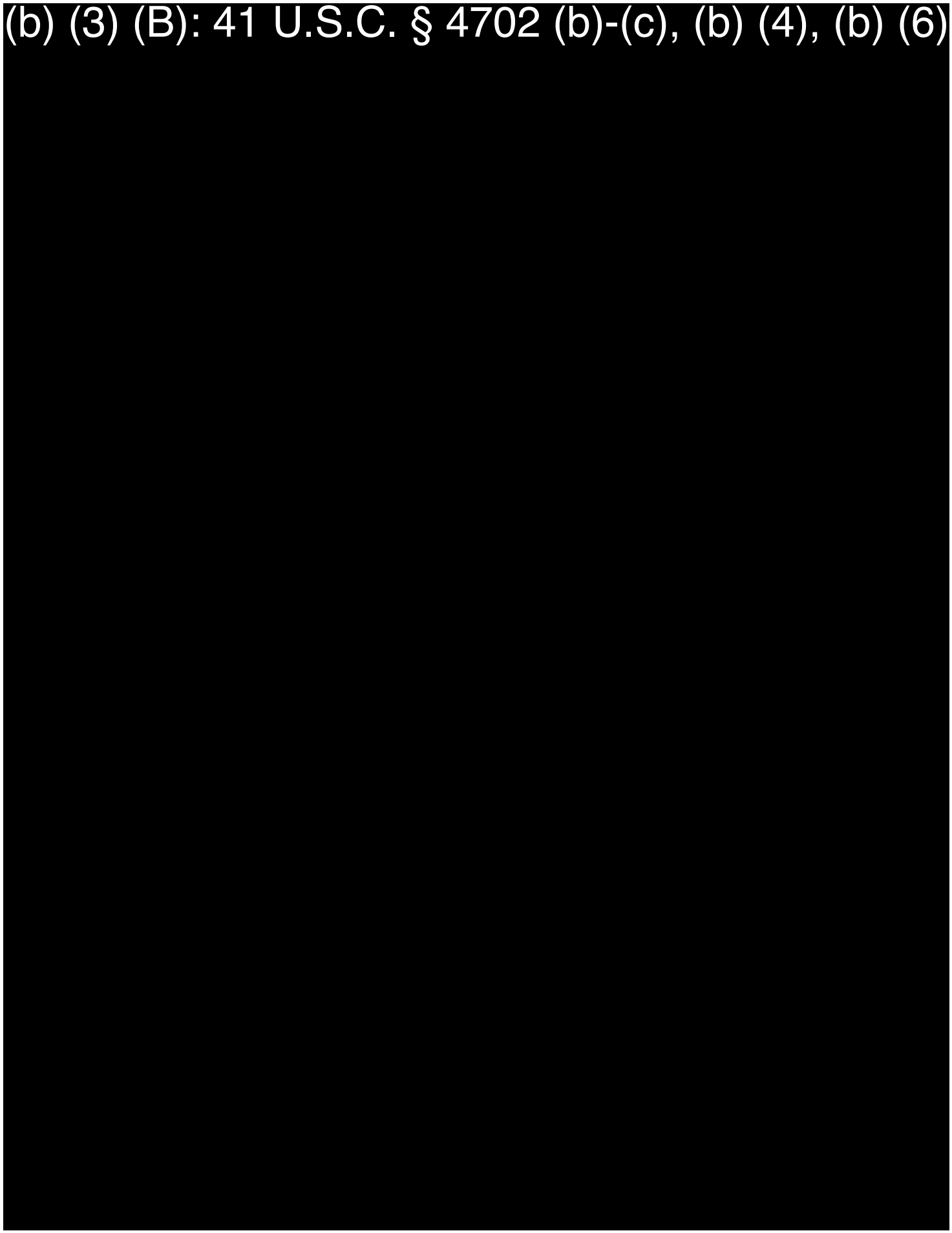
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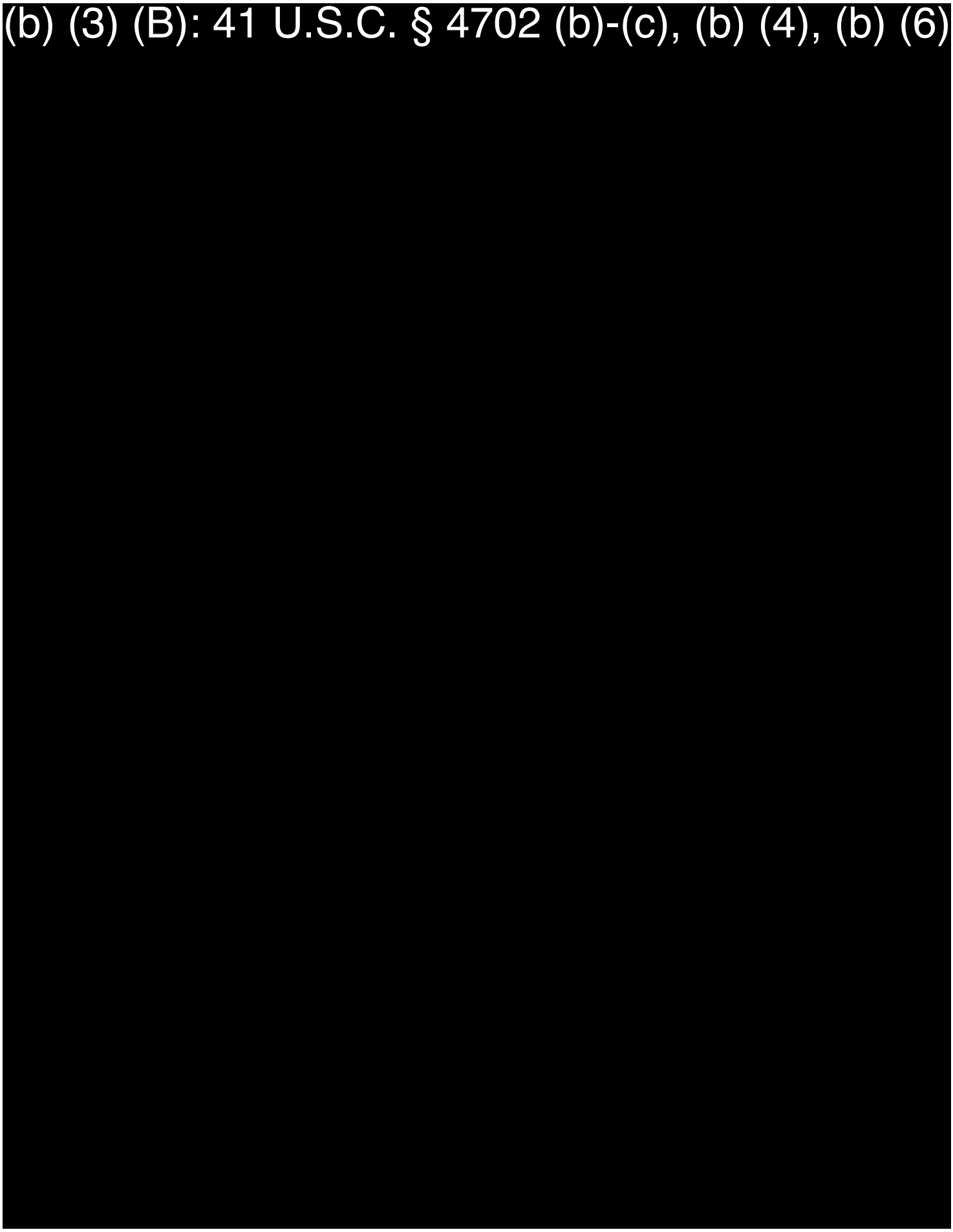
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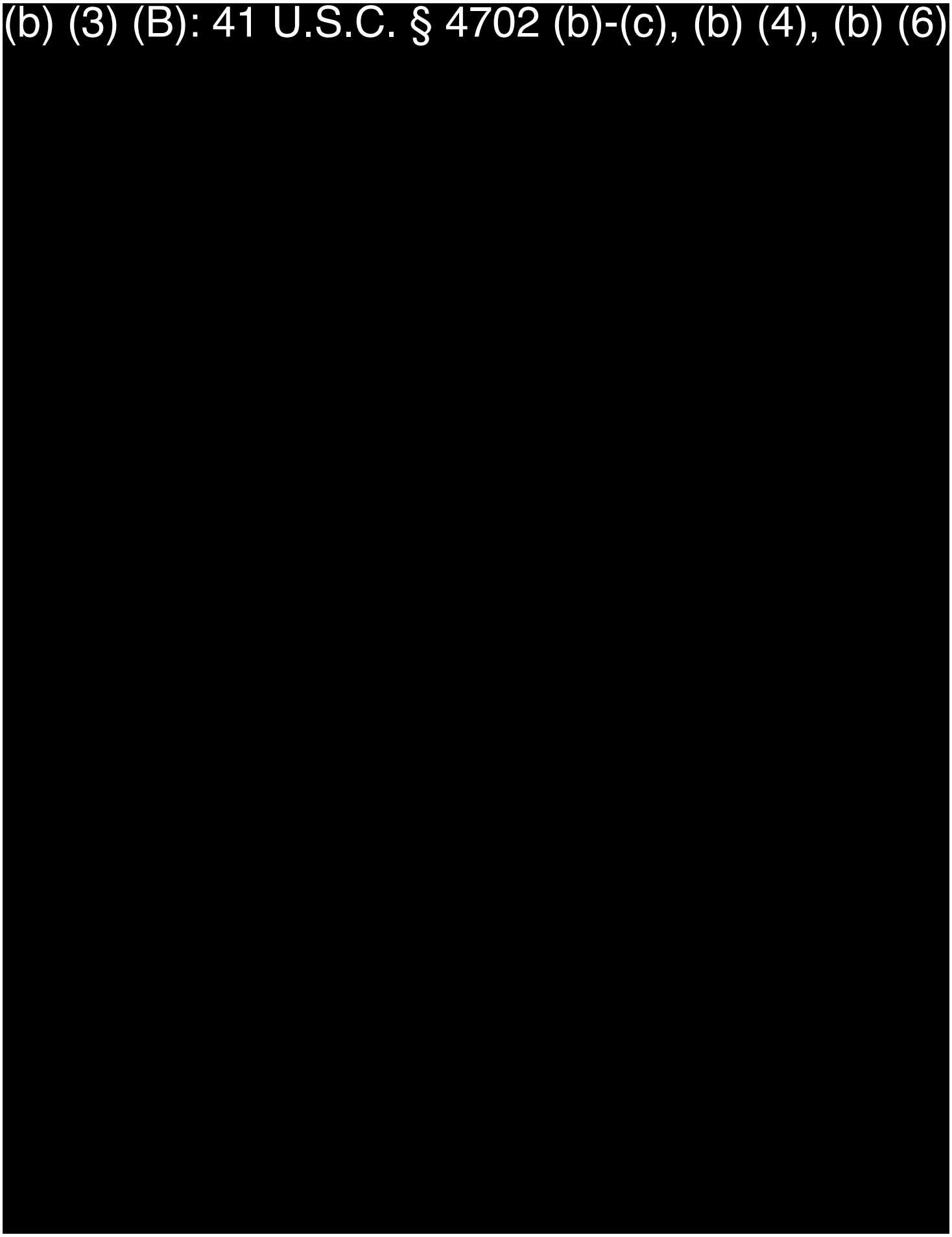
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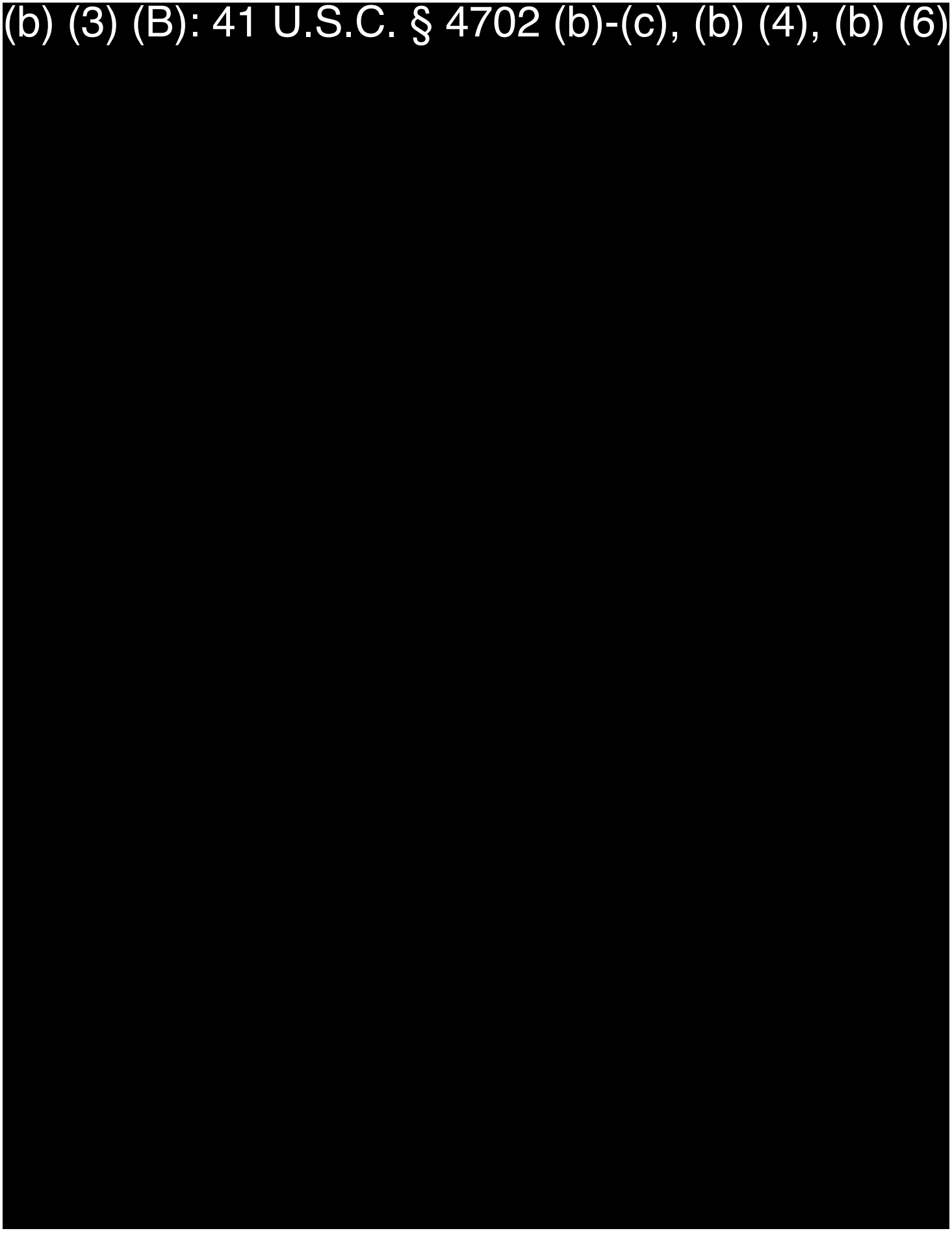
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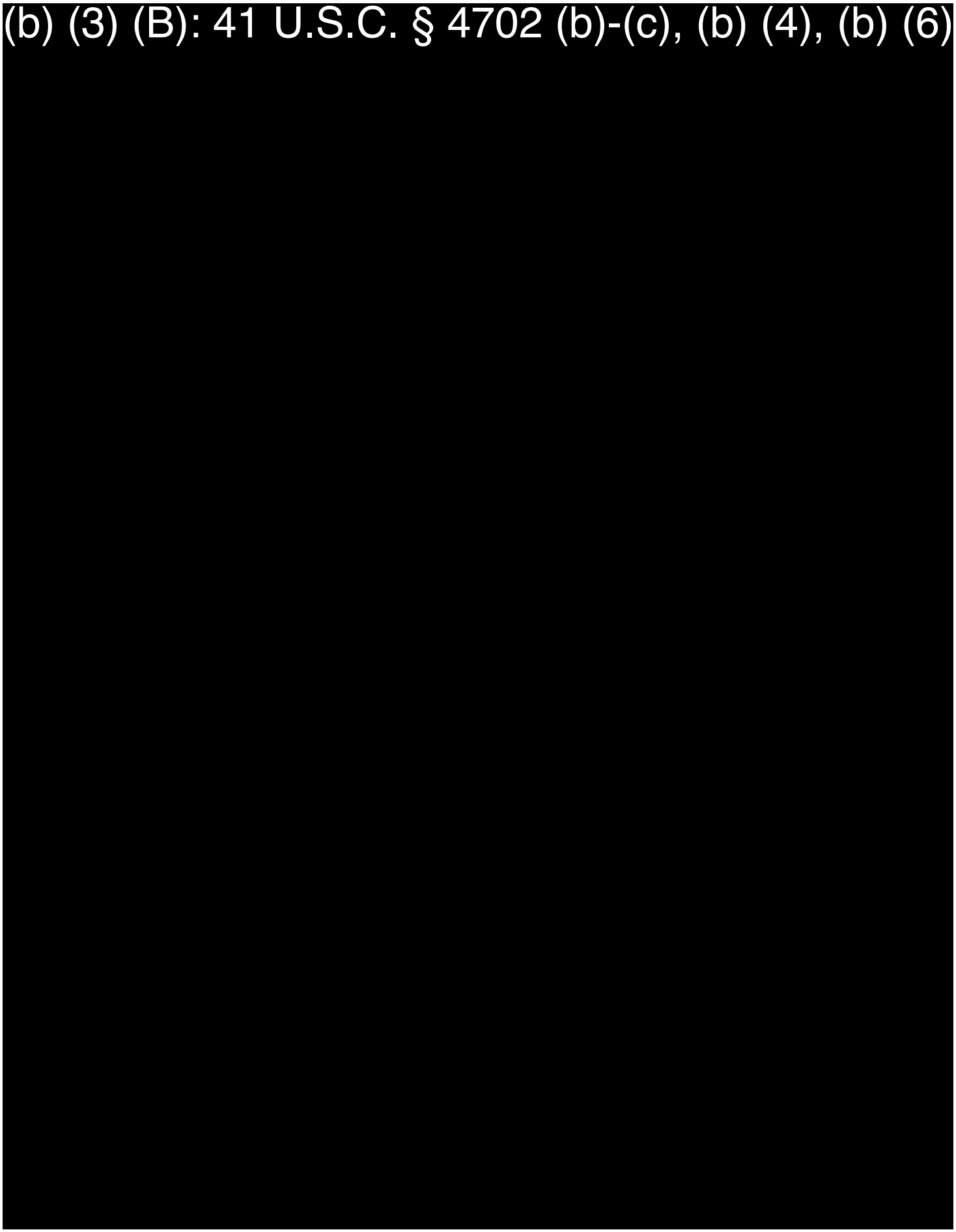
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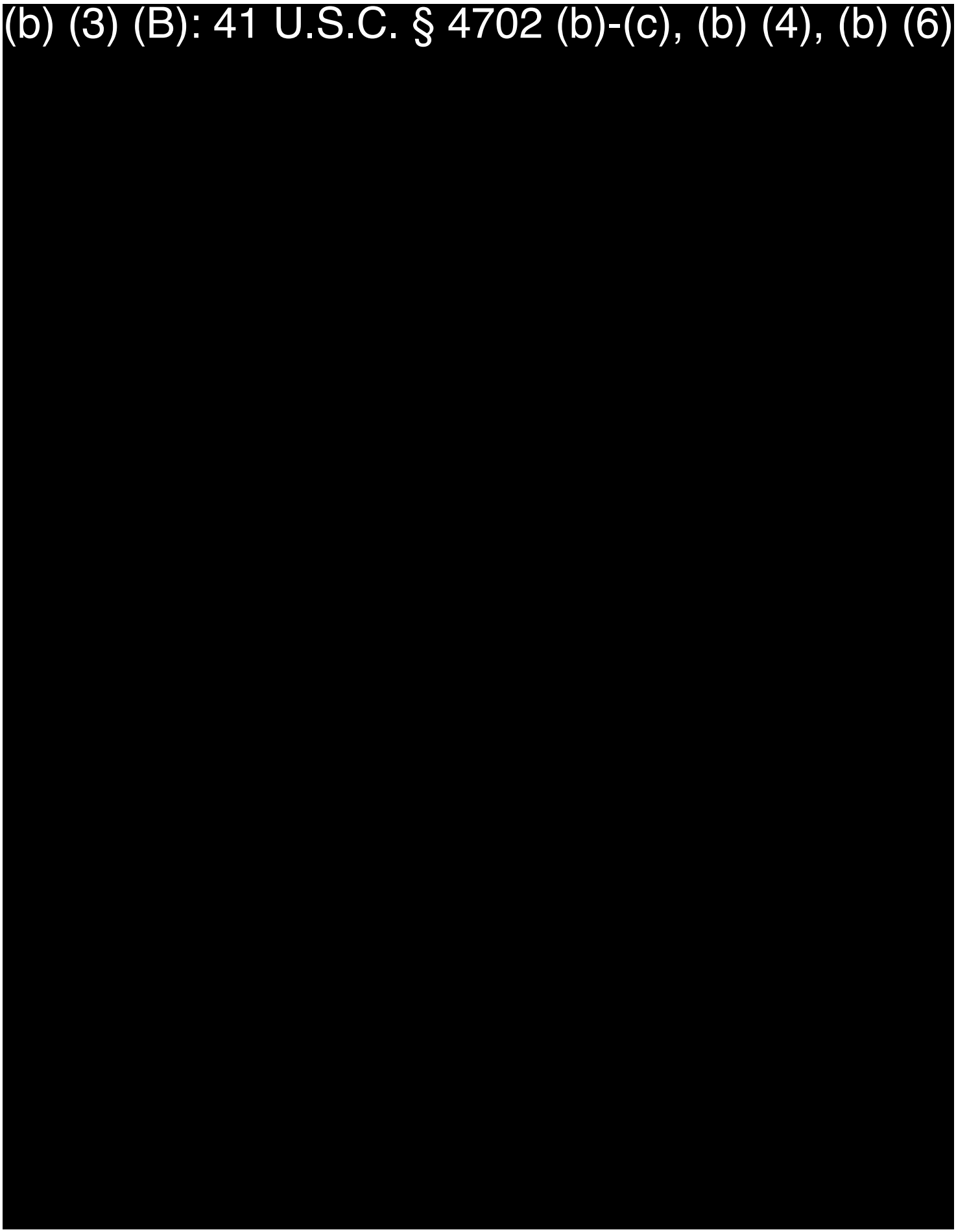
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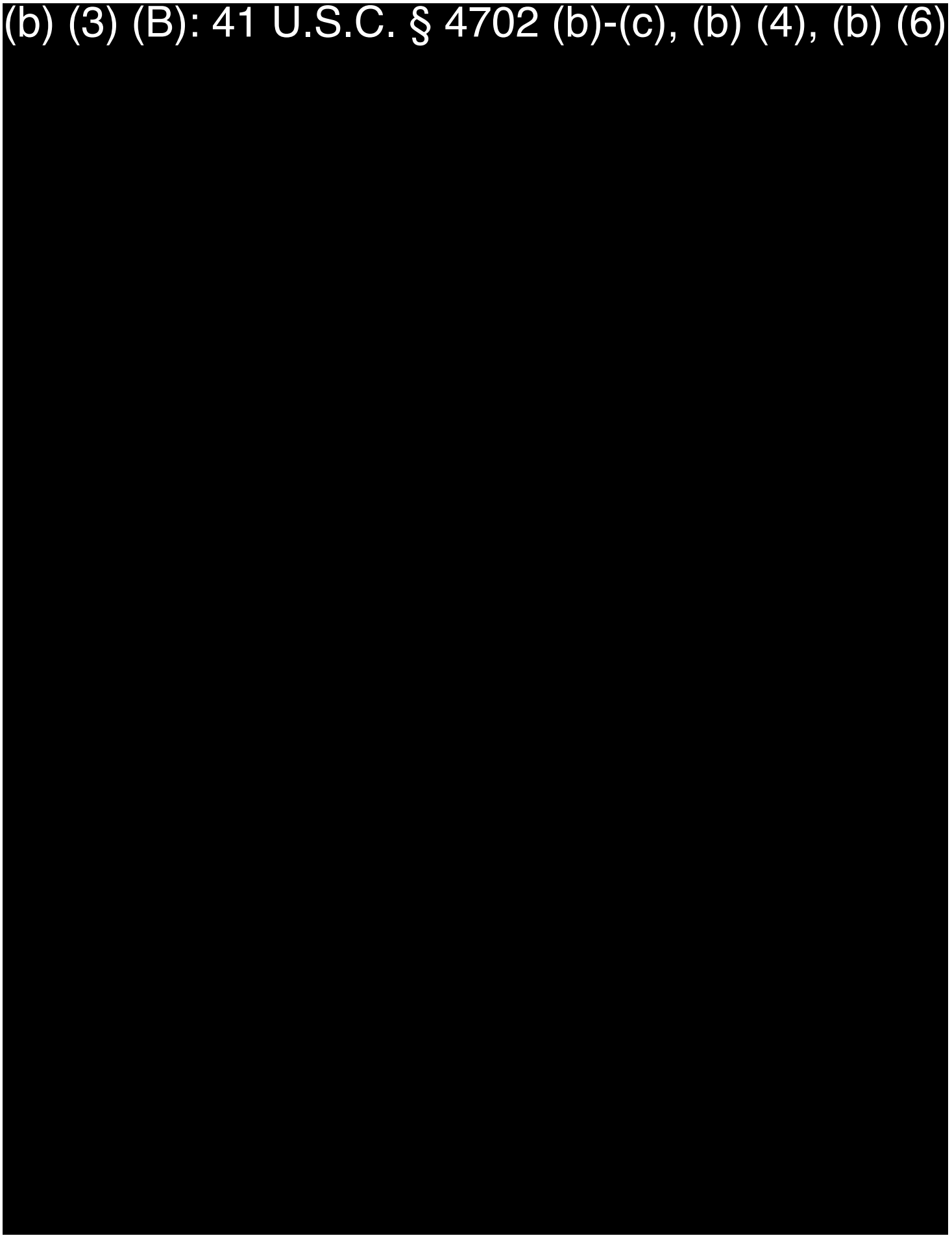
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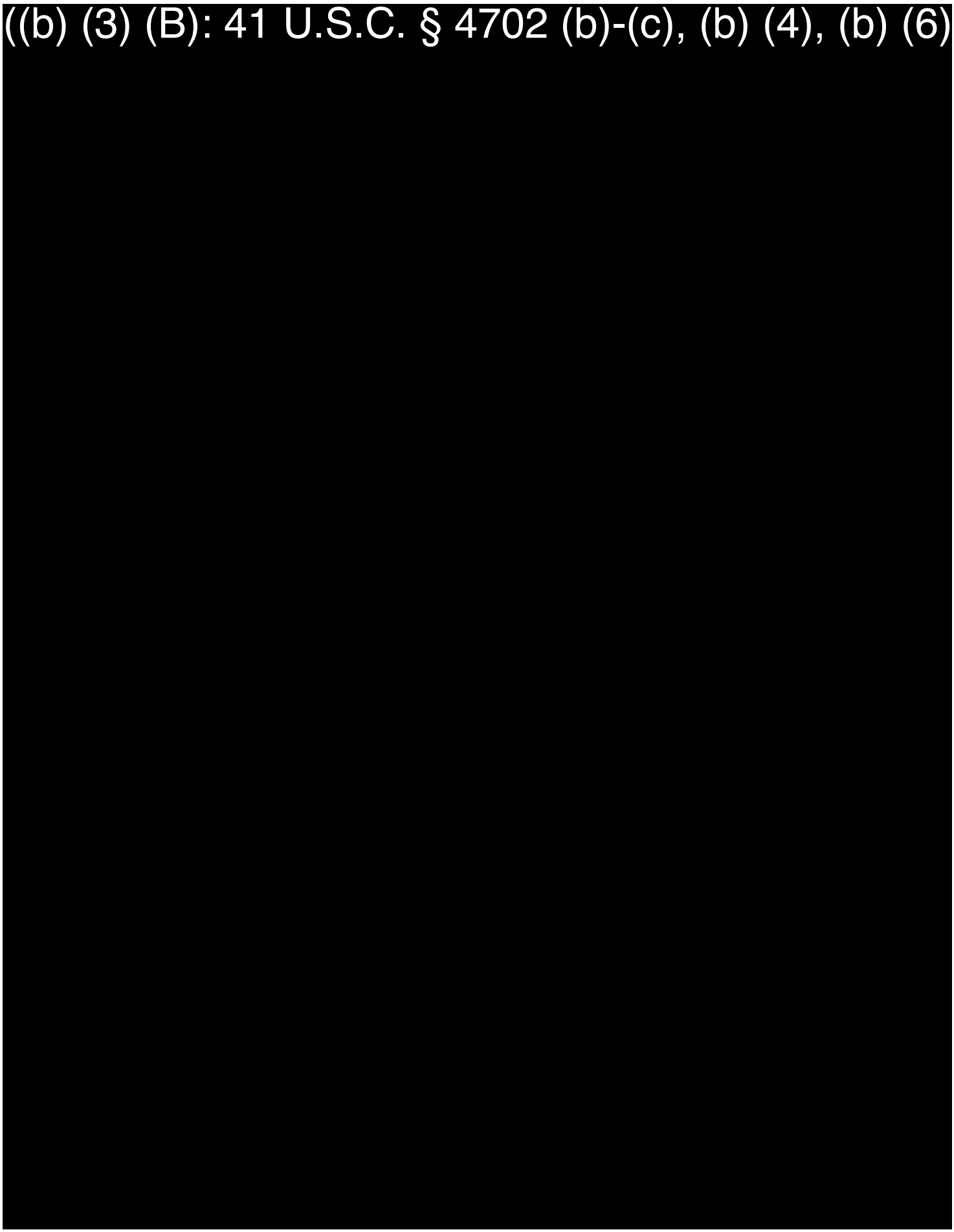
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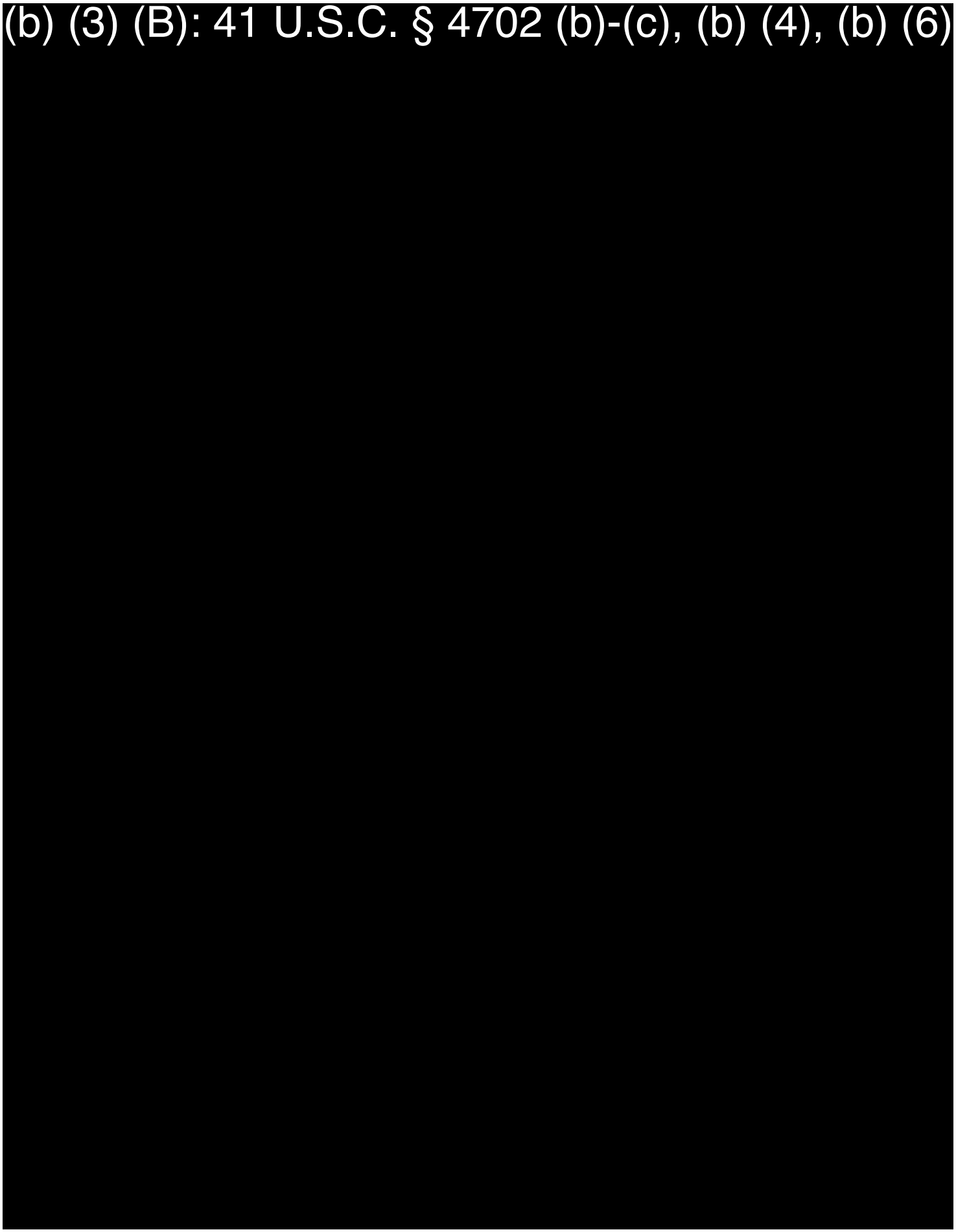
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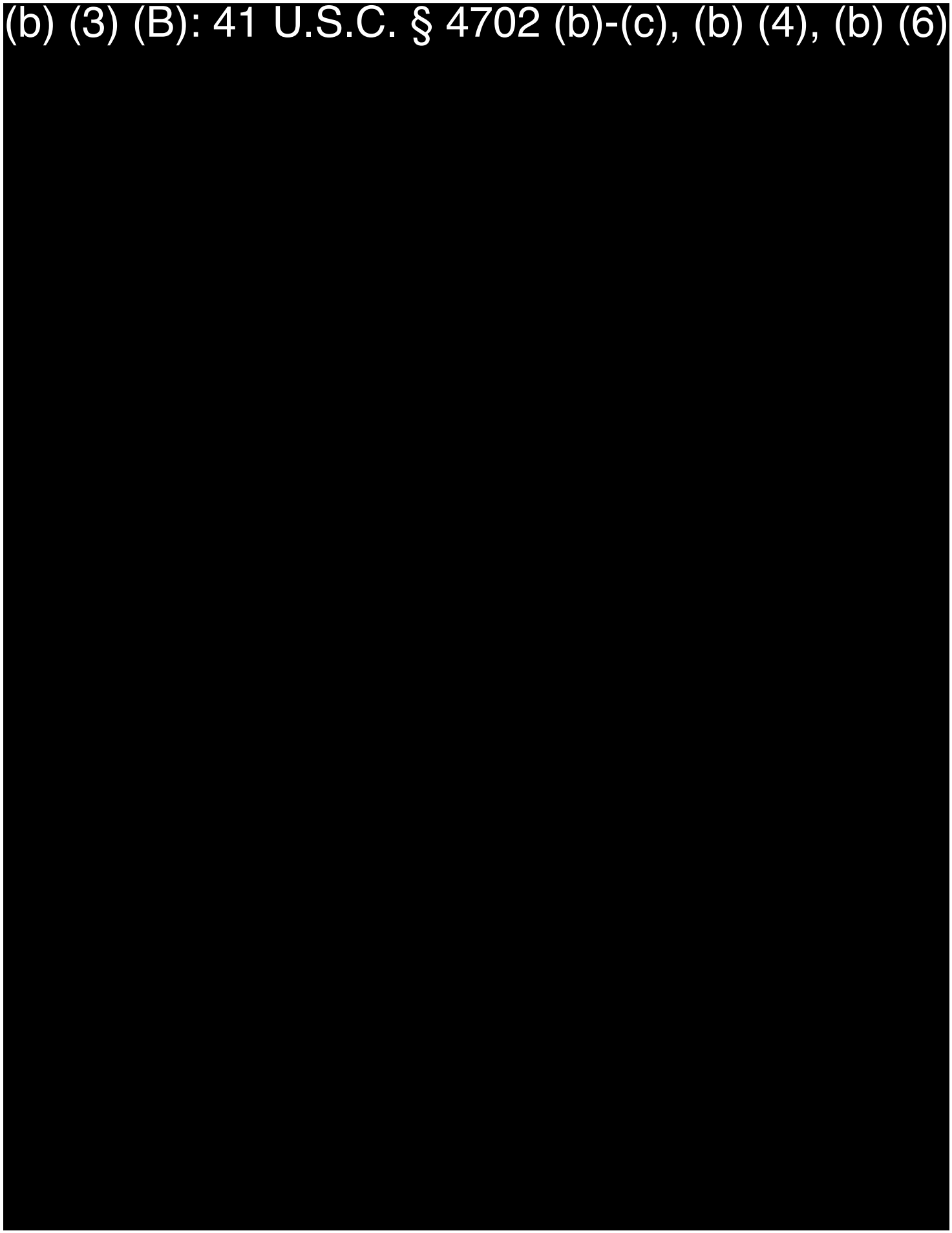
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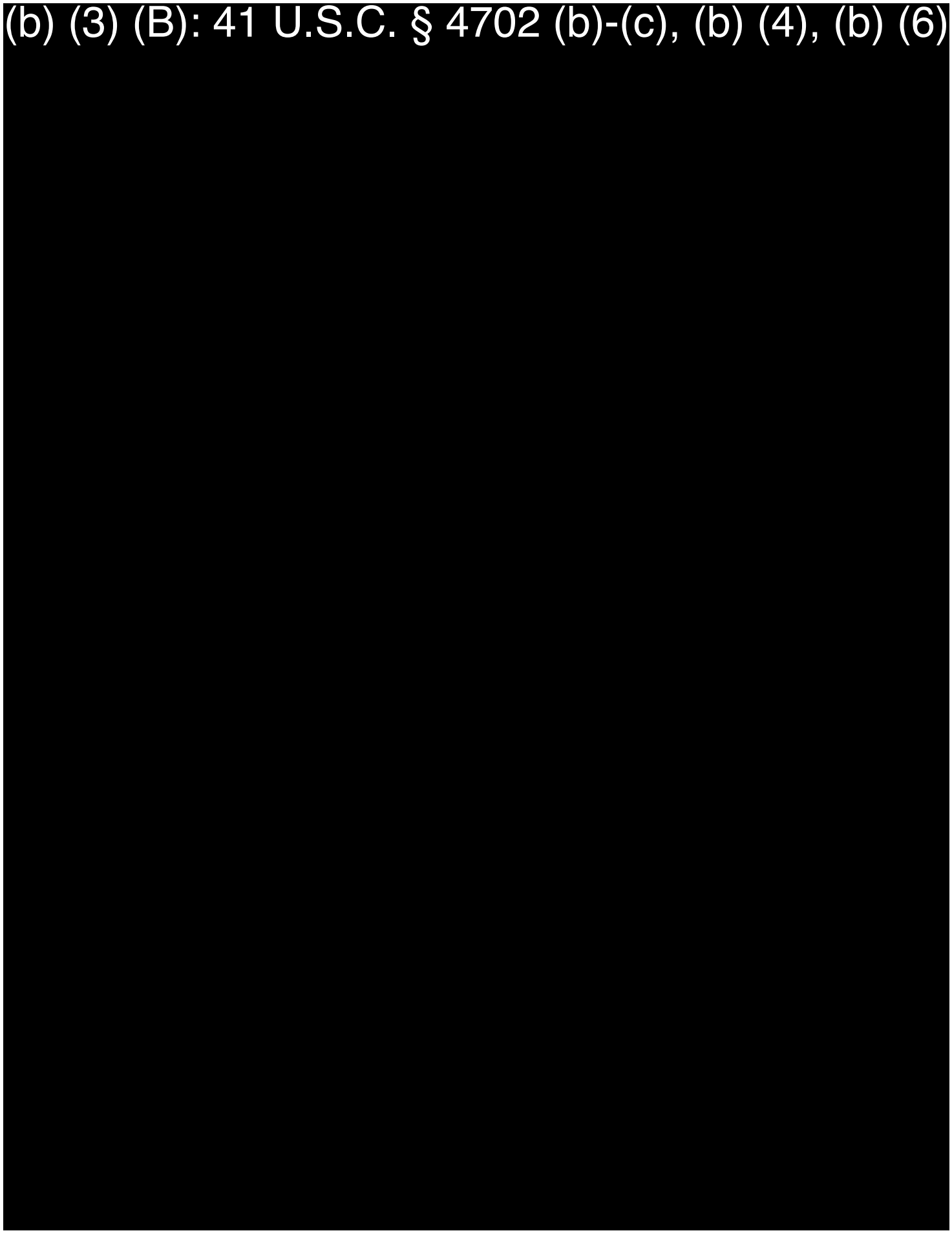
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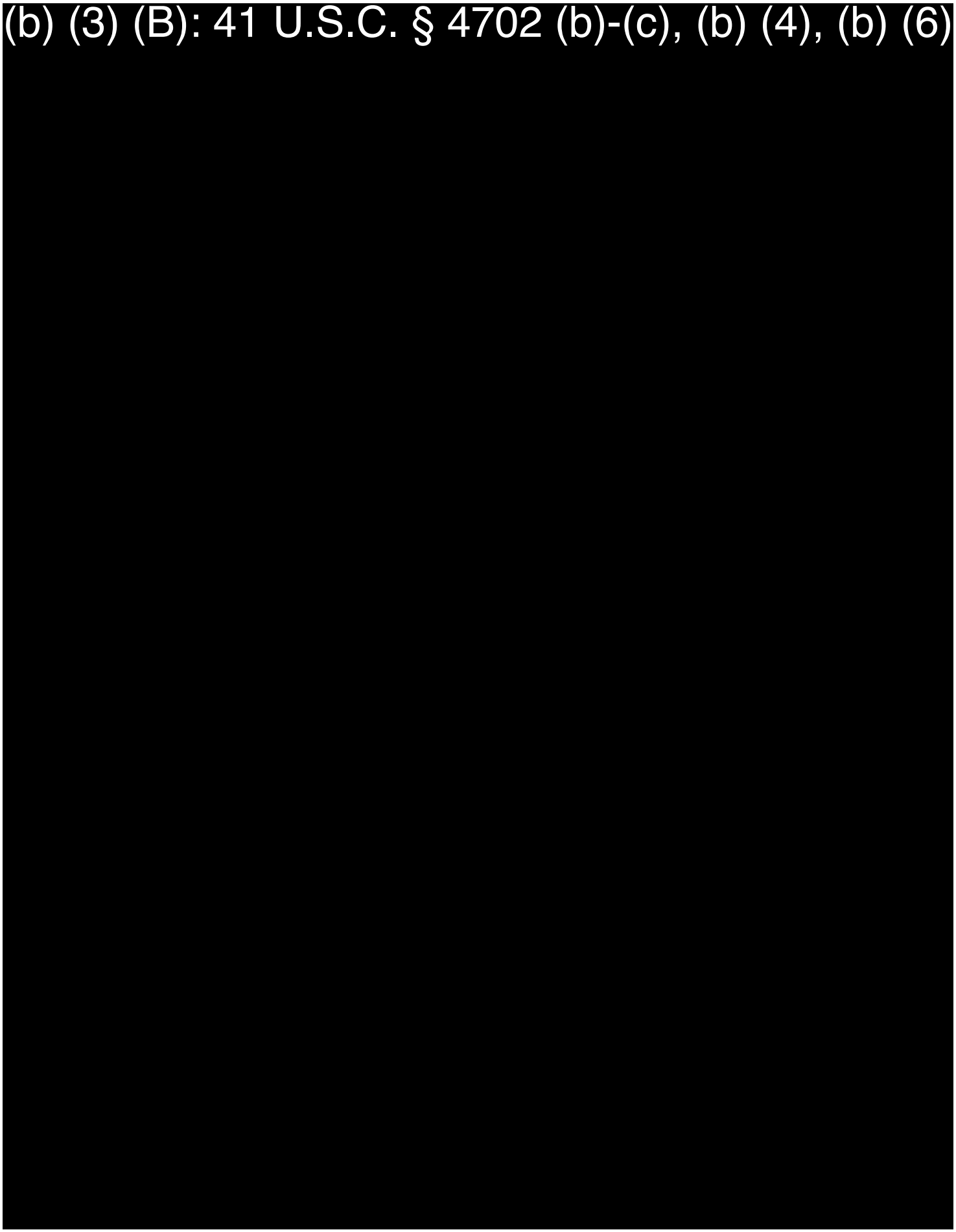
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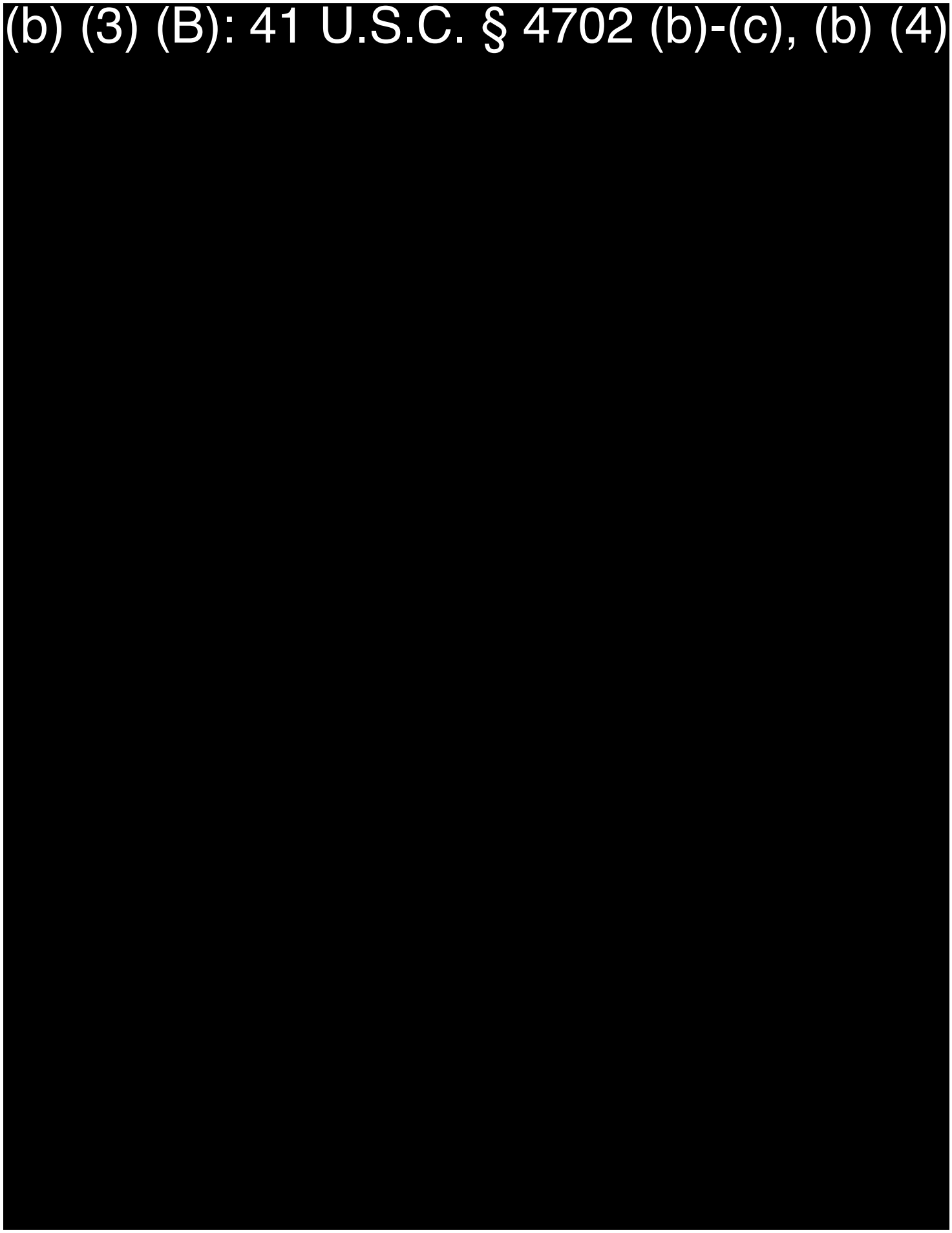
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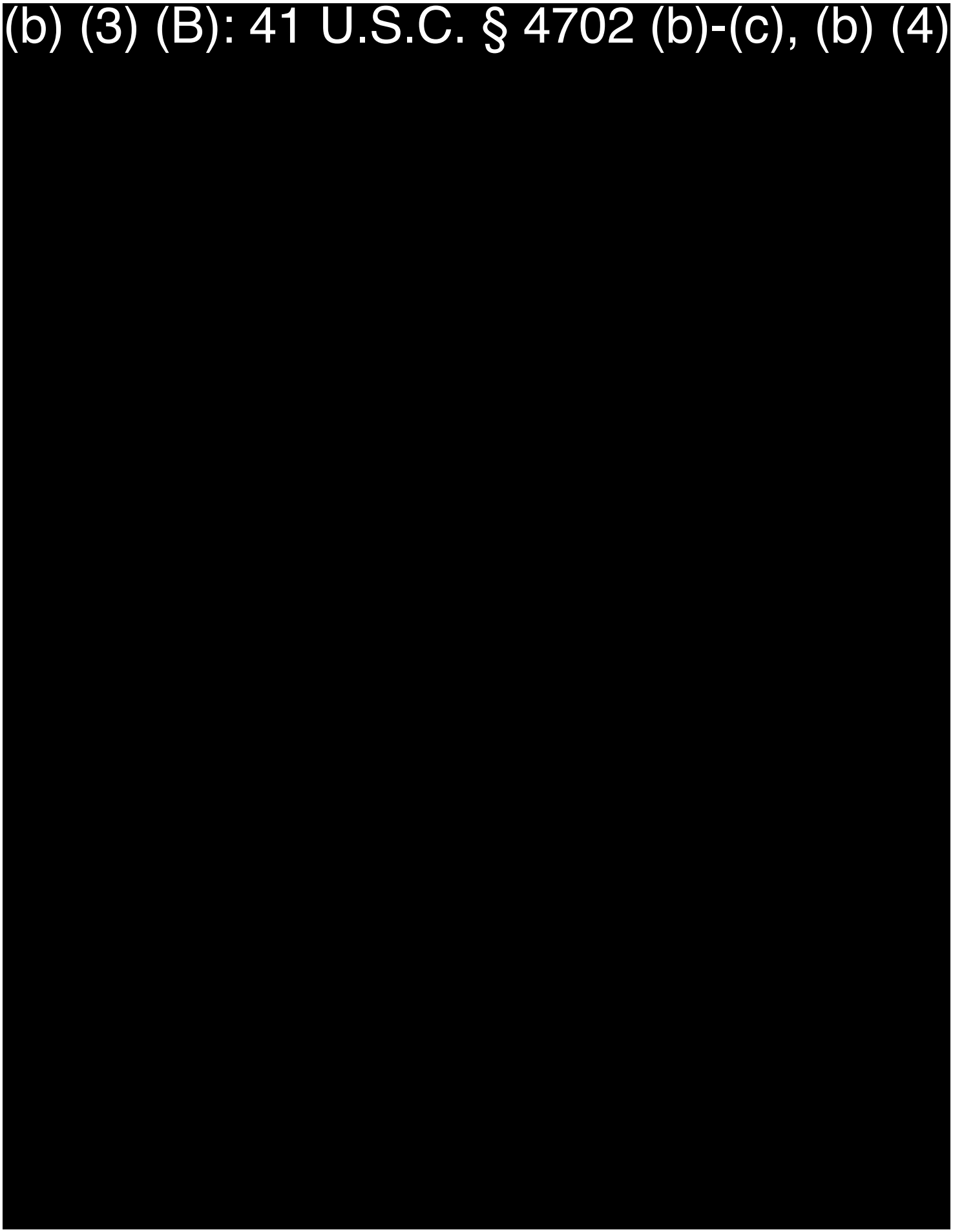
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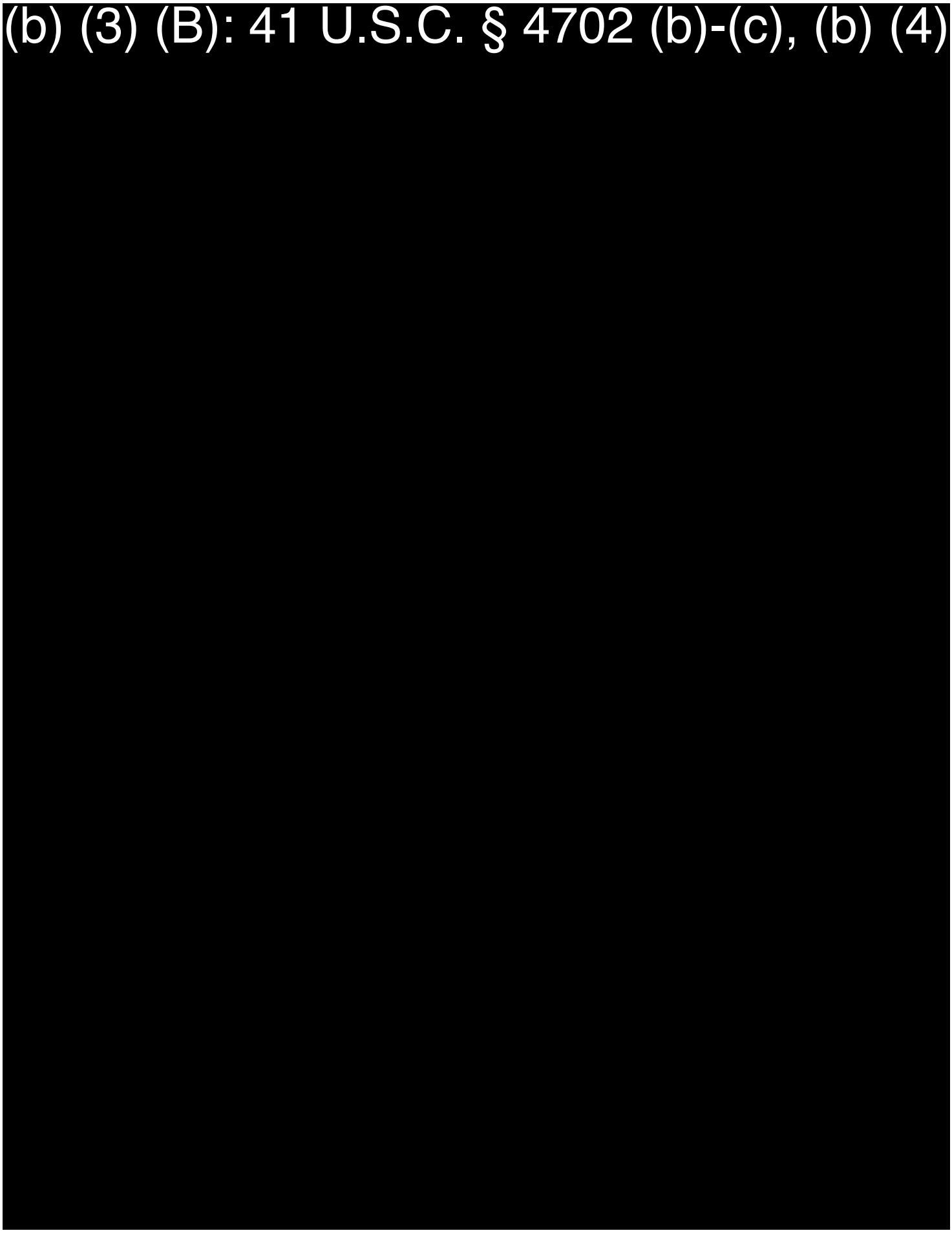
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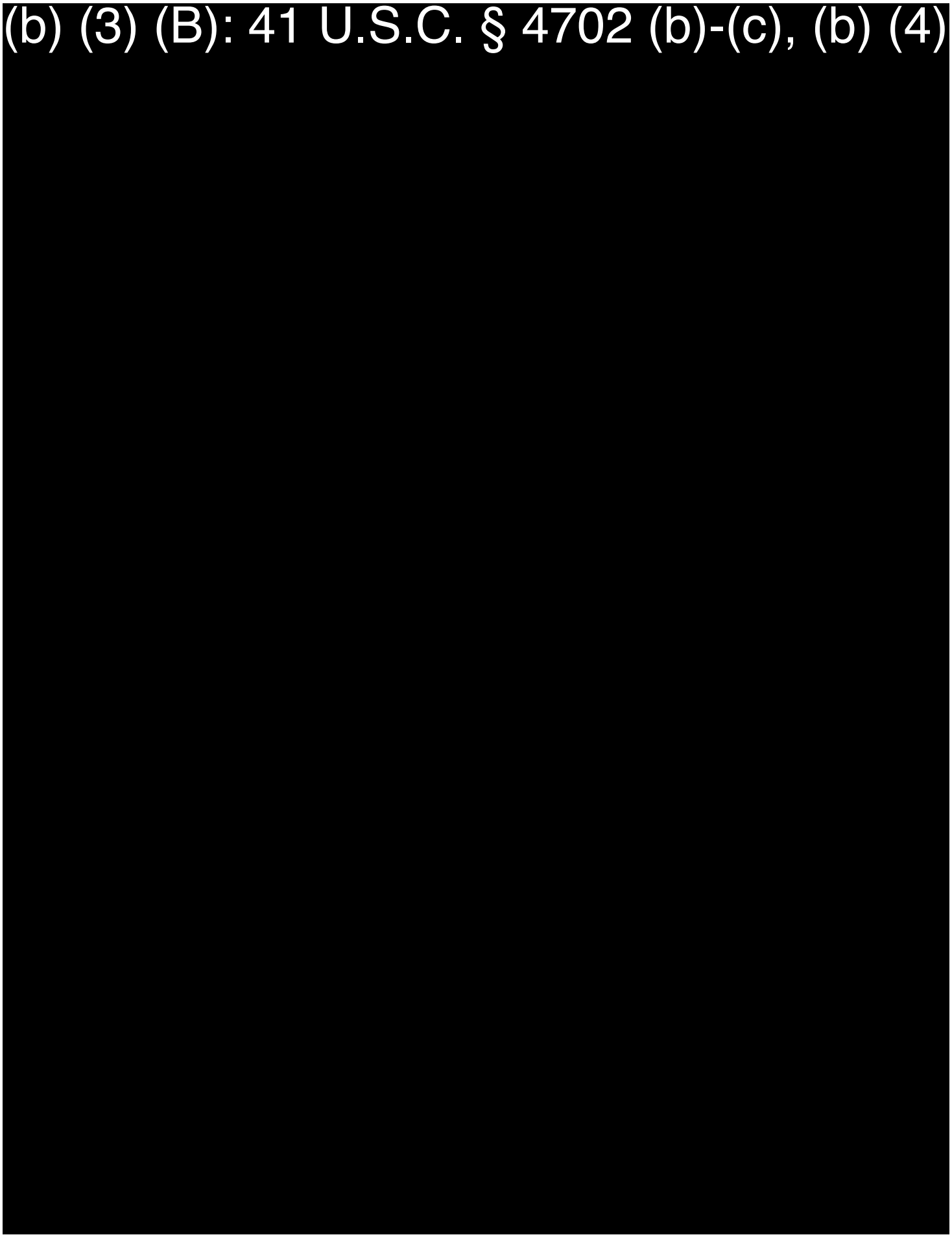
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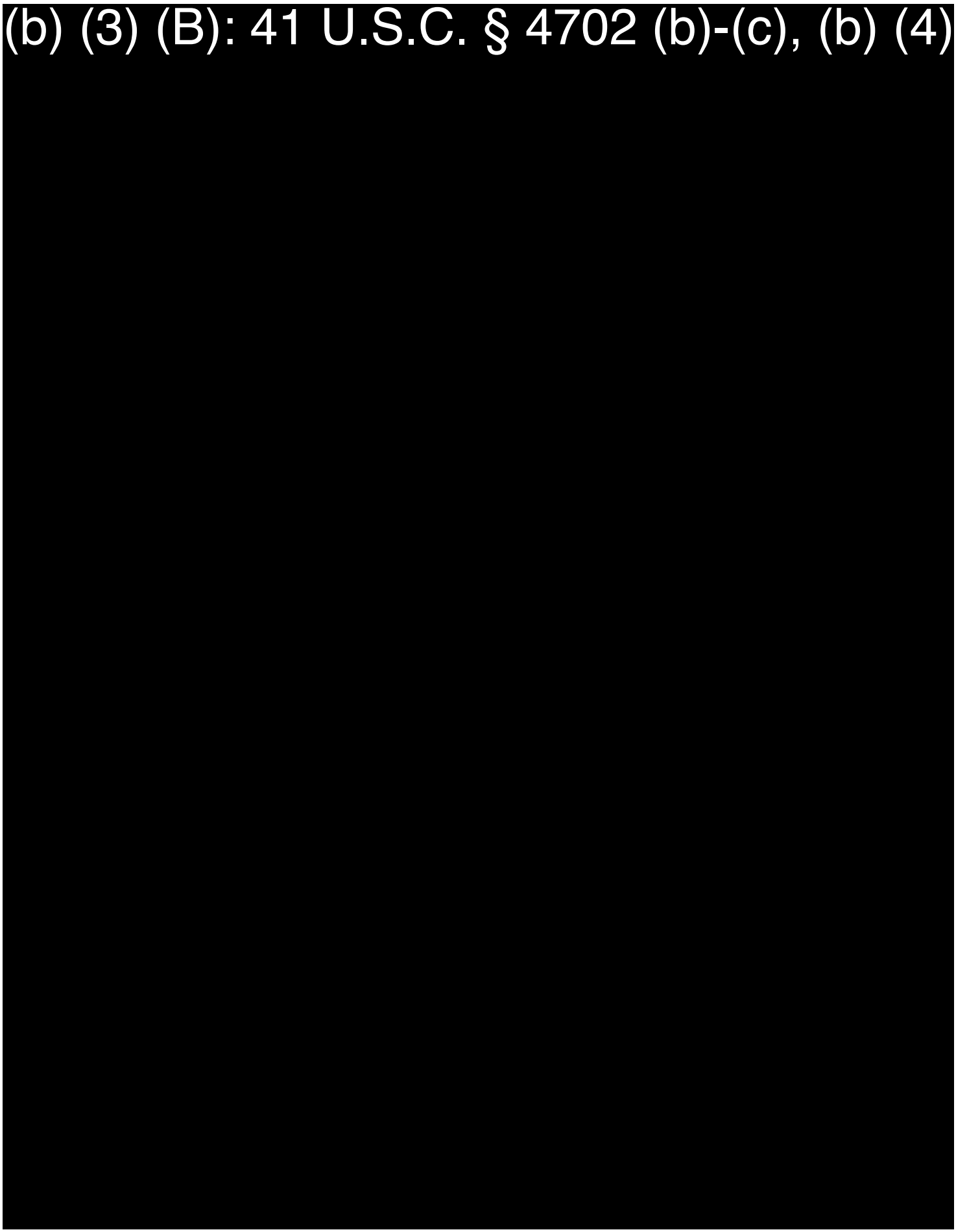
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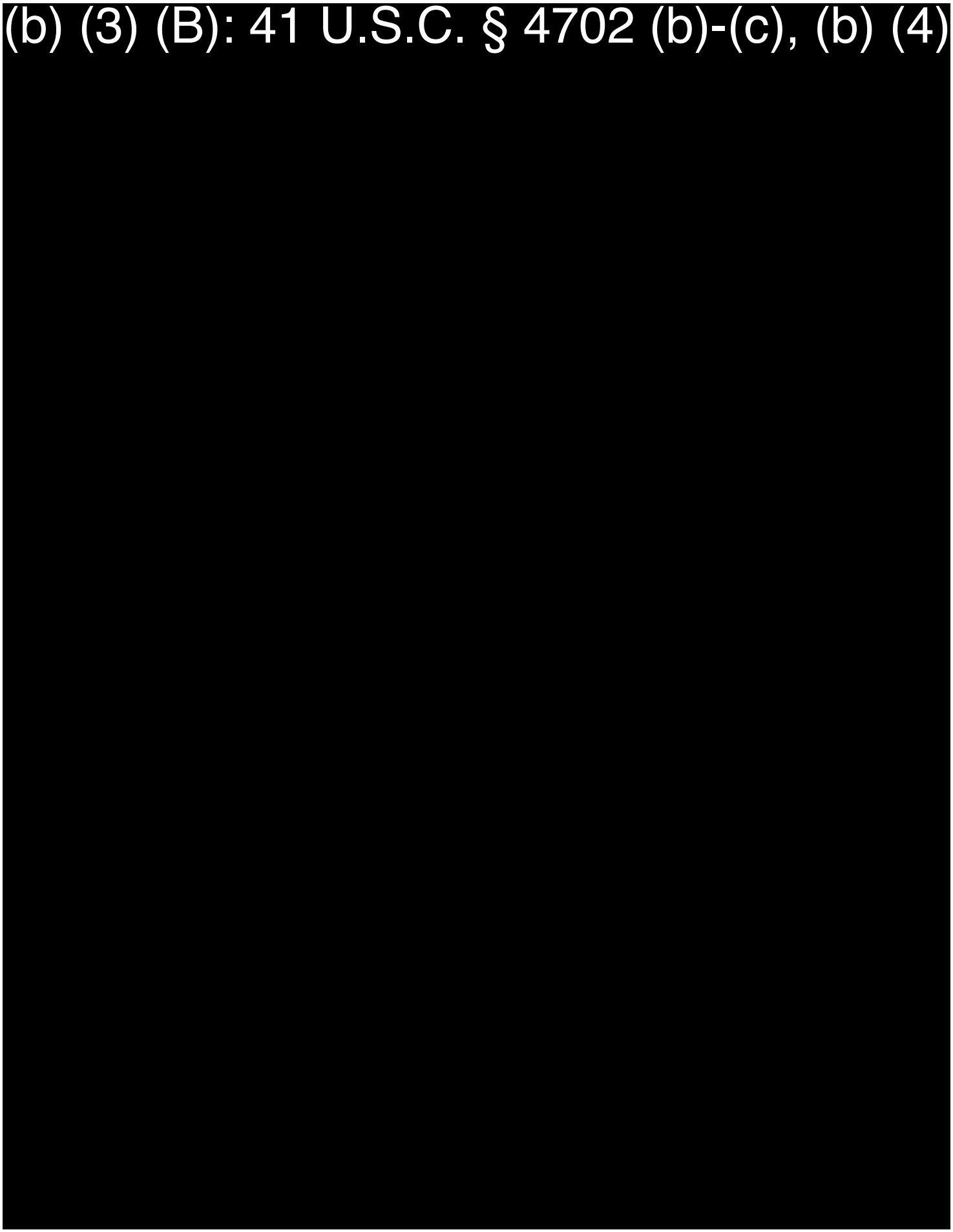
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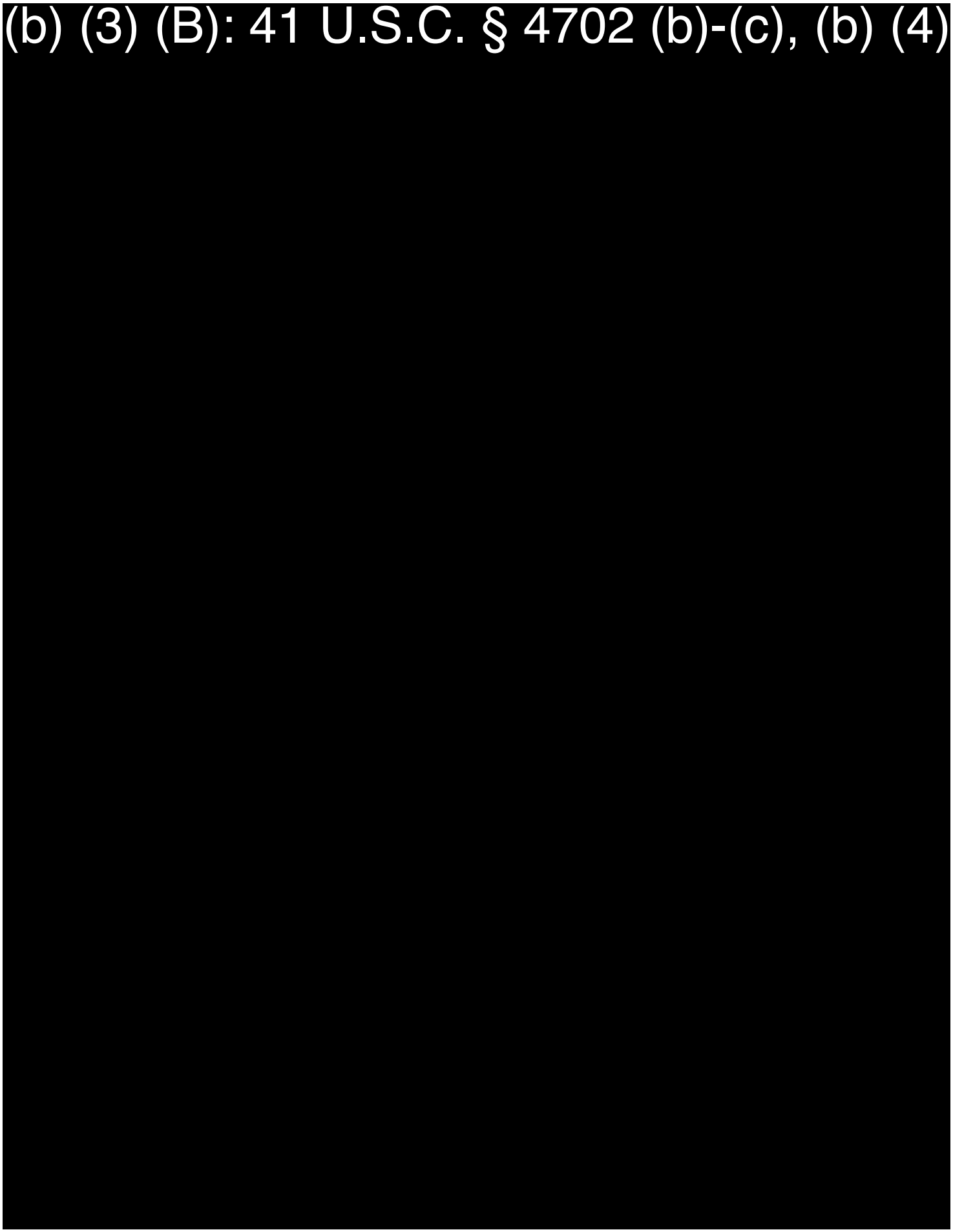
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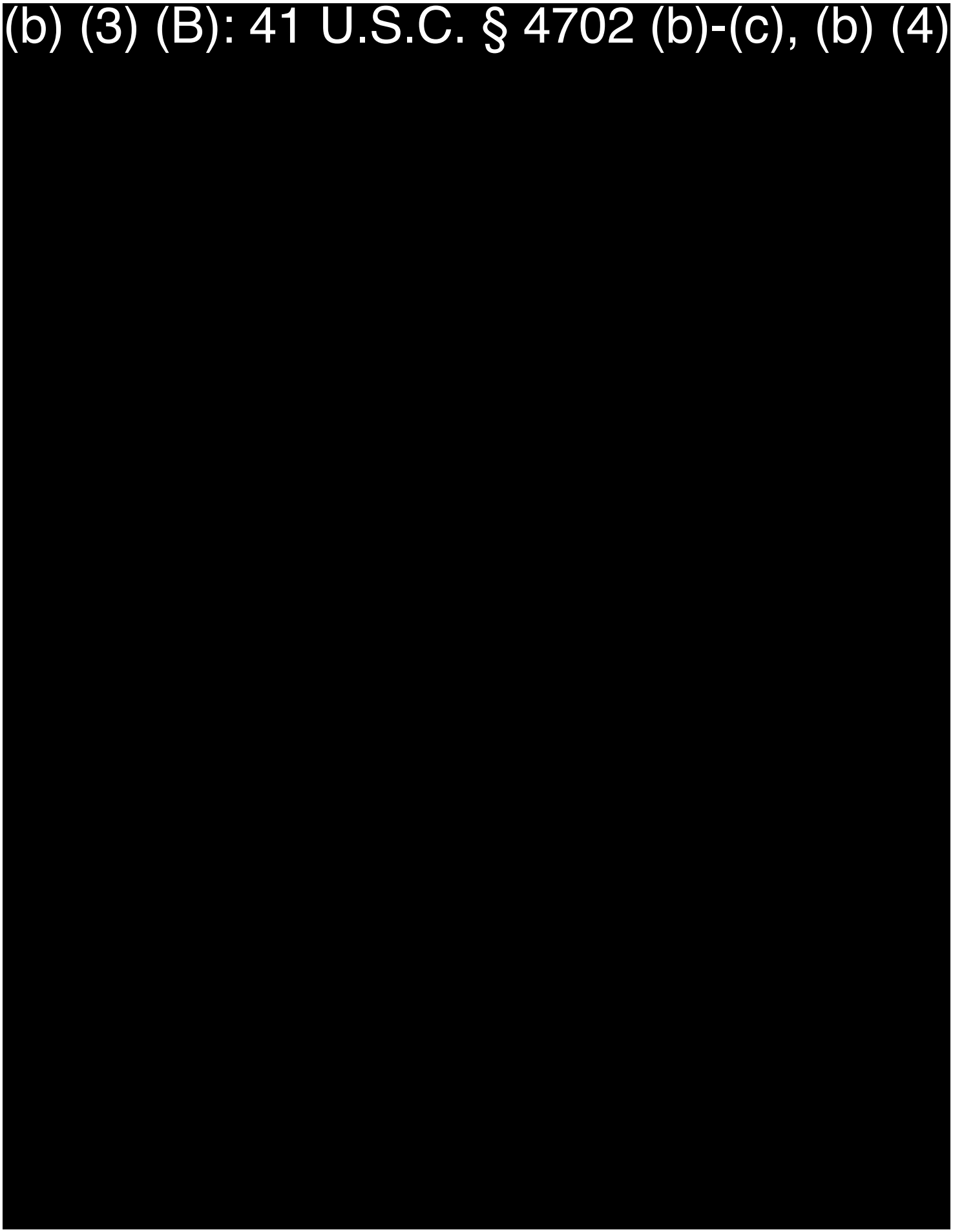
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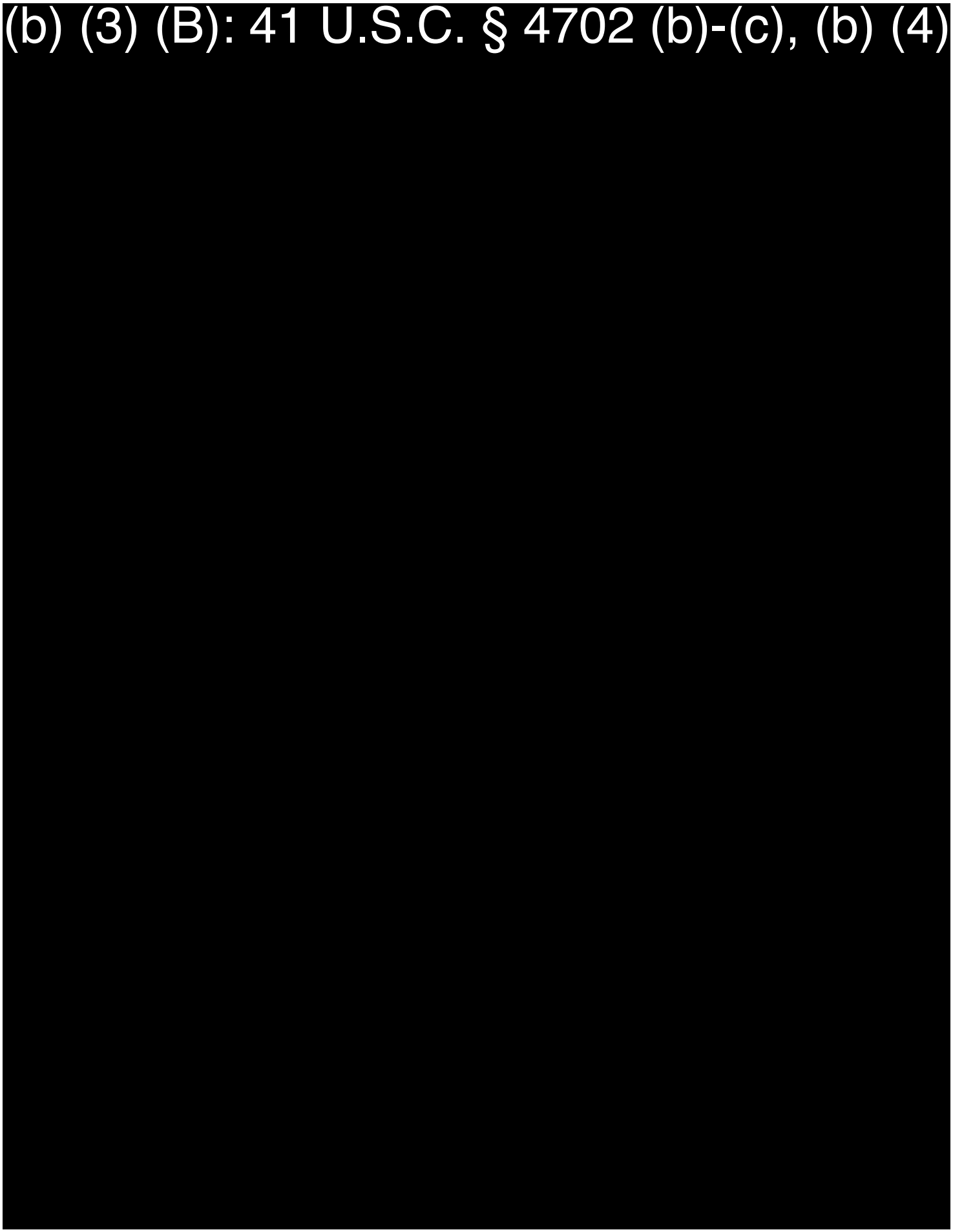
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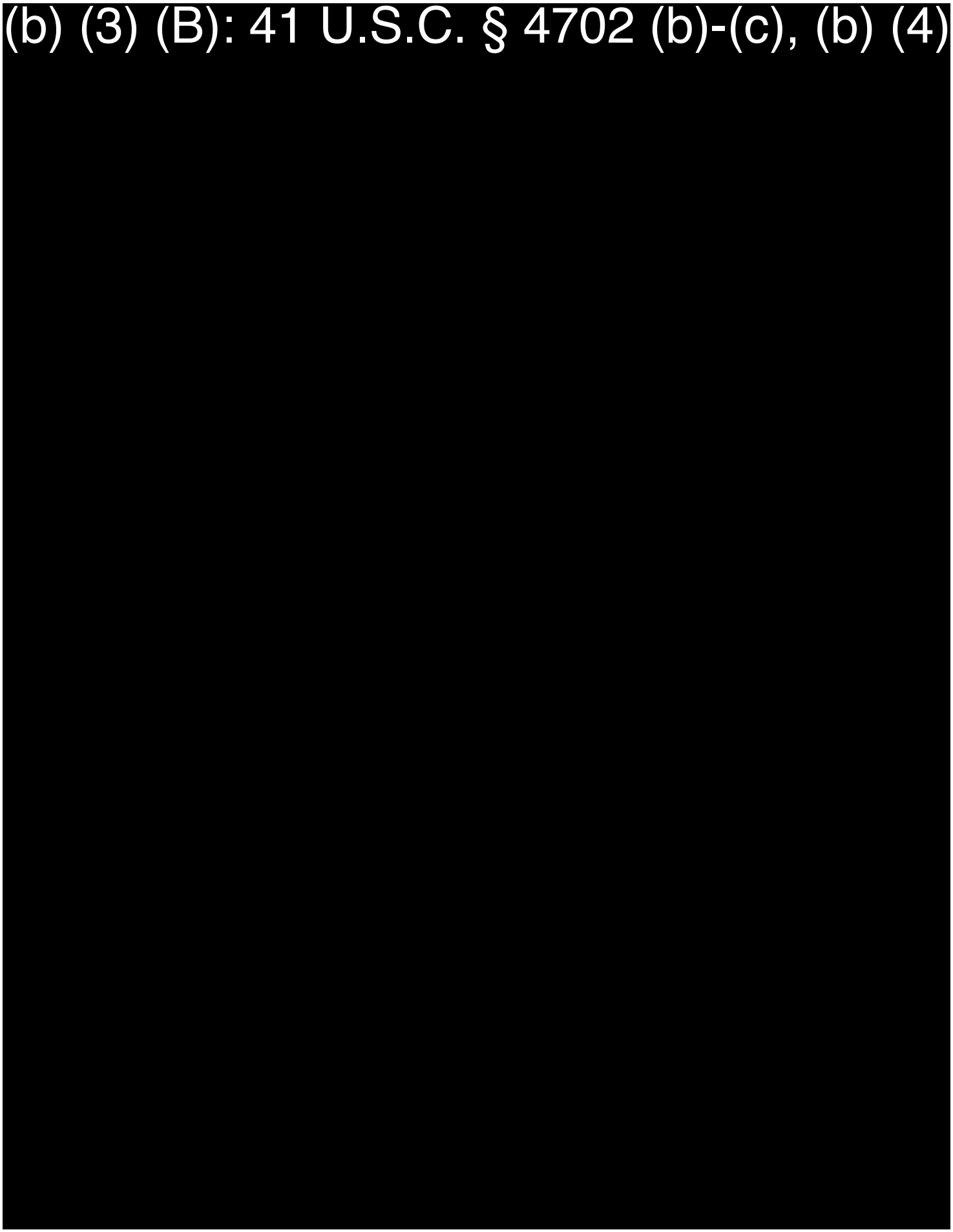
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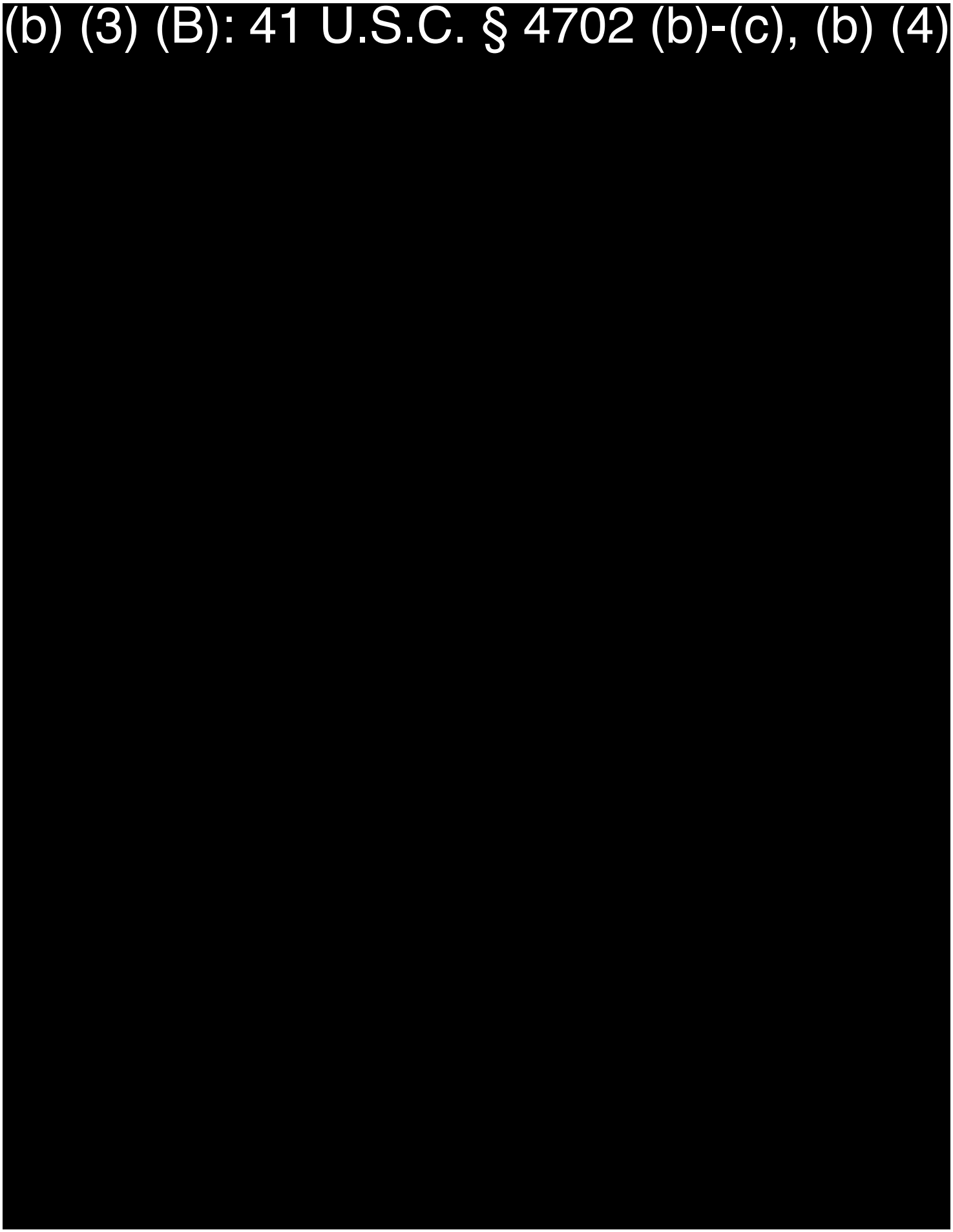
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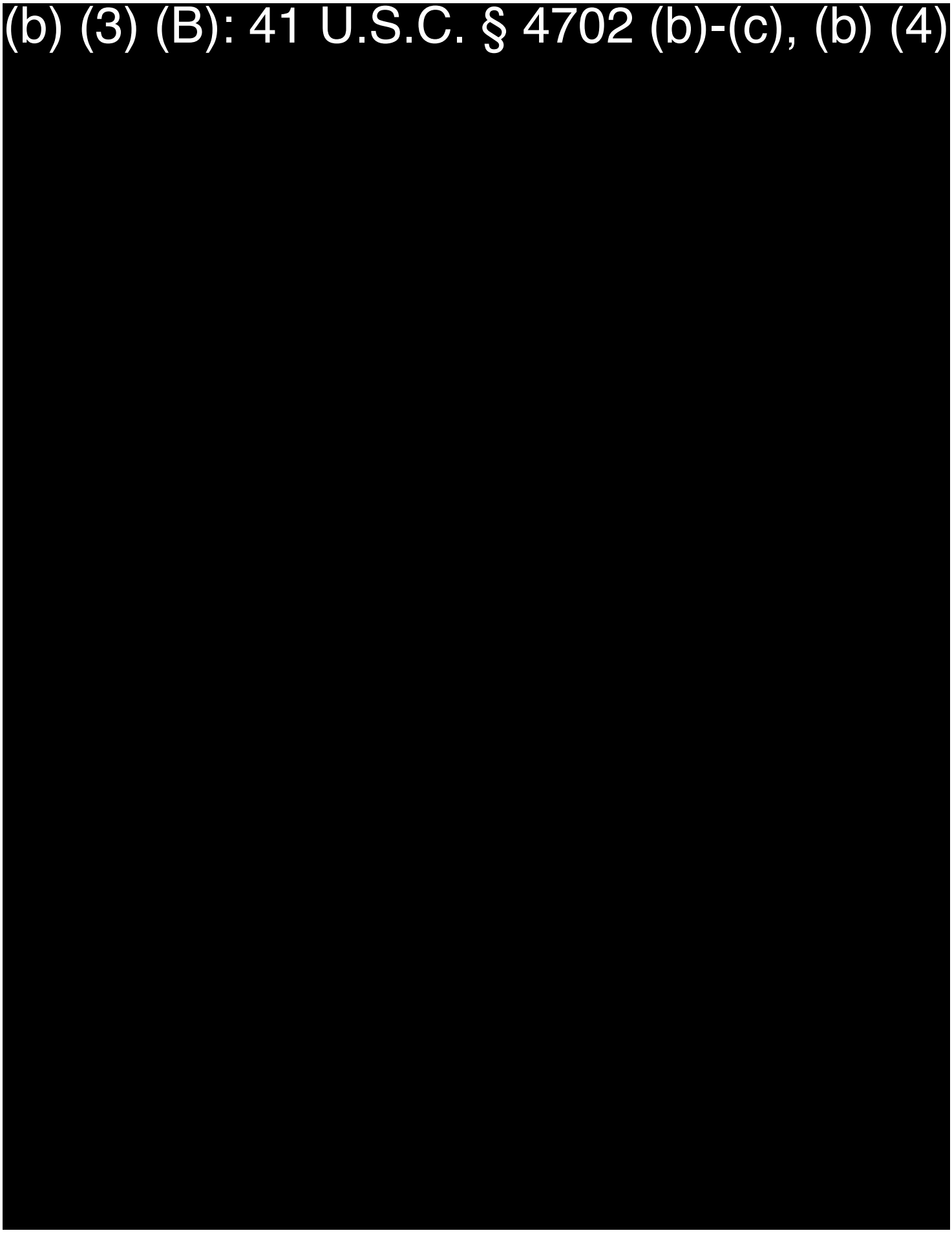
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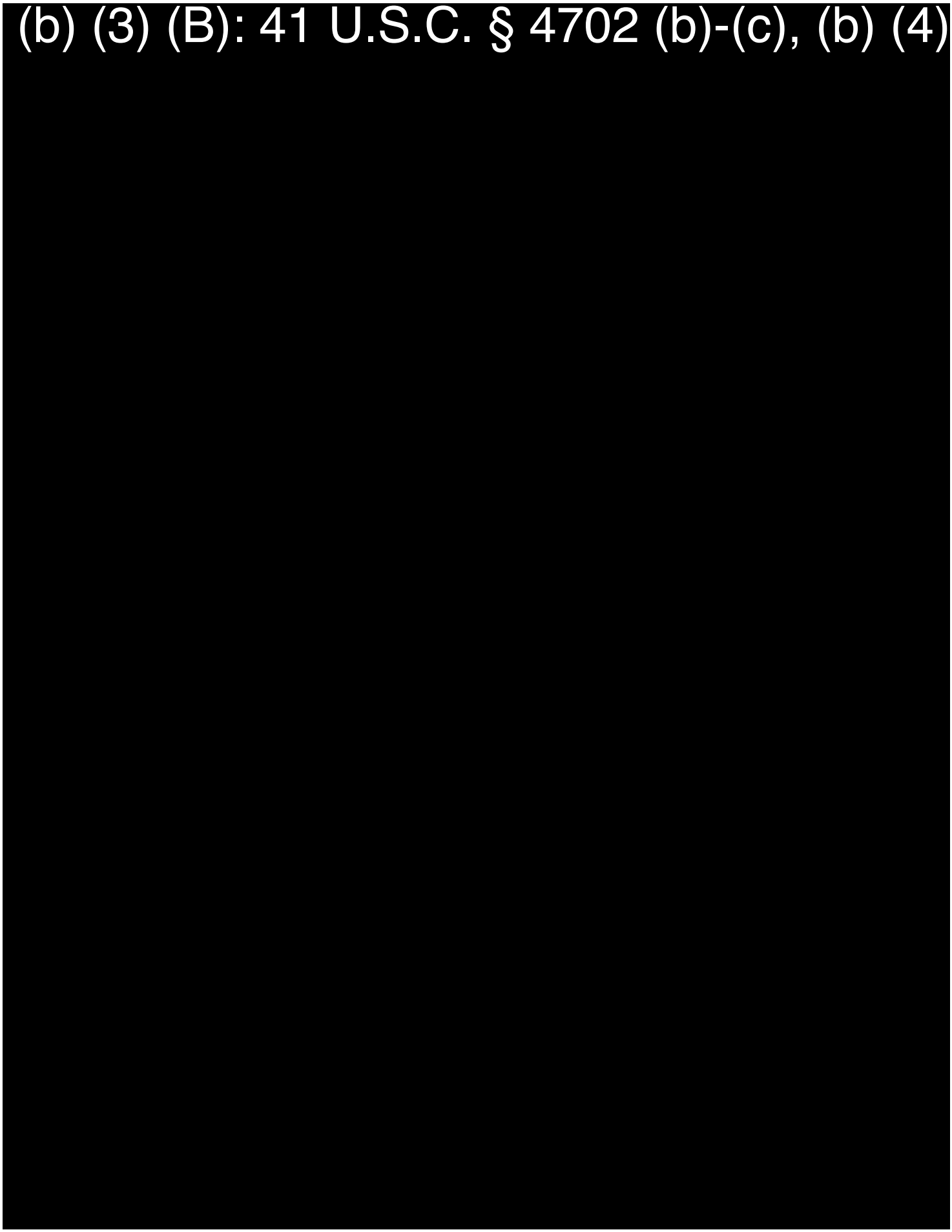
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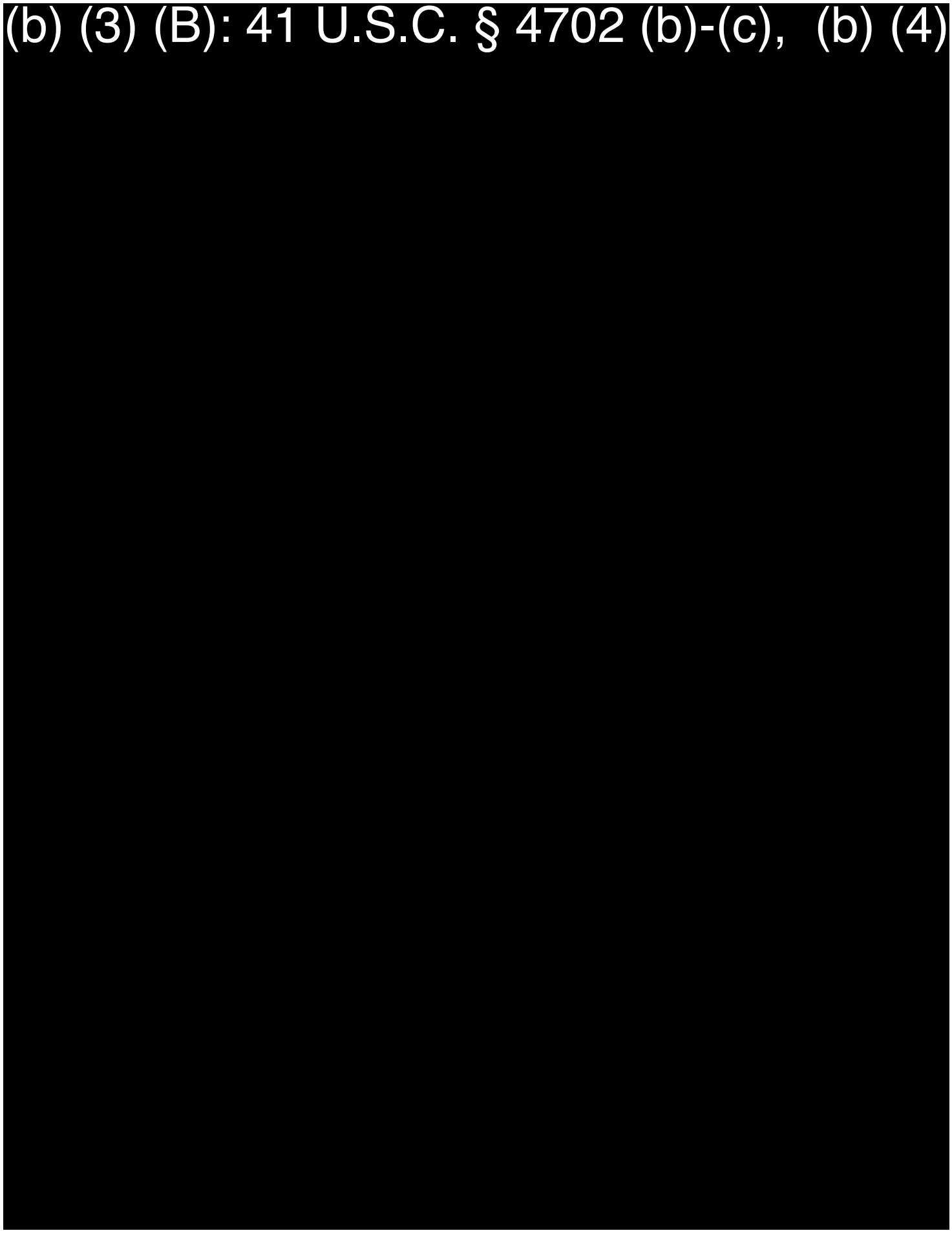
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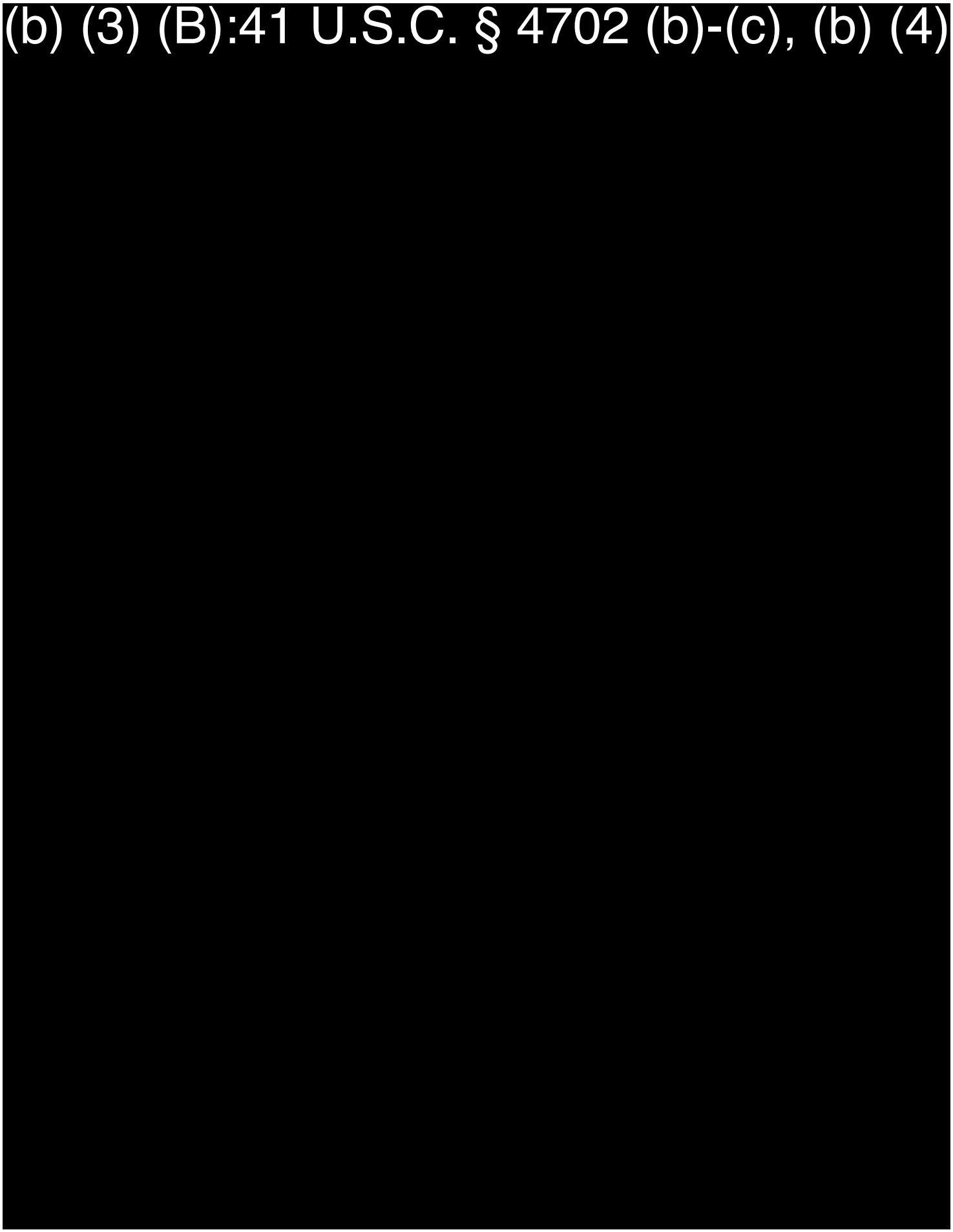
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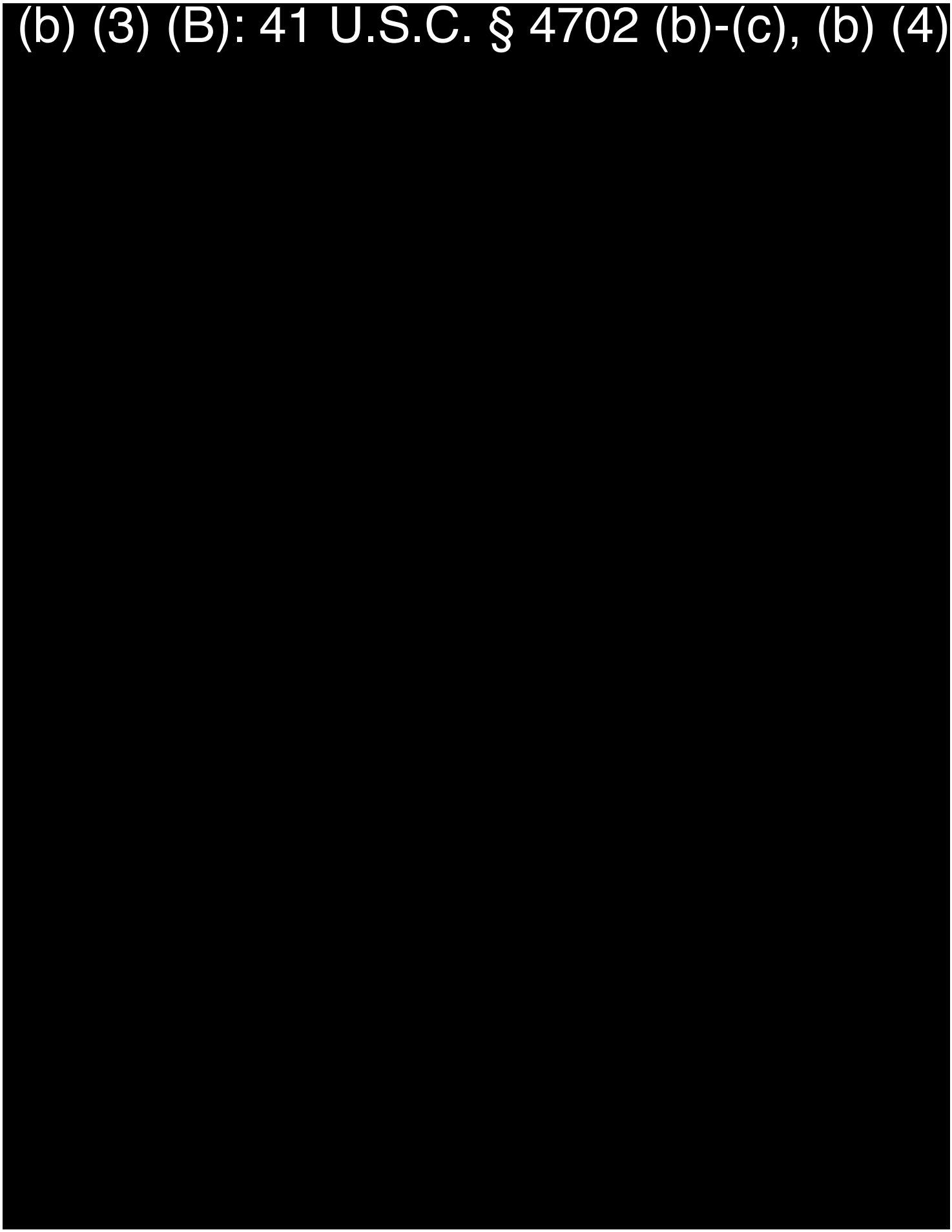
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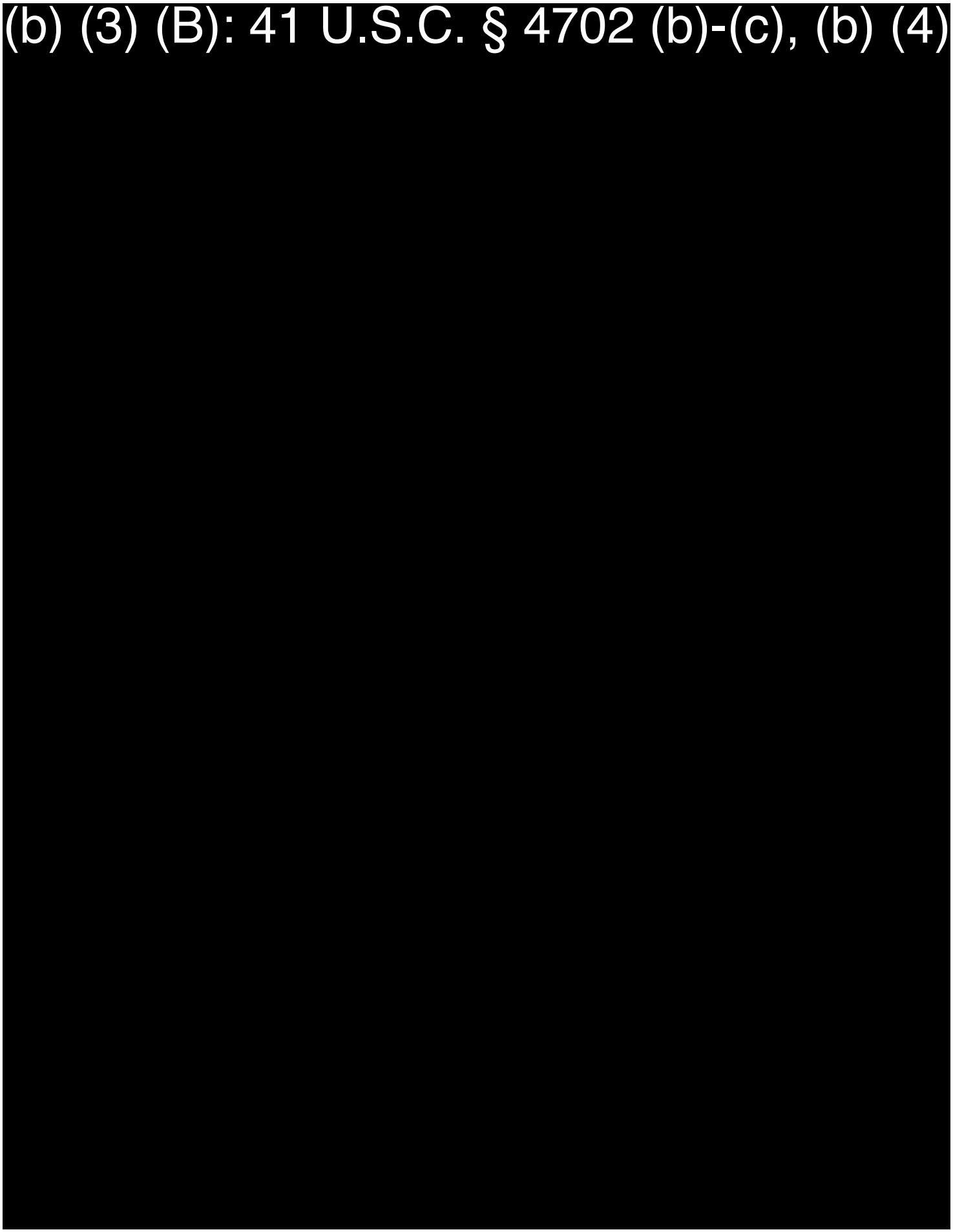
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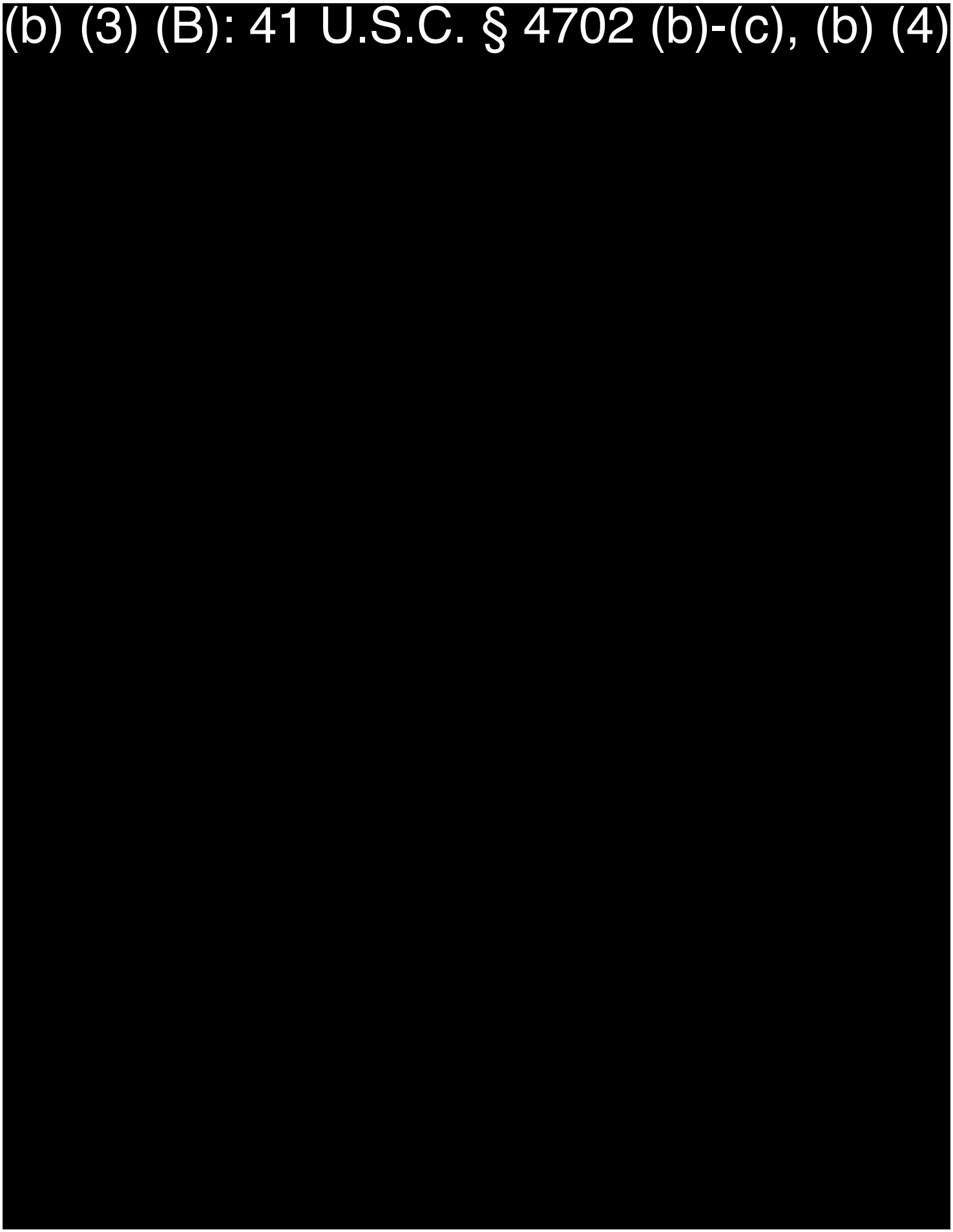
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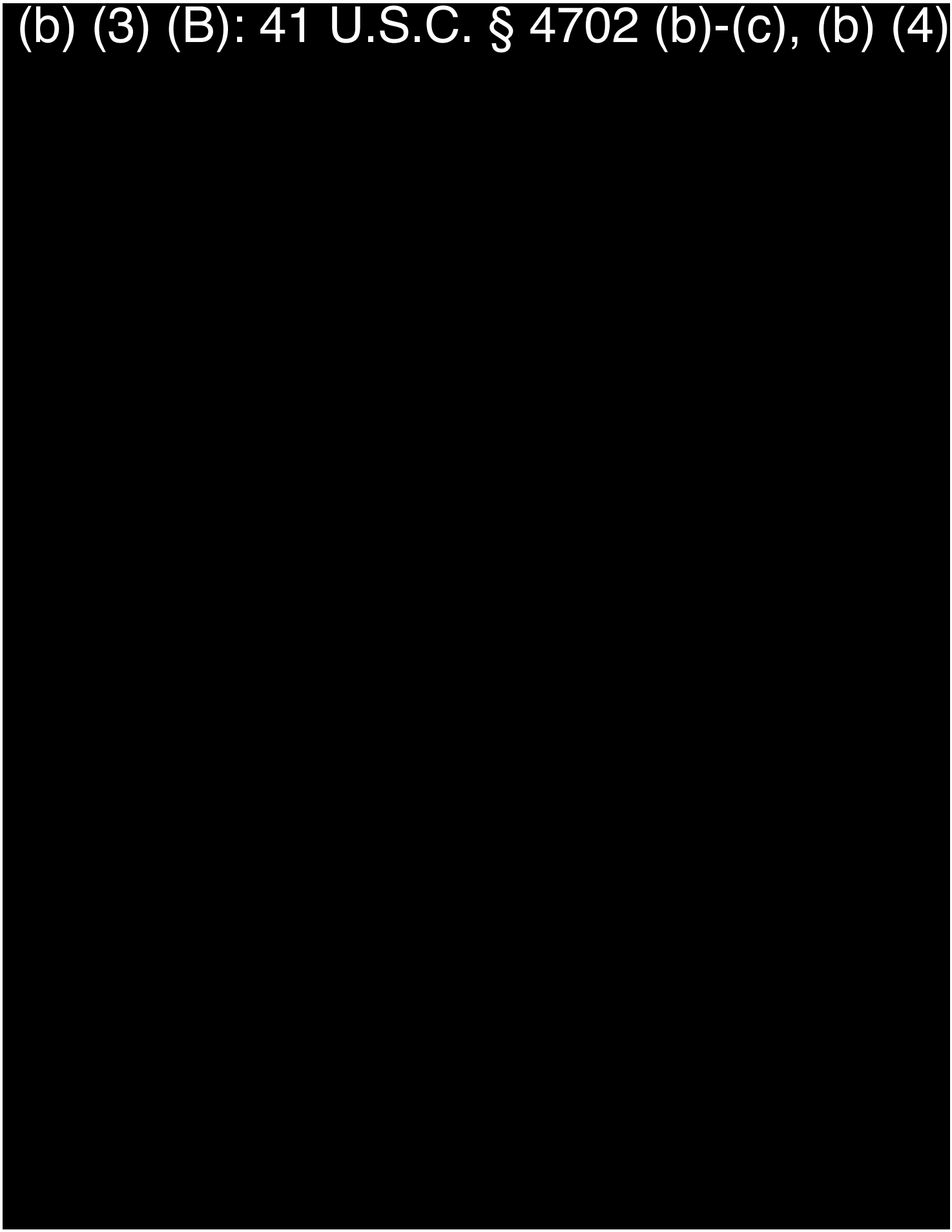
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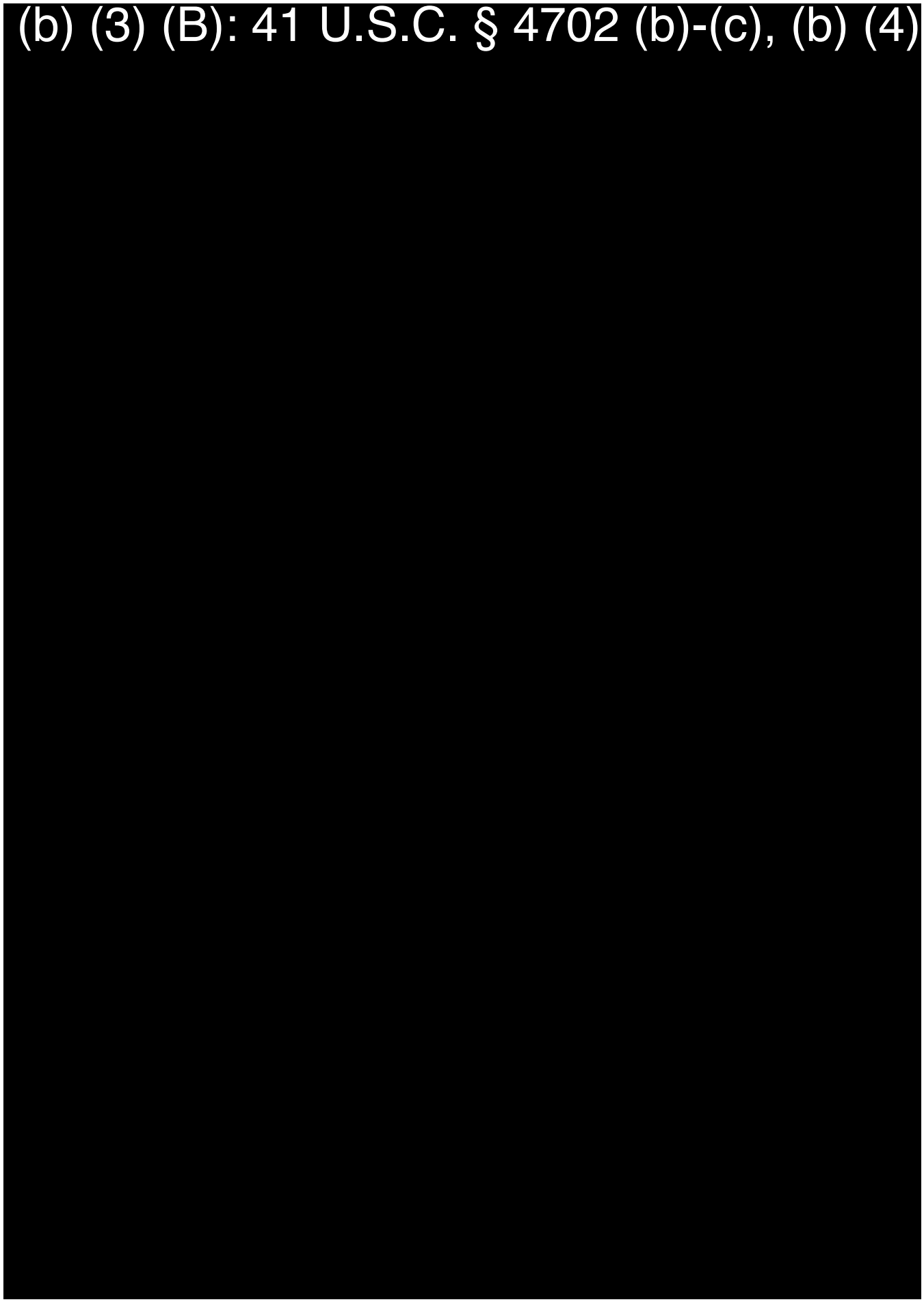
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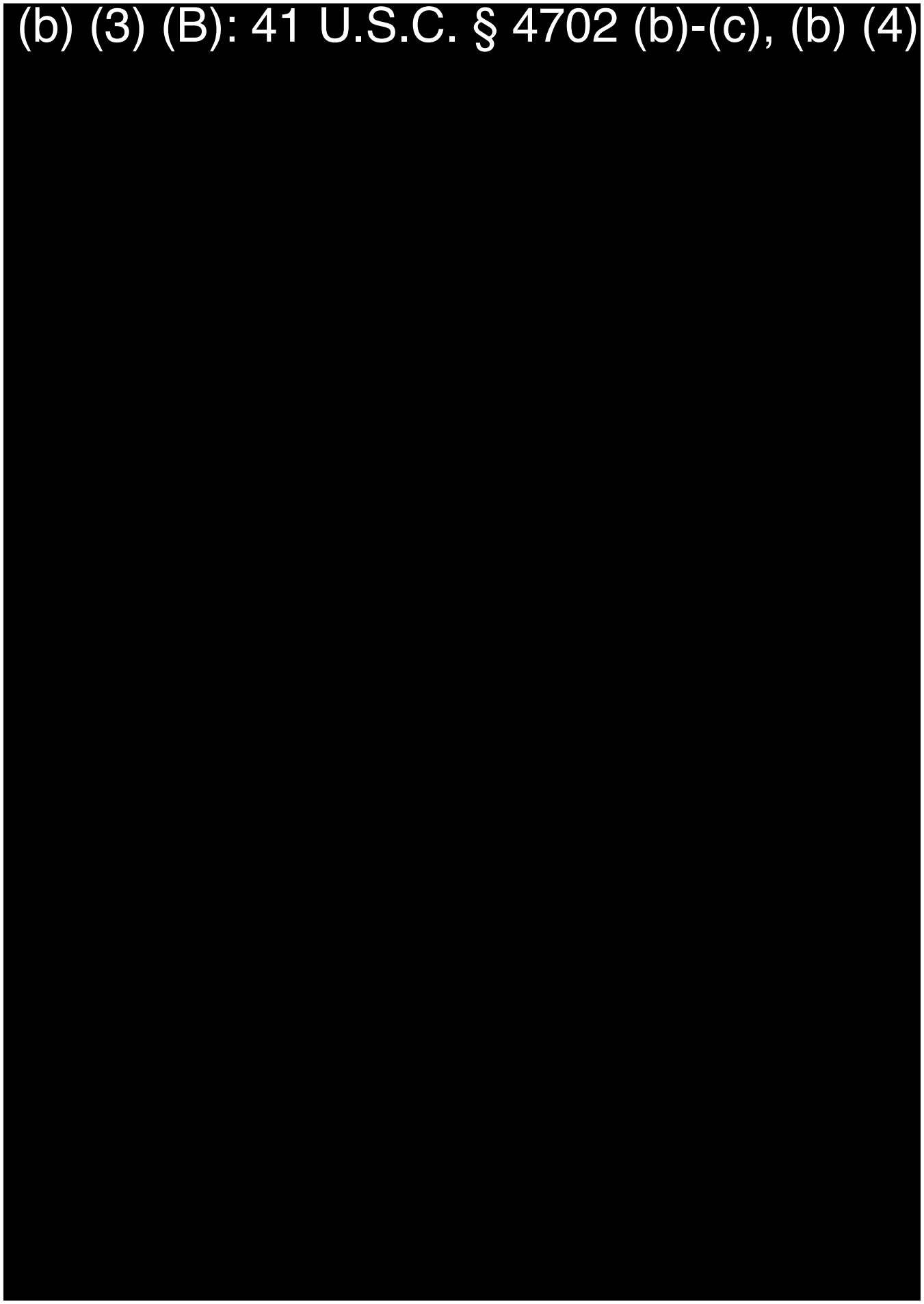
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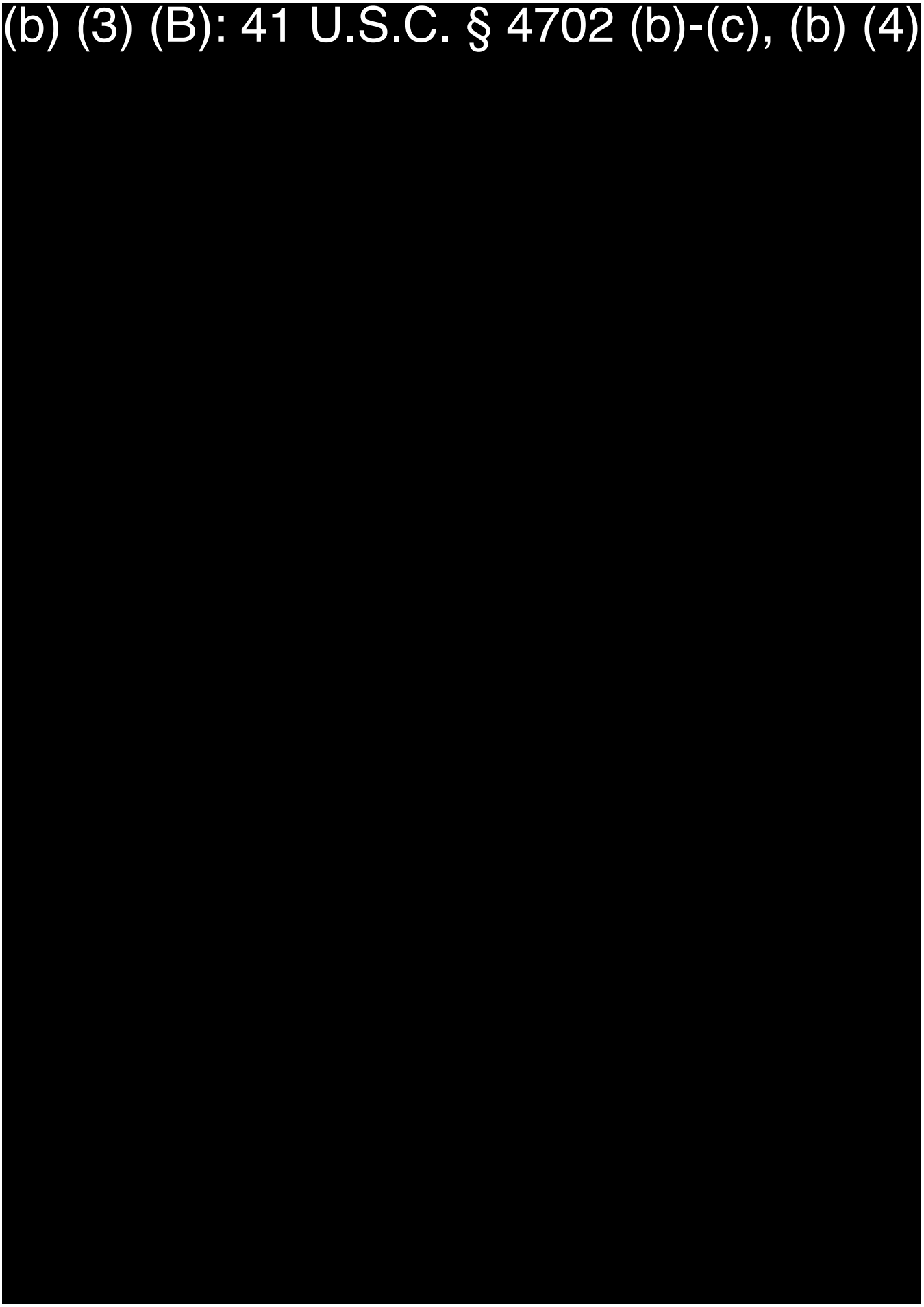
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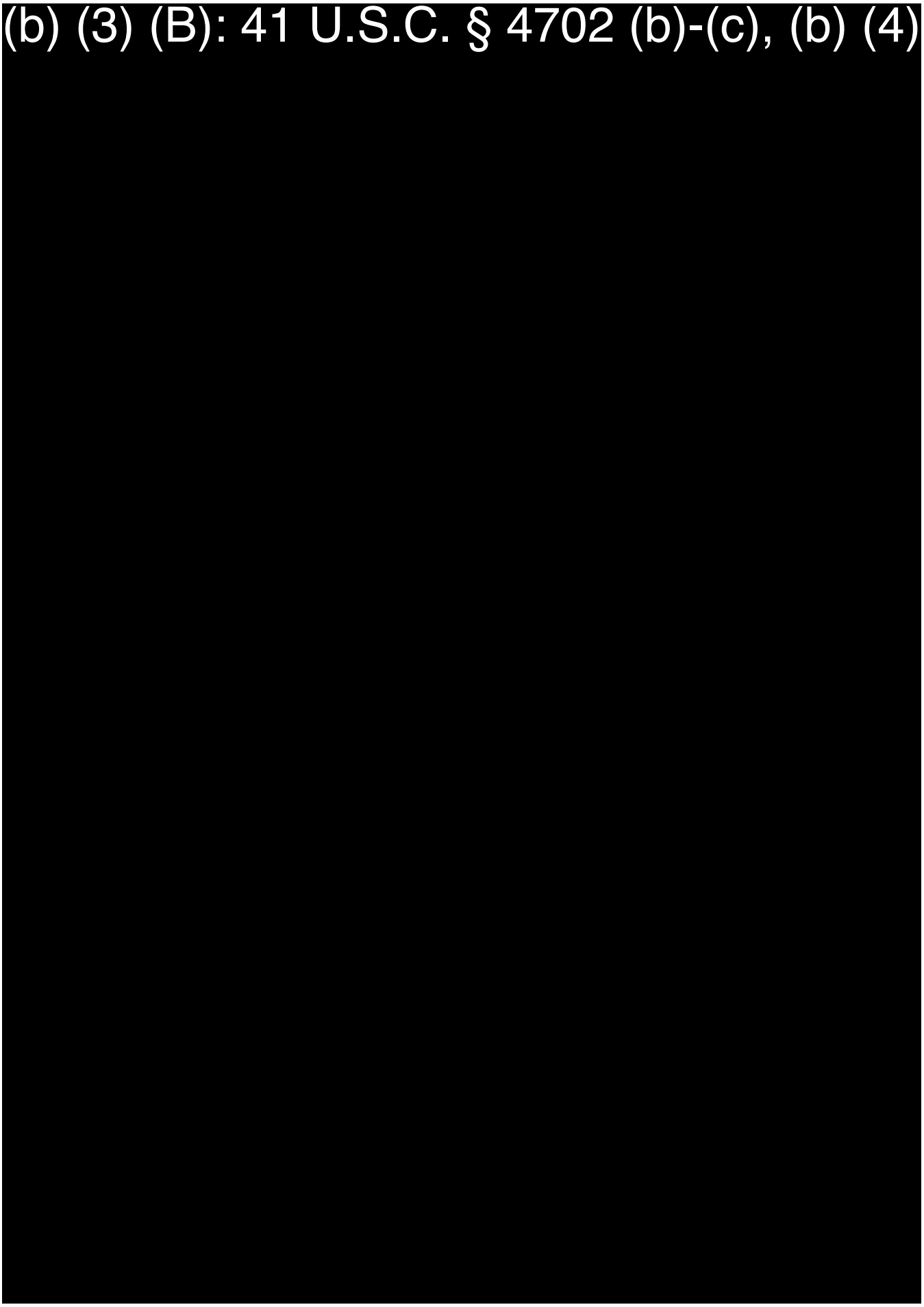
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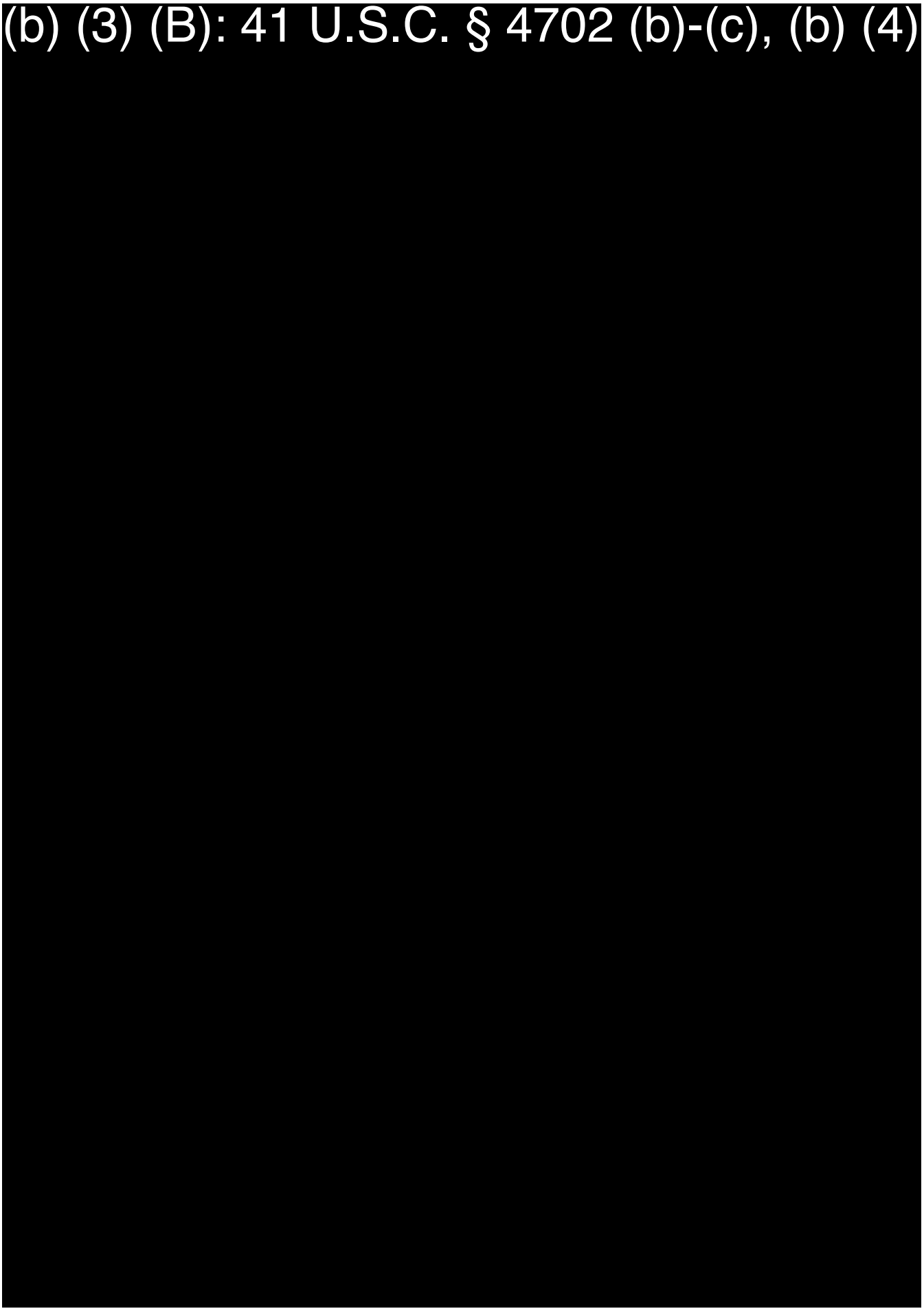
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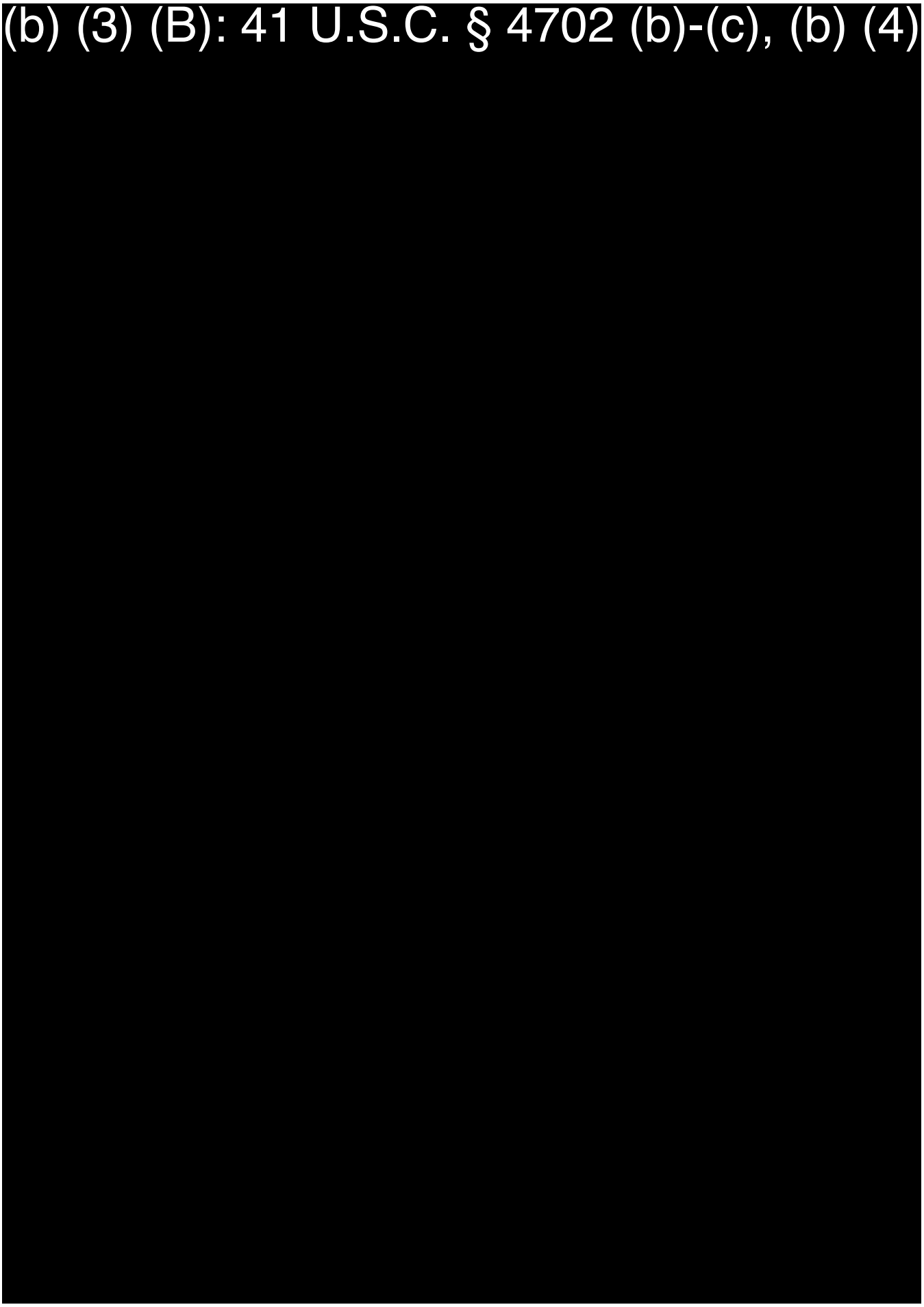
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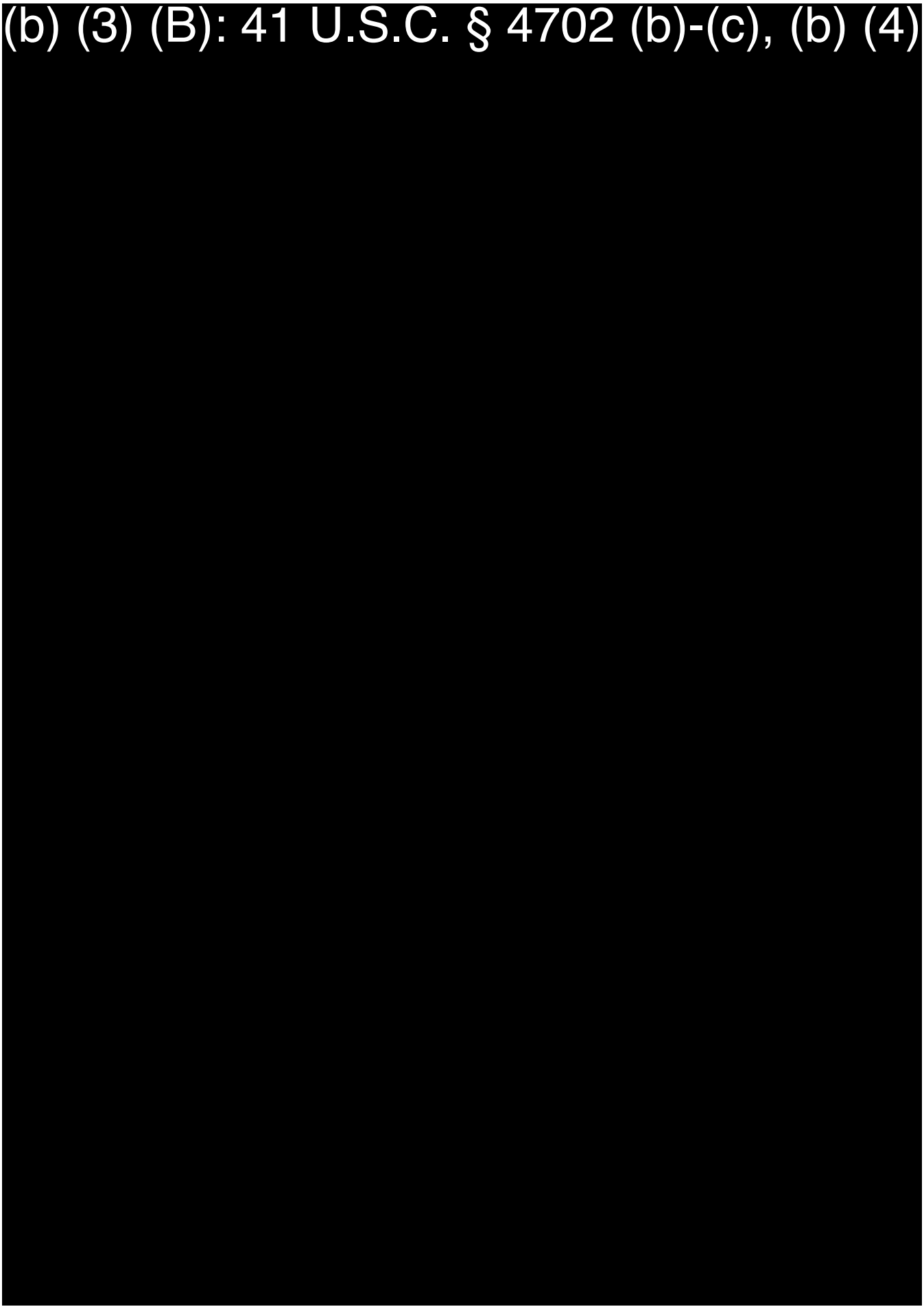
(b) (3) (B): 41 U.S.C. § 4702 (b)-(c), (b) (4)

## ARC - aerosols

William B. Karesh (b) (6) @gmail.com>
Fri 2/2/2018 12:34 PM
**To:** Rocke, Tonie E <trocke@usgs.gov>; Peter Daszak <daszak@ecohealthalliance.org>
**Cc:** Luke Hamel <hamel@ecohealthalliance.org>

1 attachments (438 KB)
PARC.pdf;

parc
A Xerox Company

3333 Coyote Hill Road
Palo Alto, CA 94304 USA
+1 650 812 4000
engage@parc.com
www.parc.com

**Project Overview**

- PARC developed a unique spray technology for large area and high throughput aerosol delivery of highly viscous and concentrated fluids. These fluids can include a range of solutions, e.g., bioactive formulations. This technology has a potential application in large area inoculation of animals/humans with bioengineered formulations for pre-emptive reduction of disease transfer.
- PARC has expertise in fluid/aerosol delivery, leveraging the unique spray method that can aerosolize fluids independent of viscosity or bioactive concentration. This technique enables partners in the biological space to deliver bioactive formulations to animal models with improved chance of efficacy/bioavailability. Potential technical challenges to overcome will be systems integration with rapid development/preparation of pre-emptive agents (potentially with on-demand concentration and composition) and in testing the biological response with animal models.
- PARC can have significant involvement in Technical Area 2 of a PRE-EMPT project: development of a scalable aerosol delivery method for wide-scale inoculation of animal models.

**Teaming Overview and Objectives**

- PARC has worked with both commercial and university partners for applications of this technology.
- PARC has expertise in fluid delivery, droplet generation, and device and systems integration drawing on our long history with developing printing systems (ink-on-paper). PARC will leverage both previous and on-going work and our related IP portfolio on fluid delivery using platform technologies (spray, transdermal delivery) to meet the PRE-EMPT program objectives.
- PARC has the institutional assets to develop and fabricate new systems for spraying, as well as the background to help improve spray formulation for uptake in mucosal and other targeted membranes.
- PARC is well-positioned to advance its unique spray technology for the PRE-EMPT program, given its demonstrated scalability and wide applicability across different fluids (ranging from low to very high viscosity and independent of bioactive concentration/loading). PARC is looking for collaborators who will investigate disease transmission across animal species and develop the necessary pre-emptive biologicals to prevent such transmission. These engineered biologicals can then be delivered to animal models using the spray technology with maximum chance for efficacy and bioavailability.

**Contact Information**

Dr. Jerome Unidad; email: Jerome.Unidad@parc.com; telephone: 650-812-4209

## First (rough) draft of the DARPA abstract - Project DEFUSE


# Peter Daszak <daszak@ecohealthalliance.org>


Wed 2/7/2018 8:51 PM

**To:** Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
**Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.
Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

**Peter Daszak**
*President*

EcoHealth Alliance
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www.ecohealthalliance.org

*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

**DARPA – PREEMPT – HR001118S0017**

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**Abstract Submission Requirements**:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

*Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."*

B. Executive Summary Slide:

*Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at* http://www.fbo.gov.

****See slide template at bottom of document.**

**PROJECT DEFUSE**

C. Goals and Impact:

*Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:*

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with high viral loads due to unique damping of their immune systems, likely as an evolutionary adaptation to flight. We will use this to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Decide which of following parts to talk about:

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

**Commented [PD1]:** Check on the duration of PREEMPT

D. Technical Plan:
*Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.*
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve the program goal"**
Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

**Overview**

*Rationale for the SE Asian SARS-related CoV* – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making

**Commented [PD2]:** I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

**TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region**

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic

distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

The modeling team will use these data to build models of 1) risk of viral

**Commented [PD3]:** Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

**Commented [PD4]:** Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

**TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.**

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-

regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered

by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We will conduct initial experiments on a lab colony of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting infection experiments on this bat genus …(details and citation if possible). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets

collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

*A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.*

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

*Team:*
*Lead Organization: EcoHealth Alliance, New York*
*PI:* Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
*Key Personnel:*
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

*Subcontract #1: University of North Carolina Medical School*
*Organizational Lead:* Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

*Subcontract #2: USGS National Wildlife Health Center*
*Organizational Lead:* Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

*Subcontract #3: Duke NUS, Singapore*
*Organizational Lead:* Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

*Subcontract #4: Wuhan Institute of Virology, China*
*Organizational Lead:* Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography

*Links to relevant papers, reports, or resumes of key performers.*

Do not include more than two resumes as part of the abstract.

****Resumes count against the abstract page limit.**

**Dr. Peter Daszak** is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

**Prof. Ralph Baric** is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

**Commented [PD5]:** I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

**1) 5.1.1. Overall Scientific and Technical Merit**

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies

major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

**2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission**

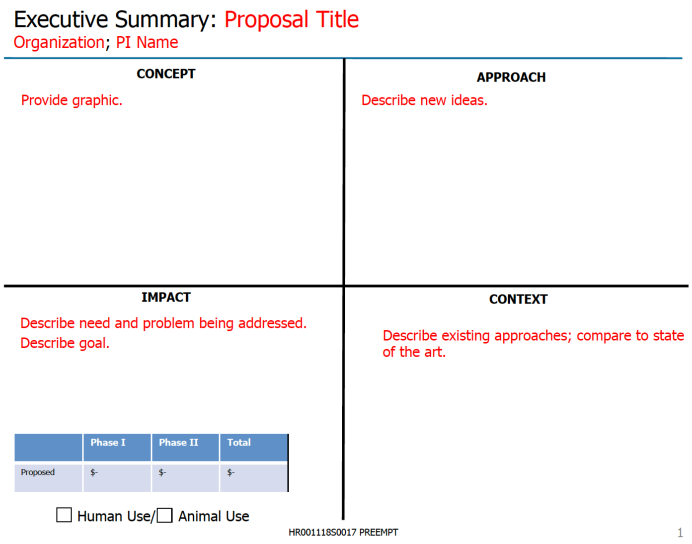
The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

**3) 5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

**Commented [EA6]:** Please note




**Attachment 1: Executive Summary Slide template**

**Citations**


1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

## RE: First (rough) draft of the DARPA abstract - Project DEFUSE

# Wang Linfa <linfa.wang@duke-nus.edu.sg>

Thu 2/8/2018 6:24 AM

**To:** Peter Daszak <daszak@ecohealthalliance.org>; Ralph Baric (rbaric@email.unc.edu) <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
**Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

See my brief notes/edits in the attached.

I am working on a large grant here in SG and won't be able to spend too much time until next week.

LF

***Linfa (Lin-Fa) WANG, PhD FTSE***
**Professor & Director**
**Programme in Emerging Infectious Disease**
**Duke-NUS Medical School,**
**8 College Road, Singapore 169857**
**Tel: +65 6516 8397**

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**From:** Peter Daszak [mailto:daszak@ecohealthalliance.org]
**Sent:** Thursday, 8 February, 2018 10:51 AM
**To:** Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
**Cc:** Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
**Subject:** First (rough) draft of the DARPA abstract - Project DEFUSE
**Importance:** High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.
Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

**Peter Daszak**
*President*

EcoHealth Alliance
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New York, NY 10001

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www.ecohealthalliance.org

*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

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intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

**DARPA – PREEMPT – HR001118S0017**

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**Abstract Submission Requirements**:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

*Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."*

B. Executive Summary Slide:

*Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at* http://www.fbo.gov.

****See slide template at bottom of document.**

**PROJECT DEFUSE**

C. Goals and Impact:

*Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:*

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

**Commented [L1]:** My understanding is that the project will have two parts: A) better risk assessment and modeling and B) risk defusing.

Do we need to say anything about A here?!

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely as an evolutionary adaptation to flight. We will use this new finding to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

**Deleted:** high viral loads

**Deleted:** their

**Commented [L2]:** This will become important late: while we are specifically targeting SARS-realted CoVS, this strategy will be applicable to ALL bat-borne viruses in future

4. What are the key technical challenges in your approach and how do you plan to overcome these?

Decide which of following parts to talk about:

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

Commented [L3]: I have highlighted the ones which are most challenging and novel for this proposal

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5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

Commented [PD4]: Check on the duration of PREEMPT

D. Technical Plan:
*Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.*
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve the program goal"**
Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

**Overview**

Commented [PD5]: I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

*Rationale for the SE Asian SARS-related CoV* – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making

sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

**Commented [L6]:** We need to provide background info about bat immunity and the track record of this group in the field

**Commented [L7]:** Peng: I am working on an important grant here in Singapore. Can you add a few points here? Thanks

**TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region**

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic

distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

The modeling team will use these data to build models of 1) risk of viral

**Commented [PD8]:** Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

**Commented [PD9]:** Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

**TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.**

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-

regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered

by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar bats for experimental use (one of very few experimental bat breeding colonies in the world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

**Deleted:** We will conduct initial experiments on a lab colony of wild-caught

**Deleted:** on this bat

**Deleted:** …(details and citation if possible).

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in

Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

*A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.*

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years'

experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

*Team:*
*Lead Organization: EcoHealth Alliance, New York*
*PI:* Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
*Key Personnel:*
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

*Subcontract #1: University of North Carolina Medical School*
*Organizational Lead:* Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

*Subcontract #2: USGS National Wildlife Health Center*
*Organizational Lead:* Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

*Subcontract #3: Duke NUS, Singapore*
*Organizational Lead:* Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

*Subcontract #4: Wuhan Institute of Virology, China*
*Organizational Lead:* Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
*Links to relevant papers, reports, or resumes of key performers.*
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

**Dr. Peter Daszak** is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

**Prof. Ralph Baric** is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

**Commented [PD10]:** I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

1) **5.1.1. Overall Scientific and Technical Merit**

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) **5.1.2. Potential Contribution and Relevance to the DARPA Mission**

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

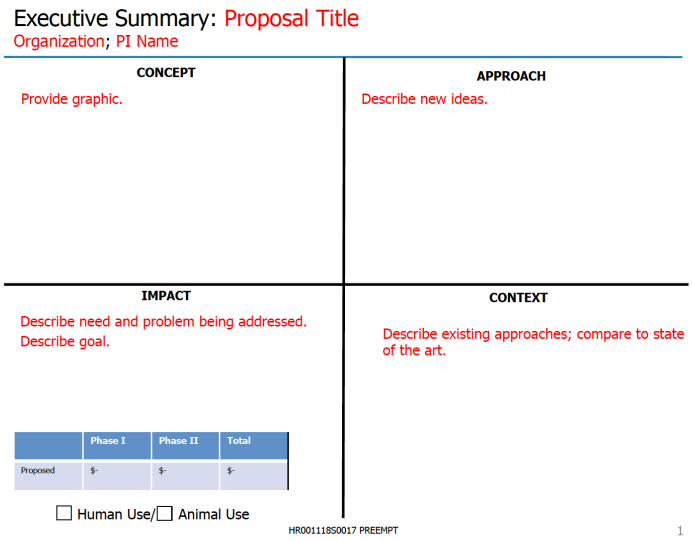
3) **5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to**

**offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

**Commented [EA11]:** Please note




**Attachment 1: Executive Summary Slide template**

**Citations**


1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).

6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).
8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

## RE: First (rough) draft of the DARPA abstract - Project DEFUSE

# Baric, Ralph S <rbaric@email.unc.edu>

Thu 2/8/2018 10:22 AM

**To:** Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>
**Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

I have built in my comments atop of Linfa's comments. ralph

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**From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
**Sent:** Thursday, February 8, 2018 7:25 AM
**To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
**Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
**Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE

See my brief notes/edits in the attached.

I am working on a large grant here in SG and won't be able to spend too much time until next week.

LF

***Linfa (Lin-Fa) WANG, PhD FTSE***
**Professor & Director**
**Programme in Emerging Infectious Disease**
**Duke-NUS Medical School,**
**8 College Road, Singapore 169857**
**Tel: +65 6516 8397**

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**From:** Peter Daszak [mailto:daszak@ecohealthalliance.org]
**Sent:** Thursday, 8 February, 2018 10:51 AM
**To:** Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
**Cc:** Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
**Subject:** First (rough) draft of the DARPA abstract - Project DEFUSE
**Importance:** High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my

hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.
Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

**Peter Daszak**
*President*

EcoHealth Alliance
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New York, NY 10001

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www.ecohealthalliance.org

*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

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**DARPA – PREEMPT – HR001118S0017**

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**Abstract Submission Requirements**:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

*Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."*

B. Executive Summary Slide:

*Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at* http://www.fbo.gov.

****See slide template at bottom of document.**

**PROJECT DEFUSE**

C. Goals and Impact:

*Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:*

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely in part as an evolutionary adaptation to flight. We will use this new finding to design strategies like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

**Commented [L1]:** My understanding is that the project will have two parts: A) better risk assessment and modeling and B) risk defusing.

Do we need to say anything about A here?!

**Deleted:** high viral loads

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**Commented [L2]:** This will become important late: while we are specifically targeting SARS-realted CoVS, this strategy will be applicable to ALL bat-borne viruses in future

**Commented [BRS3]:** I thought we were also going to use innate immune antagonists to boost baseline immunity, which should attenuate virus burden in animals?

Isn't this supposed to be a two pronged approach that are complementary, e.g., in that innate immune agonists will also boost immunity to recombinant spike vaccines.

Decide which of following parts to talk about:
Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea, Vietnam and Cambodia?). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SARS and related prepandemic zoonotic strains), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

D. Technical Plan:
*Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.*
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (even if risky) plan to achieve**

**Commented [L4]:** I have highlighted the ones which are most challenging and novel for this proposal

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**the program goal"**

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
  - DARPA wants to know who has experience w/ ACURO IACUC work.
    - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
    - IRB also in place, just has to be modified

**Overview**

*Rationale for the SE Asian SARS-related CoV* – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially highly heterogeneous zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at fixed, known locations, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs evaluated in people and rodents may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind and replicate efficiently in primary human lung airway cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have

**Commented [PD6]:** I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

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**Commented [BRS7]:** About 90,000 of the 550,000 deployed US military are in se asian, mostly japan and south korea.

**Commented [BRS8]:** What abbreviation mean

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evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified SARS-like strains in a single cave in Yunnan that harbor every gene found in the human SARS-CoV strains detected during the 2002-2003 epidemic. Within this bat population, an ideal evolutionary soup exists which can produce new human strains by high frequency RNA recombination and presents a perfect target for 21st generation intervention strategies.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas gene drives that are likely to be far less effective in bats because most bats are long-lived relative to their small size, long inter-generational periods (2-5 yrs) and low progeny (~1-2 pups). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

**TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region**

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of the 2002-2003 epidemic SARS-CoV distributed across a

**Commented [BRS9]:** These viruses can either be cultured and/or recovered using reverse genetic strategies.

**Commented [BRS10]:** Filoviruses pretty diverse, although not anywhere near as diverse as cov. Is this a sampling thing or not likely remains unclear?

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**Commented [L12]:** We need to provide background info about bat immunity and the track record of this group in the field

**Commented [L13]:** Peng: I am working on an important grant here in Singapore. Can you add a few points here? Thanks

bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

**Commented [BRS14]:** Is surveillance in these other caves equally robust over the past 8 yrs?

**Commented [PD15]:** Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of

samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Their group have also devised new strategies to culture SARS-like bat coronaviruses, allowing biological characterization of both high risk strains that can replicate in primary human cells and low risk strains that can only replicate in the presence of exogenous enhancers. Viral spike glycoproteins that bind receptor will then be inserted into SARS-CoV backbones, and inoculated into human cells and humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV ((PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993), The Baric group has also demonstrated that a nucleoside analogue inhibitor, GS-5734 (Gilead Inc), blocks epidemic, preepidemic and zoonotic SARS-CoV and SARS-like bat coronavirus replication in primary human airway cells and in mice (PMC5567817). Consequently, they will evaluate the ability of this drug to block replication of newly disovered pre-epidemic and zoonotic high risk strains. As the drug has been used to effectively treat Ebola virus infected patients (PMC4967715, PMC5583641) as well and has potent activity against Nipha and Hendra viruses (PMC5338263), an alternative intervention for military personnel is prophylactic treatment treatment prior to deployment into high risk settings.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model

**Commented [PD16]:** Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well...

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**Commented [BRS17]:** IN the US, these recombinant SARS CoV are studied under BSL3, not BSL2, especially important for those that are able to bind and replicate in primary human cells.
In china, might be growin these virus under bsl2. US reseachers will likely freak out.

the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

**TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.**

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon

**Commented [BRS18]:** Like what

**Commented [BRS19]:** Transient low level Chronic inflammation sounds better

treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar bats for experimental use (one of very few experimental bat breeding colonies in the

**Commented [BRS20]:** This could easily take longer than 3 years. Poly ic, IFN or any type of TLR agonist might be more robust. Might want to test in captive bats infected with SARS or select SARS like viruses, like SHC014, which we could provide.

**Commented [BRS21]:** We have several papers showing importance of TLR3 and TLR4 signaling in control of SARS pathogenesis. PMC4447251, PMC5473747

**Commented [BRS22]:** Don't attack the other arm of the program. And I disagree that its superior to vaccination, which potentially provides long-term immunity.

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**Commented [BRS23]:** The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (PMC5584442). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (PMC4058772, PMC5423355, PMC2883479, PMC5578707, PMC3014161). Initially, we will test various delivery vehicles in controlled conditions in bats in a laboratory setting, taking the best candidate forward for testing in the field.
The Baric laboratory has built recombinant S pike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad based immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, thereby reducing disease risk in these animals for multiple years (PMC3977350, PMC2588415).

world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

**Deleted:** We will conduct initial experiments on a lab colony of …

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E. Capabilities:

*A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and*

*responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.*

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

*Team:*
*Lead Organization: EcoHealth Alliance, New York*
*PI:* Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
*Key Personnel:*
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year

TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

*Subcontract #1: University of North Carolina Medical School*
*Organizational Lead:* Prof. Ralph Baric Ph.D., 2 months/year
Dr. Tim Sheahan (6 months/yr)
Dr. Amy Sims (4 months/yr)
Sarah Leist, Postdoctoral fellow (4 months/yr)
Boyd Yount, Research Analyst, 12 months/year
Trevor Scobey, Research Technician, 6 months/yr

*Subcontract #2: USGS National Wildlife Health Center*
*Organizational Lead:* Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

*Subcontract #3: Duke NUS, Singapore*
*Organizational Lead:* Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

*Subcontract #4: Wuhan Institute of Virology, China*
*Organizational Lead:* Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
*Links to relevant papers, reports, or resumes of key performers.*
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

**Dr. Peter Daszak** is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering

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**Commented [PD24]:** I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

**Prof. Ralph Baric** is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology and Department of Microbiology and Immunology . His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission and pathogenesis. Dr. Baric and his group have developed a platform strategy to access the potential "preepidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

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****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

**1) 5.1.1. Overall Scientific and Technical Merit**

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

**2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission**

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

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**3) 5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

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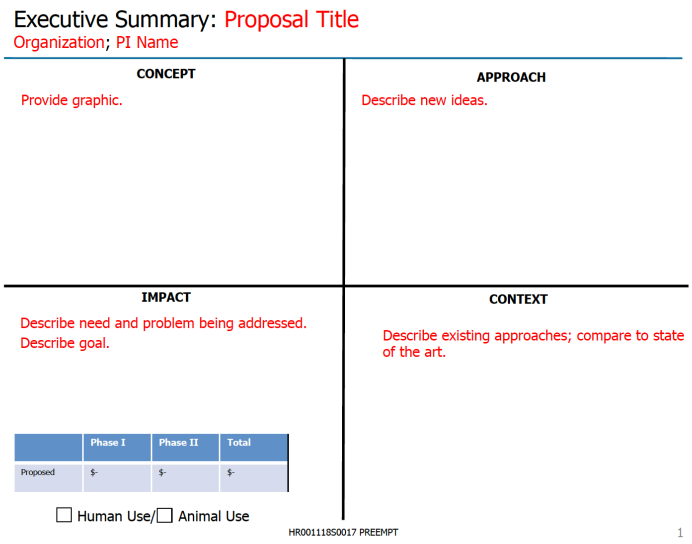


**Attachment 1: Executive Summary Slide template**

**Citations**


1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

**From:** Rocke, Tonie <trocke@usgs.gov>
**Sent:** Thursday, February 8, 2018 10:01 AM
**To:** Baric, Ralph S
**Cc:** Wang Linfa; Peter Daszak; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Hongying Li
**Subject:** Re: First (rough) draft of the DARPA abstract - Project DEFUSE
**Attachments:** DARPA (PREEMPT) Abstract EcoHealth Alliance DEFUSE 1st Draft-LW180208-rsb-ter.docx

Likewise, I added my comments to Ralph's document. I added some detail, but not too much, so let me know if you want more. Best -Tonie

On Thu, Feb 8, 2018 at 10:22 AM, Baric, Ralph S <rbaric@email.unc.edu> wrote:

> I have built in my comments atop of Linfa's comments. ralph
>
> **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> **Sent:** Thursday, February 8, 2018 7:25 AM
> **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
>
> See my brief notes/edits in the attached.
>
> I am working on a large grant here in SG and won't be able to spend too much time until next week.
>
> LF
>
> ***Linfa (Lin-Fa) WANG, PhD FTSE***

**Professor & Director**

**Programme in Emerging Infectious Disease**

**Duke-NUS Medical School,**

**8 College Road, Singapore 169857**

**Tel: +65 6516 8397**

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**From:** Peter Daszak [mailto:daszak@ecohealthalliance.org]
**Sent:** Thursday, 8 February, 2018 10:51 AM
**To:** Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
**Cc:** Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
**Subject:** First (rough) draft of the DARPA abstract - Project DEFUSE
**Importance:** High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

**Peter Daszak**

*President*

EcoHealth Alliance

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New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

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Tonie E. Rocke
USGS National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711
608-270-2451
trocke@usgs.gov

**DARPA – PREEMPT – HR001118S0017**

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**Abstract Submission Requirements**:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

*Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."*

B. Executive Summary Slide:

*Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at* http://www.fbo.gov.

****See slide template at bottom of document.**

**PROJECT DEFUSE**

C. Goals and Impact:

*Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:*

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely in part as an evolutionary adaptation to flight. We will use this new finding to design strategies, like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists, to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

Decide which of following parts to talk about:
Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea, Vietnam and Cambodia?). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SARS and related prepandemic zoonotic strains), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms and devastating the industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

D. Technical Plan:
*Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.*
****Note: "The technical plan should demonstrate a deep understanding of the technical challenges and present a credible (<u>even if risky</u>) plan to achieve**

**the program goal"**

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

**Overview**

*Rationale for the SE Asian SARS-related CoV* – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially highly heterogeneous zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at fixed, known locations, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs evaluated in people and rodents may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind and replicate efficiently in primary human lung airway cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have

evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case- making validated prevention of spillover challenging. These viruses also show little strain diversity ,which also makes it more difficult to model which evolutionary lines are high-risk. Conversely, SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified SARS-like strains in a single cave in Yunnan that harbor every gene found in the human SARS-CoV strains detected during the 2002-2003 epidemic. Within this bat population, an ideal evolutionary soup exists that could produce new human strains by high frequency RNA recombination, and thus, it presents a perfect target for 21$^{st}$ generation intervention strategies.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas gene drives that are likely to be far less effective in bats because most bats are long-lived relative to their small size, have long inter-generational periods (2-5 yrs) and low progeny (~1-2 pups). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

**<u>TA1</u>: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region**

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of the 2002-2003 epidemic SARS-CoV distributed across a

bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in that cave. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of

samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Their group have also devised new strategies to culture SARS-like bat coronaviruses, allowing biological characterization of both high risk strains that can replicate in primary human cells and low risk strains that can only replicate in the presence of exogenous enhancers. Viral spike glycoproteins that bind receptor will then be inserted into SARS-CoV backbones, and inoculated into human cells and humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV ((PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). The Baric group has also demonstrated that a nucleoside analogue inhibitor, GS-5734 (Gilead Inc), blocks epidemic, preepidemic and zoonotic SARS-CoV and SARS-like bat coronavirus replication in primary human airway cells and in mice (PMC5567817). Consequently, they will evaluate the ability of this drug to block replication of newly disovered pre-epidemic and zoonotic high risk strains. As the drug has been used to effectively treat Ebola virus infected patients (PMC4967715, PMC5583641) as well and has potent activity against Nipha and Hendra viruses (PMC5338263), an alternative intervention for military personnel is prophylactic treatment treatment prior to deployment into high risk settings.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches, the data above, and other biological and ecological data to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be more effective to treat. We will then model

the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

**<u>TA2:</u> Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.**

Our goal is to test? two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators designed to up-regulate their naïve immunity and assess their ability to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will conduct inoculation trials with live bats to assess suppression of replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against

filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar

bats for experimental use (one of very few experimental bat breeding colonies in the world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. <u>These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.</u>

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the US Geological Survey, National Wildlife Health Center, who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using locally acquired insectiverous bats like *Tadarida brasiliensis* or *Eptesicus fuscus* (*10, 11*) as proxies, Dr. Rocke will further develop and assess delivery vehices (mediums) and methods of delivery for the molecules, inocula proposed above, including: 1) transdermally applied nanoparticles; 2) sticky edible gels that bats will groom from themselves and each other; 3) aerosolization via spayers that could be used in cave settings; and 4) automated sprays triggered by timers and movement detectors at critical cave entry points. Simple gels have already been used to vaccinate big brown bats against rabies (11) in a laboratory setting, and hand delivery of these gels containing biomarkers (no vaccine) to vampire bats (*Desmodus rotundus*) in Peru and Mexico have shown they are readily consumed and transferred between bats. Methods to improve uptake (different gels, nanoparticles) and mechanize delivery methods (aerosolization) will be tested first in a laboratry setting, and secondly in local field settings using the biomarker, rhodamine B (which marks hair and whiskers upon consumption) to assess uptake by bats. The most optimal approaches will then be tested on live bats in our three cave sites in Yunnan Province with the most successful immunomodulators developed in TA1?. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods tested. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental

inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

*A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.*

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

*Team:*

*Lead Organization: EcoHealth Alliance, New York*

*PI:* Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year

*Key Personnel:*
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

*Subcontract #1: University of North Carolina Medical School*
*Organizational Lead:* Prof. Ralph Baric Ph.D., 2 months/year
Dr. Tim Sheahan (6 months/yr)
Dr. Amy Sims (4 months/yr)
Sarah Leist, Postdoctoral fellow (4 months/yr)
Boyd Yount, Research Analyst, 12 months/year
Trevor Scobey, Research Technician, 6 months/yr

*Subcontract #2: USGS National Wildlife Health Center*
*Organizational Lead:* Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

*Subcontract #3: Duke NUS, Singapore*
*Organizational Lead:* Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

*Subcontract #4: Wuhan Institute of Virology, China*
*Organizational Lead:* Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography

*Links to relevant papers, reports, or resumes of key performers.*
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

**Dr. Peter Daszak** is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

**Prof. Ralph Baric** is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology and Department of Microbiology and Immunology . His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission and pathogenesis. Dr. Baric and his group have developed a platform strategy to access the potential "preepidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

**1) 5.1.1. Overall Scientific and Technical Merit**
The proposed technical approach is innovative, feasible, achievable, and complete.

Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

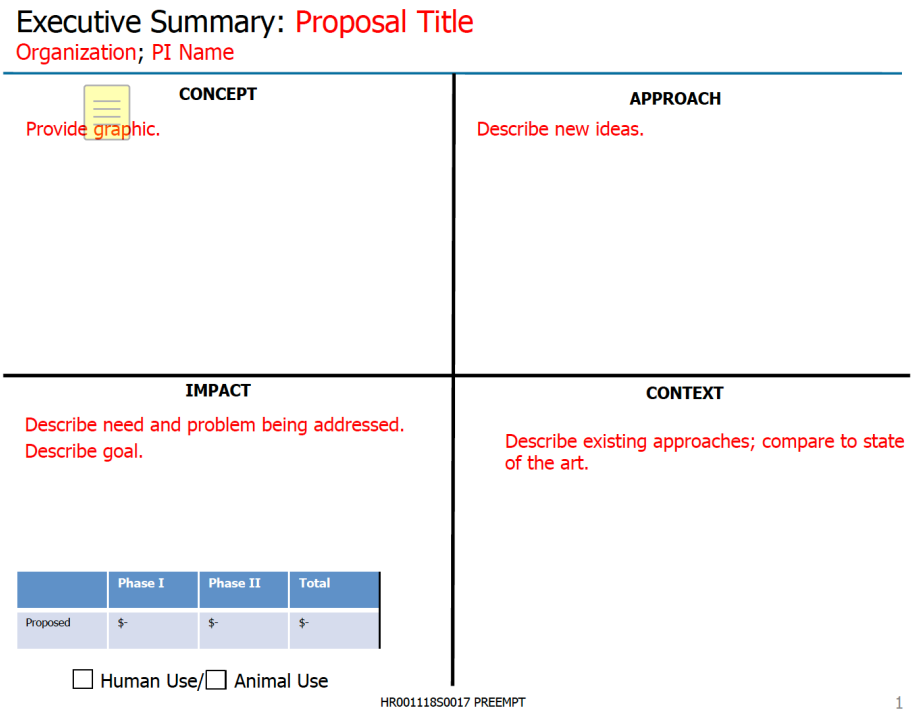
**2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission**

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

**3) 5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**




**Attachment 1: Executive Summary Slide template**

## Citations


1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).

8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

## Re: First (rough) draft of the DARPA abstract - Project DEFUSE

# Noam Ross <ross@ecohealthalliance.org>

Thu 2/8/2018 11:24 AM

**To:** Baric, Ralph S <rbaric@email.unc.edu>
**Cc:** Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie E <trocke@usgs.gov>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

My changes and comments attached. They primarily
1) Clarify the the modeling components (genotype-phenotype, evolutionary, ecological)
2) Emphasize validation against actual spillover events in the human population

These changes are on Peter's original draft but shouldn't conflict with Linfa and Ralph's.

Noam

On Thu, Feb 8, 2018 at 11:22 AM Baric, Ralph S <rbaric@email.unc.edu> wrote:

> I have built in my comments atop of Linfa's comments. ralph
>
> ---
>
> **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> **Sent:** Thursday, February 8, 2018 7:25 AM
> **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
>
> See my brief notes/edits in the attached.
>
> I am working on a large grant here in SG and won't be able to spend too much time until next week.
>
> LF
>
> ***Linfa (Lin-Fa) WANG, PhD FTSE***

**Professor & Director**

**Programme in Emerging Infectious Disease**

**Duke-NUS Medical School,**

**8 College Road, Singapore 169857**

**Tel: +65 6516 8397**

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**From:** Peter Daszak [mailto:daszak@ecohealthalliance.org]
**Sent:** Thursday, 8 February, 2018 10:51 AM
**To:** Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
**Cc:** Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
**Subject:** First (rough) draft of the DARPA abstract - Project DEFUSE
**Importance:** High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

**Peter Daszak**

*President*

EcoHealth Alliance

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Tel. +1 212-380-4473

www.ecohealthalliance.org

*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

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**Dr. Noam Ross**
*Senior Research Scientist*

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***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

**DARPA – PREEMPT – HR001118S0017**

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**Abstract Submission Requirements**:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

*Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."*

B. Executive Summary Slide:

*Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at* http://www.fbo.gov.

****See slide template at bottom of document.**

**PROJECT DEFUSE**

C. Goals and Impact:

*Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:*

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?
Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with high viral loads due to unique damping of their immune systems, likely as an evolutionary adaptation to flight. We will use this to design strategies to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?
Decide which of following parts to talk about:

Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts
Model validation

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SADS), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). In the region directly surrounding our study site, these bat hosts currently roost in unoccupied military bases that may be used by troops at a future time. Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like SADS-CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
42 months.

Deleted: 46 months

D. Technical Plan:
*Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.*
****Note: "The technical plan should demonstrate a deep understanding of the**

**technical challenges and present a credible (<u>even if risky</u>) plan to achieve the program goal"**

<u>Key Terms/Aspects to Emphasize in Abstract</u>

- IACUC/IRB
    - DARPA wants to know who has experience w/ ACURO IACUC work.
        - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
        - IRB also in place, just has to be modified

**Overview**

*Rationale for the SE Asian SARS-related CoV* – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous and roost in dense colonies at a fixed, known location, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs trialed out in people may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group has worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for over 10 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and characterization of their ability to bind with human cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have

**Commented [PD3]:** I know this is too long. I'll edit later this weekend, but want to keep this text for the full proposal

evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic. This also gives us the unique ability to validate our models on a significant number of actual spillover events, not only experimental infections; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified a single cave in Yunnan that harbors every gene from the SARS-CoV in a diversity of SARSr-CoVs within the bat population, making it an ideal evolutionary soup to target for intervention.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas are likely to be far less effective in bats because most bats are long-lived relative to their small size, with long inter-generational periods (2-5 years). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

**TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region**

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and the surrounding region and includes a cave in which we have identified all the genetic components of SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have

not yet identified the high-risk SARSr-CoVs in those caves. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- and satellite telemetry to identify the home range of each species of bat in the caves, to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build environmental niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARSr-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to assess the feasibility of surveys using pooled fresh fecal and urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike

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**Commented [PD4]:** Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

**Commented [PD5]:** Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Proteins that bind will then be inserted into SARS-CoV backbones, and inoculated into humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV (REF).

Using both samples from our previous work and new sampling of the human population in the region surrounding our sites, we will determine which viral strains in addition to SARS-CoV have successfully jumped into humans.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy. First, based on binding and infection assays in mouse models, we will develop genotype-to-phenotype models to predict viral ability to infect host cells based on genetic traits. Secondly, data on diversity of bat spike proteins, prevalence of recombinant CoVs, and flow of genes within each bat cave via bat movement and migration, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Finally, ecological data, including viral host species and species home ranges will be used to estimate the likelihood of spillover into human populations.

Because of our unique collaboration among world-class modelers, and coronavirologists, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-2 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra, Nipah), which require BSL-4 level facilities for cell culture. In addition, the high frequency of SARSr-CoV spillover events into the human population in this region gives us the allows us to validate models to a degree not possible in systems where spillover events are extremely rare.

We will use stochastic simulation modeling approaches to characterize the dynamics of viral circulation in these bat populations using the data above and other biological and ecological data. Using this model, we will estimate the frequency, efficacy, and population coverage required for our intervention approaches to effectively suppress the viral population. We will determine the seasons, locations within a cave, and different delivery methods (spray, swab, cave mouth automated) that will be most effective. Finally we will determine the time frame the treatment will be effective until re-colonization or evolution will cause a return of a high-risk SARSr-CoV to the population.

**TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.**

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**Deleted:** Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve.

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**Deleted:** to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming.

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**Deleted:** , what proportion of a population needs to be reached to have effective viral dampening, and whether specific

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Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating

dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We will conduct initial experiments on a lab colony of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting infection experiments on this bat genus …(details and citation if possible). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be trialed out on live bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

*A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.*

****Note: While <u>only the proposal requires</u> an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project.

*Team:*
*Lead Organization: EcoHealth Alliance, New York*
*PI:* Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
*Key Personnel:*
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

*Subcontract #1: University of North Carolina Medical School*
*Organizational Lead:* Prof. Ralph Baric Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year

*Subcontract #2: USGS National Wildlife Health Center*
*Organizational Lead:* Tonie Rocke Ph.D., 2 months/year, no salary requested

TBD Research Technician, 9 months/year

*Subcontract #3: Duke NUS, Singapore*
*Organizational Lead:* Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

*Subcontract #4: Wuhan Institute of Virology, China*
*Organizational Lead:* Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography
*Links to relevant papers, reports, or resumes of key performers.*
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

**Dr. Peter Daszak** is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

**Prof. Ralph Baric** is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission. His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

**Commented [PD6]:** I'm planning to use my resume and Ralph's. Linfa/Zhengli, I realize your resumes are also very impressive, but I am trying to downplay the non-US focus of this proposal so that DARPA doesn't see this as a negative.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

**1) 5.1.1. Overall Scientific and Technical Merit**

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

**2) 5.1.2. Potential Contribution and Relevance to the DARPA Mission**

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In
addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

**3) 5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed

subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

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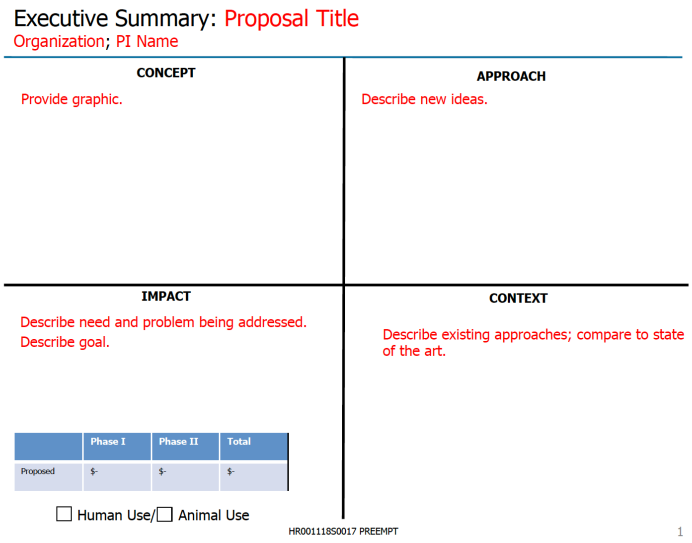


**Attachment 1: Executive Summary Slide template**

**Citations**


1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).

3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).
7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).
8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

## Re: First (rough) draft of the DARPA abstract - Project DEFUSE

# Jon Epstein <epstein@ecohealthalliance.org>

Thu 2/8/2018 1:46 PM

**To:** Rocke, Tonie E <trocke@usgs.gov>
**Cc:** Baric, Ralph S <rbaric@email.unc.edu>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; Peter Daszak <daszak@ecohealthalliance.org>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>

Attached are my comments.

Cheers,
Jon

On Thu, Feb 8, 2018 at 1:00 PM, Rocke, Tonie <trocke@usgs.gov> wrote:

> Likewise, I added my comments to Ralph's document. I added some detail, but not too much, so let me know if you want more. Best -Tonie
>
> On Thu, Feb 8, 2018 at 10:22 AM, Baric, Ralph S <rbaric@email.unc.edu> wrote:
>
> > I have built in my comments atop of Linfa's comments. ralph
> >
> > ---
> >
> > **From:** Wang Linfa [mailto:linfa.wang@duke-nus.edu.sg]
> > **Sent:** Thursday, February 8, 2018 7:25 AM
> > **To:** Peter Daszak <daszak@ecohealthalliance.org>; Baric, Ralph S <rbaric@email.unc.edu>; Zhengli Shi (zlshi@wh.iov.cn) <zlshi@wh.iov.cn>; William B. Karesh <karesh@ecohealthalliance.org>; Rocke, Tonie <trocke@usgs.gov>
> > **Cc:** Luke Hamel <hamel@ecohealthalliance.org>; Jonathon Musser <musser@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Kevin Olival, PhD <olival@ecohealthalliance.org>; Jon Epstein <epstein@ecohealthalliance.org>; Noam Ross <ross@ecohealthalliance.org>; Aleksei Chmura <chmura@ecohealthalliance.org>; Anna Willoughby <willoughby@ecohealthalliance.org>; Hongying Li <li@ecohealthalliance.org>
> > **Subject:** RE: First (rough) draft of the DARPA abstract - Project DEFUSE
> >
> > See my brief notes/edits in the attached.
> >
> > I am working on a large grant here in SG and won't be able to spend too much time until next week.
> >
> > LF

***Linfa (Lin-Fa) WANG, PhD FTSE***

**Professor & Director**

**Programme in Emerging Infectious Disease**

**Duke-NUS Medical School,**

**8 College Road, Singapore 169857**

**Tel: +65 6516 8397**

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**From:** Peter Daszak [mailto:daszak@ecohealthalliance.org]
**Sent:** Thursday, 8 February, 2018 10:51 AM
**To:** Ralph Baric (rbaric@email.unc.edu); Wang Linfa; Zhengli Shi (zlshi@wh.iov.cn); William B. Karesh; Rocke, Tonie
**Cc:** Luke Hamel; Jonathon Musser; Anna Willoughby; Kevin Olival, PhD; Jon Epstein; Noam Ross; Aleksei Chmura; Anna Willoughby; Hongying Li
**Subject:** First (rough) draft of the DARPA abstract - Project DEFUSE
**Importance:** High

Dear All,

I've attached a first rough draft of the DARPA abstract. Apologies for the delay. Unfortunately, edits to my Science paper came through on Friday and took many hours to do, so this delayed me. I'm right now in Geneva in my hotel at 3 am finishing these off before flying back to NYC from a WHO meeting.

Some important points:

1) Zhengli, Linfa, Ralph – Billy and I spoke with Tonie Rocke on Friday. Tonie is at the National Wildlife Health Center, Madison USA, and has worked on wildlife vaccines: plague in prairie dogs, rabies in Jamaican fruit bats, white nose syndrome in US bats. We needed someone with expertise in delivery of molecules/vaccines to wildlife because DARPA specifically lay that out. As you'll see, Tonie is perfect for our project and will be able to do work at USGS NWHC and with Zhengli in China to help with TA2

2) Zhengli and Linfa – After I spoke with you both, I had a great conversation with Ralph Baric. He proposed to work on recombinant chimeric spike proteins as a second line of attack. I think that is a perfect fit because 1) it's his expertise and he has published on it, 2) it

will act as an alternative to the blue-sky and risky immune boosting work that Linfa/Peng have proposed. I hope you agree!

3) Ralph, Zhengli, Linfa, Tonie – as you can see, I have mangled the language/technical details for most of your sections. Pardon my lack of knowledge, and please draft a couple of paragraphs each to make your sections look correct. Thanks to Peng for giving me some text anyway – very useful, but please check what I've done with it.

4) All – please add some names and details on the team part so we get clarity in this on what staff you will need to do the work.

5) Please don't worry about keeping this to the 8 page limit. Just add text here and there, references, and edit to make what I've written correct, and more exciting. I will work on this on Saturday, Sunday and Monday to bring it down to 8 pages of very crisp, super-exciting text. I also want as many of your good ideas in here, so that I can use this draft to build on for the full proposal.

6) Finally – please edit rapidly using tracked changes in word. If you don't want to mess up endnote, please just insert references as comment boxes and we'll pull them off the web.

Aleksei and Anna: please read the draft and work on some draft image designs that sum up the project flow. I'll call you Thursday afternoon to discuss so you can finish them off.

Luke – please have a go at a first draft of the executive summary slide. I'll pick up from what you've done once you send it to me.

Thanks again to all of you for agreeing to collaborate on this proposal. From what I know of the competition, what DARPA wants, and what we're offering, I think we have an extremely strong team, so I'm looking forward to getting the full proposal together and winning this contract!

Cheers,

Peter

**Peter Daszak**

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*EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.*

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***EcoHealth Alliance leads cutting-edge scientific research into the critical connections between human and wildlife health and delicate ecosystems. With this science, we develop solutions that prevent pandemics and promote conservation.***

**DARPA – PREEMPT – HR001118S0017**

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**Abstract Submission Requirements**:

**8 pages with 12 point font or higher (smaller font may be used for figures, tables and charts)

**Page limit includes all figures, tables, charts and the Executive Summary Slide

**Copies of all documents submitted must be clearly labeled with the following:

-DARPA BAA number

-Proposer Organization

-Proposal title/Proposal short title

-Submission letter is optional and does not count towards page limit

A. Cover Sheet (does not count towards page limit):

*Include the administrative and technical points of contact (name, address, phone, fax, email, lead organization). Also include the BAA number, title of the proposed project, primary subcontractors, estimated cost, duration of project, and the label "ABSTRACT."*

B. Executive Summary Slide:

*Provide a one slide summary in PowerPoint that effectively and succinctly conveys the main objective, key innovations, expected impact, and other unique aspects of the proposed project. Use the slide template provided at* http://www.fbo.gov.

****See slide template at bottom of document.**

**PROJECT DEFUSE**

C. Goals and Impact:

*Clearly describe what is being proposed and what difference it will make (qualitatively and quantitatively), including brief answers to the following questions:*

1. What is the proposed work attempting to accomplish or do?

We aim to **defuse the potential for emergence of novel bat-origin high-zoonotic risk SARS-related coronaviruses** in Southeast Asia. We envisage a scenario whereby the US warfighter is called on to intervene in a security hotspot in SE Asia for a period of 3-6 months. As planners begin choosing sites for the mission, they will use an app we will design to assess the background risk of a site harboring dangerous zoonotic viruses. If

there is no alternative to a high-risk site, a tactical forward team will deploy automated delivery technology we will develop in caves that harbor bats carrying these viruses. These devices will release immune boosting molecules and chimeric polyvalent spike protein immune priming inocula to lower viral shedding from bats at the site for a few weeks or months, allowing our warfighters to execute the operation at lowered risk for spillover.

2. How is it done today? And what are the limitations?

Currently, there is no available technology to reduce the risk of exposure to novel coronaviruses from bats, other than avoid the regions where bats harbor these viruses. This includes large areas of southeast Asia where SARS-related CoVs are endemic in bats, which roost in caves during the day, but forage over wide areas at night, shedding virus in their feces and urine. The limitations of this lack of capacity are significant – we have shown evidence of recent spillover of SARS-related CoVs into people in southern China, and have identified viruses in this region that are capable of producing SARS-like illness in humanized mice, with no available vaccines or countermeasures. These viruses are a clear-and-present danger to our military personnel, and to global health security.

3. What is innovative in your approach and how does it compare to current practice and state-of-the-art (SOA)?

****Note: DARPA wants to know, "how the proposed project is revolutionary and how it significantly rises above the current state of the art**

Our group has shown that bats harbor the highest proportion of potential zoonoses of any mammal group, and that they are able to live with the host without causing diseases due to unique damping of certain pathways in their immune systems, likely in part as an evolutionary adaptation to flight. We will use this new finding to design strategies like small molecule Rig like receptor (RLR) or Toll like receptor (TLR) agonists to upregulate their immune response in their cave roosts, down-regulate viral replication, and reduce the risk of viral shedding and spillover (broad immune boosting strategy). At the same time, we will inoculate bats with novel chimeric polyvalent recombinant spike proteins to enhance their immune response against replication of specific, high-risk viruses (targeted immune priming strategy). We will use our innovative modeling to design apps that identify the likelihood of any region harboring high-risk bat viruses. We will design novel, automated approaches to deliver both types of inoculum remotely into caves to reduce exposure risk during decontamination.

4. What are the key technical challenges in your approach and how do you plan to overcome these?

**Commented [L1]:** My understanding is that the project will have two parts: A) better risk assessment and modeling and B) risk defusing.

Do we need to say anything about A here?!

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**Commented [L2]:** This will become important late: while we are specifically targeting SARS-realted CoVS, this strategy will be applicable to ALL bat-borne viruses in future

**Commented [BRS3]:** I thought we were also going to use innate immune antagonists to boost baseline immunity, which should attenuate virus burden in animals?

Isn't this supposed to be a two pronged approach that are complementary, e.g., in that innate immune agonists will also boost immunity to recombinant spike vaccines.

Decide which of following parts to talk about:
Modeling bat suitability
Inventory of caves
Sampling/testing
Reverse engineering, binding assays, mouse expts
Modeling viral risk of evolution and spillover
Modeling inoculation/defusing strategy
Immune modulation
Immune Booster recombinant production
Gain-of-function issue.
Inoculum delivery
Mesocosm expts
Cave expts

5. Who will care and what will the impact be if you are successful?
This will have direct relevance to the warfighter. The potential for deployment to the region in which bat hosts of SARS-related CoVs exist is high – countries include security hotspots (Myanmar, Bangladesh, Pakistan, Lao, Korea, Vietnam and Cambodia?). The ability to decontaminate and defuse these viruses will be useful in preventing potentially devastating illness. Furthermore, these technologies, if successful, can be adapted to hosts of other bat-origin CoVs (MERS, SARS and related prepandemic zoonotic strains), and potentially other zoonotic bat-origin viruses (Hendra, Nipah, EBOV). Finally, our approach is directly applicable to public health measures in the region to reduce the risk of spillover into the general population, as well as for food security by reducing the risk of viruses like Severe Acute Diarrheal Syndrome CoV spilling over from bats into intensive pig farms, and devastating and industry, leading to potential civil unrest.

6. How much will it cost and how long will it take?
Will insert this later after calculating and brainstorming.
46 months

D. Technical Plan:
*Outline and address all technical challenges inherent in the approach and possible solutions for overcoming potential problems. This section should provide appropriate specific milestones (quantitative, if possible) at intermediate stages of the project to demonstrate progress and a brief plan for accomplishment of the milestones.*
****Note: "The technical plan should demonstrate a deep understanding of the**

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**technical challenges and present a credible (even if risky) plan to achieve the program goal"**

Key Terms/Aspects to Emphasize in Abstract

- IACUC/IRB
  - DARPA wants to know who has experience w/ ACURO IACUC work.
    - EHA has multiple ACURO IACUC proposals (either approved or undergoing approval)
    - IRB also in place, just has to be modified
  - EHA has more than 50 years experience with IACUC (Jon, Billy, Linfa, & Ralph combined, including free-ranging and captive bat species) proposals and currently we have 3 DoD funded projects approved or undergoing ACURO review.

**Overview**

*Rationale for the SE Asian SARS-related CoV* – Rhinolophus *bat target system, and immune priming/boosting:* 1) Our group has shown that bats harbor a higher proportion of potentially highly heterogeneous zoonotic viruses than any other mammalian group (*1*), so that proof-of-concept for blocking viral spillover from this host group may lead to a bigger impact on global health security; 2) The *Rhinolophus* bats that harbor SARS like-CoVs are insectivorous, common, have a broad geographic range throughout Asia; roost in dense colonies at fixed, known locations, yet disperse each night over wide distances from these sites. Defusing the risk of viral shedding in the roost will also defuse the risk of viral shedding over the population range. This would be difficult for rodent or primate reservoirs; 3) Bats are mammalian hosts, therefore immune modulating drugs evaluated in people and rodents may also work on bats. This would be less likely for an insect vector; 4) Members of our collaborative group have worked together on bats and their viruses for over 15 years, with a total of >100 yrs experience focused on bat-origin zoonoses among the key personnel. We have published much of the seminal work on the bat origins of SARS, Nipah, Hendra, and MERS viruses, and have opened new boundaries in studies of bat host-viral relationships ecologically, immunologically and virologically; 5) The South and Southeast Asian region where these bats occur is a security hotspot, with active political and ethnic conflicts, and displaced populations in Bangladesh, Pakistan, Myanmar, Thailand, Indonesia, Philippines and other countries. This is a likely potential site for US warfighter deployment; 6) We have worked for 15 years on the SARS-related CoV – *Rhinolophus* bat system in China, demonstrating the origin of SARS-CoV within this host, the presence of SARSr-CoVs with remarkable sequence identity in the spike protein to SARS-CoV, their isolation and

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**Commented [BRS7]:** About 90,000 of the 550,000 deployed US military are in se asian, mostly japan and south korea.

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**Commented [BRS8]:** What abbreviation mean

characterization of their ability to bind and replicate efficiently in primary human lung airway cells. We have demonstrated that chimeric SARS-CoV backbone with spike protein from SARSr-CoVs from our cave sites in Yunnan Province can infect a humanized mouse model and cause SARS-like illness, and that clinical signs are not reduced with SARS monoclonal therapy or vaccination. Finally, we have demonstrated that people living up to 6 kilometers from our cave site have evidence of SARSr-CoV antibodies (3% seroprevalence in 200+ cohort), suggesting active spillover, and marking these viruses as a clear-and-present danger of a new SARS-like pandemic; 7) SARSr-CoVs are transmitted among bats via fecal-oral route, making sampling relatively easy (collection of fresh fecal pellets) and molecule or vaccine approaches feasible; 8) Proof-of-concept in this system may be rapidly scalable to other bat-coronavirus systems, e.g. MERS-CoV, SADS-CoV, and to other cave bat origin viruses.

Other important bat-origin zoonotic viruses (e.g. filoviruses, henipaviruses) have very rare spillover events - usually to a single index case, which makes validated prevention of spillover challenging. These viruses also show little strain diversity which makes modeling which evolutionary lines will be more high-risk, a challenge. SARSr-CoVs are diverse, with recombinants regularly identified in the field and lab. Furthermore, we have identified SARS-like strains in a single cave in Yunnan that harbor every gene found in the human SARS-CoV strains detected during the 2002-2003 epidemic. Within this bat population, an ideal evolutionary soup exists which can produce new human strains by high frequency RNA recombination and presents a perfect target for 21st generation intervention strategies.

Finally, we believe that alternative approaches to transmission blocking, e.g. CRISPER-Cas gene drives that are likely to be far less effective in bats because most bats are long-lived relative to their small size, long inter-generational periods (2-5 yrs) and low progeny (~1-2 pups per year). Gene drives would likely take many decades to run through a population, so that proof-of-concept of transmission blocking in the DARPA time scale wouldn't be possible. Furthermore, many bat species' populations mix readily or migrate which would disperse the impact of gene drives, whereas targeting a small number of caves in a region for molecule or vaccine delivery would cover a very large dispersal area.

**TA1: Develop and validate integrated, multiscale models that quantify the likelihood a human-capable virus will emerge from an animal reservoir residing in a "hot spot" geographic region**

The DEFUSE modeling and analytics team will develop models to evaluate the likelihood of bat caves harboring high-risk SARSr-CoVs, evaluate the probability of specific SARS-related CoV spillover, and identify the most effective strategy for inoculation of immune

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**Commented [BRS9]:** These viruses can either be cultured and/or recovered using reverse genetic strategies.

**Commented [J10]:** However, we may want to highlight that our approach of immune modulation may also reduce the viral load of filoviruses and henipaviruses in cave bat populations. Our work has found Ebola Reston in cave bats in the Philippines (Mineopterus spp.) and henipaviruses and filoviruses have been identified in insectivorous bats in China.

**Commented [BRS11]:** Filoviruses pretty diverse, although not anywhere near as diverse as cov. Is this a sampling thing or not likely remains unclear?

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**Commented [BRS12]:** Is this correct?

**Commented [J13]:** Yes, correct

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**Commented [L14]:** We need to provide background info about bat immunity and the track record of this group in the field

**Commented [L15]:** Peng: I am working on an important grant here in Singapore. Can you add a few points here? Thanks

boosting molecules and chimeric spike protein immune priming inocula.

We will collect specific data to inform our model building, validate assumptions and refine predictions. At the start of Yr 1, we will conduct a full inventory of host and virus distribution within our field sites, two caves in Yunnan Province, China. This builds on 8 years of surveillance in these caves and includes a cave in which we have identified all the genetic components of the 2002-2003 epidemic SARS-CoV distributed across a bat population. Two other caves will act as controls/comparison sites, in that we have not yet identified the high-risk SARSr-CoVs in these caves. We will assess: the population density, distribution and segregation of individual bats; changes in these daily, weekly and by season; viral prevalence and intensity in individuals; distribution and seasonal shedding of low- and high-risk SARSr-CoV strains, and how readily these are transmitted among bat species, age classes, genders; and using mark-recapture to assess metapopulation structure. To assess geographic distribution of bat hosts, we have access to biological inventory data on all bat caves in Southern China, as well as information on species distributions across SE Asia from the literature and museum records. We will use radio- GPS telemetry to identify the home range of each species of bat in the caves, to identify additional roost sites; to assess how widely the viral 'plume' could contaminate surrounding regions, and therefore how wide the risk zone is for the warfighter positioned close to bat caves.

We will build ecological niche models using the data above, and environmental and ecological correlates, and traits of cave species communities (eg. phylogenetic and functional diversity), to predict the species composition of bat caves across Southern China, South and SE Asia. We will validate these with data from the current project and data from PREDICT sampling in Thailand, Indonesia, Malaysia and other SE Asian countries. We will then use our unique database of bat host-viral relationships updated from our recent *Nature* paper (*1*) to assess the likelihood of low- or high-risk SARS-CoVs being present in a cave at any site across the region. At the end of Yr 1, we will use these analyses to produce a prototype app for the warfighter that identifies the likelihood of bats harboring dangerous viral pathogens based on these analyses. **The 'high-risk bats near me' app** will be updated as new host-viral surveillance data comes on line from our project and others, to ground-truth and fine-tune its predictive capacity. Specifically, our telemetry data on bat movement will be used to assess how often and how far bats from high-risk caves migrate to other colonies and potentially spread their high-risk strains.

The Wuhan Institute of Virology team will conduct viral testing on samples from all bat species in the caves as part of this inventory. Fecal, oral, blood and urogenital samples will be collected from bats using standard capture techniques as we have done for the last decade. In addition, tarps will be laid down in caves to collect fresh fecal and

**Commented [BRS16]:** Is surveillance in these other caves equally robust over the past 8 yrs?

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**Commented [PD17]:** Could add " We will continue monitoring the human population proximal to these caves to assess the rates of viral spillover, and ground-truth which specific CoVs are able to infect people

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urine samples. Assays will be designed to correlate viral load in an individual with viral shedding in a fecal sample. Once this is complete, surveys will continue largely on fecal samples so as not to disturb bat colonies and undermine longitudinal sampling capacity. Samples will be tested by PCR and spike proteins of all SARS-related CoVs sequenced. Analyses of phylogeny, recombination events, and further characterization of high-risk viruses (those with spike proteins close to SARS-CoV) will be carried out (REF). Isolation will be attempted on a subset of samples with novel SARSr-CoVs. Prof. Ralph Baric, UNC, will reverse engineer spike proteins in his lab to conduct binding assays to human ACE2 (the SARS-CoV receptor). Their group have also devised new strategies to culture SARS-like bat coronaviruses, allowing biological characterization of both high risk strains that can replicate in primary human cells and low risk strains that can only replicate in the presence of exogenous enhancers. Viral spike glycoproteins that bind receptor will then be inserted into SARS-CoV backbones, and inoculated into human cells and humanized mice to assess their capacity to cause SARS-like disease, and their ability to be blocked by monoclonal therapies, or vaccines against SARS-CoV ((PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993), The Baric group has also demonstrated that a nucleoside analogue inhibitor, GS-5734 (Gilead Inc), blocks epidemic, preepidemic and zoonotic SARS-CoV and SARS-like bat coronavirus replication in primary human airway cells and in mice (PMC5567817). Consequently, they will evaluate the ability of this drug to block replication of newly discovered pre-epidemic and zoonotic high risk strains. As the drug has been used to effectively treat Ebola virus infected patients (PMC4967715, PMC5583641) as well and has potent activity against Nipah and Hendra viruses (PMC5338263), an alternative intervention for military personnel is prophylactic treatment treatment prior to deployment into high risk settings.

The modeling team will use these data to build models of 1) risk of viral evolution and spillover, and 2) strategies to maximize inoculation strategy.
Data on the diversity of bat spike proteins, prevalence of recombinant CoVs, ability to bind and infect human cells, degree of clinical signs in mouse models, will be used to estimate evolutionary rates, rates of recombination, and capacity to generate novel strains capable of human infection. Using dynamic metapopulation models, we will estimate the flow of genes within each bat cave, based on the known host and viral assemblages. This will inform how rapidly new CoV strains with distinct phenotypic characteristics evolve. Because of our unique collaboration among world-class modelers, and virologists with coronavirus expertise, we will be able to test model predictions of viral capacity for spillover by conducting spike protein-based binding and cell culture experiments. The BSL-3 nature of work on SARSr-CoVs **makes our system highly cost-effective relative** to other bat-virus systems (e.g. Ebola, Marburg, Hendra,

**Commented [PD18]:** Ralph, Zhengli. If we win this contract, I do not propose that all of this work will necessarily be conducted by Ralph, but I do want to stress the US side of this proposal so that DARPA are comfortable with our team. Once we get the funds, we can then allocate who does what exact work, and I believe that a lot of these assays can be done in Wuhan as well…

**Commented [J19]:** Can we culture any bat coronaviruses? It might be good to broaden this so we can include novel beta CoVs that we may discover which look like they may be transmissible to people

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Nipah), which require BSL-4 level facilities for cell culture.

We will use modeling approaches informed by field and experimental data including the data above and other biological and ecological data, to estimate how rapidly high-risk SARSr-CoVs will re-colonize a bat population following immune boosting or priming. We will obtain model estimates of the frequency of inoculation required for both approaches, what proportion of a population needs to be reached to have effective viral dampening, and whether specific seasons, or locations within a cave would be most effective to treat. We will then model the efficacy of different delivery methods (spray, swab, cave mouth automated delivery, deliver to specific sections of a cave).

**TA2: Develop scalable approaches that target and suppress the animal virus in its reservoir(s) and/or vector(s), to reduce the likelihood of virus transmission into humans.**

Our goal is to use two approaches to defuse the potential for SARS-related CoVs to emerge in people**: 1) Immune Boosting:** using the unique immunological features of bats that our group has discovered, we will inoculate live bats in cave mesocosms with immune modulators to up-regulate their naïve immunity to suppress viral replication and shedding; **2) Immune Priming:** building on preliminary development of polyvalent chimeric recombinant molecules targeting diverse spike proteins from bat SARS-related CoVs, we will produce, and trial inoculation of live bats to suppress the replication and shedding of a broad range of dangerous SARS-related CoVs. Both lines of work will begin in Yr 1 and run parallel throughout the project.

Prof. Linfa Wang (Duke-NUS) will lead the work on immune boosting work, building on his pioneering work on bat immunity (*2*). This work provides evidence that that the long-term coexistence of bats and their viruses has led to an equilibrium between viral replication and host immunity, whereby bats have specifically down-regulated their innate immune system as part of the fitness cost of flight (the only true flying mammals) (*2*). The nature of the weakened but not entirely lost functionality of bat innate immunity factors like STING, a central DNA-interferon (IFN) sensing molecule, may have profound impact for bats to maintain the balanced state of "effective response", but not "over response" against viruses (*3*). A similar finding was also observed in bat IFNA studies, which is less abundant but was constitutively expressed without stimulation (*4*). Given native levels of SARSr-CoVs in individual bats with damped immunity, we propose to suppress bat SARSr-CoV by boosting bat innate immunity through the IFN pathway, and breaking the natural host-virus equilibrium. One of the potential problems with this approach is that it can lead to severe

**Commented [BRS20]:** IN the US, these recombinant SARS CoV are studied under BSL3, not BSL2, especially important for those that are able to bind and replicate in primary human cells.
In china, might be growin these virus under bsl2. US reseachers will likely freak out.

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**Commented [BRS21]:** Like what

**Commented [J22]:** Linfa: Do we know if these findings from Pteropus also apply to Rhinolophus? If not, we should be careful with the assertions we make…
We can make the argument that these two families of bats are more closely related than to other

**Commented [BRS23]:** Transient low level Chronic inflammation sounds better

inflammation. However, this is unlikely to occur in bats, because they also have a naturally dampened inflammation response (*5*).

Previous work has shown that aerosol spraying or intranasal inoculation of IFN or other small molecules has led to reduce viral loads in humans, ferrets and mouse models (12-14). We will therefore initially trial inoculation of live bats with synthetic double-stranded RNA (Poly I:C) and assay for reduced viral loads (DETAILS, CITATION). We will generate universal bat interferon and apply to bats in the lab. Interferon has been used extensively clinically if no viral-specific drugs are available, e.g. against filoviruses (11). Secondly, bat replication of SARSr-CoV is sensitive to interferon treatments, as has been shown in our previous work (12). We will attempt to boost bat IFN by blocking bat-specific IFN negative regulator. Bat IFNA is naturally constitutively expressed but cannot be induced to a high level (4). This is unique to bats. We think there should be a negative regulatory factor in the bat interferon production pathway. We propose using CRISPRi to find out that negative regulator and then screen for chemicals targeting at this gene. We will attempt to boost bat IFN by activating dampened bat-specific IFN production pathways which include DNA-STING-dependent and ssRNA-TLR7 dependent pathways. These changes have been proved to be bat-specific, suggesting that they are important in viruses/bats coexistence, and supported by our own work showing that a mutant bat STING restores antiviral functionality (3). By identifying small molecules to directly activate downstream of STING, we have chance to activate bat interferon and then help bats to clear viruses. Similar strategy applies to ssRNA-TLR7 dependent pathways. We will also attempt to boost bat IFN by activating functional bat IFN production pathways. We will investigate if there are other IFN production pathways in bats. We then boost bat immune responses by ligands specifically to these pathways, e.g. polyIC to TLR3-IFN pathway or 5'ppp-dsRNA to RIG-I-IFN pathway. A similar strategy has been tested successful in mouse model for SARS-CoV, IAV or HBV (*6, 7*). We believe treating wild bats with IFN-modulating small molecules by spraying is superior to other invasive strategies that might be considered by DARPA, including genome editing (CRISPR or RNAi), vaccination or DIP bats, in terms of its deployability and scalability. Finally, we will inoculate bats with fragments of non-bat Coronavirus (DETAILS).

Prof. Ralph Baric (UNC) will lead the immune priming work, building on his track record in reverse-engineering and manipulating SARS-CoV, MERS-CoV and other virus spike proteins over the last two decades . He will develop recombinant chimeric spike-proteins (*8*) based on SARSr-CoVs we have already identified, and those we will discover and characterize during project DEFUSE. RALPH – clearly I didn't really understand the details of your approach. Can you add a couple of paragraphs here and some citations please!

**Commented [BRS24]:** This could easily take longer than 3 years. Poly ic, IFN or any type of TLR agonist might be more robust. Might want to test in captive bats infected with SARS or select SARS like viruses, like SHC014, which we could provide.

**Commented [J25]:** If we're proposing experimental work with bats, we should spcify that we'll use SARS-CoV & SADS-CoV host species (Rhinolophus) which can be readily obtained by our Chinese colleagues at WIV

**Commented [BRS26]:** We have several papers showing importance of TLR3 and TLR4 signaling in control of SARS pathogenesis. PMC4447251, PMC5473747

**Commented [BRS27]:** Don't attack the other arm of the program. And I disagree that its superior to vaccination, which potentially provides long-term immunity.

**Commented [J28]:** Agree with Ralph – and this mechanism of delivery would probably be the same for vaccination attempts(intranasal or oral via grooming droplets from fur).

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**Commented [BRS29]:** The structure of the SARS-CoV spike glycoprotein has been solved and the addition of two proline residues at positions V1060P and L1061P stabilize the prefusion state of the trimer, including key neutralizing epitopes in the receptor binding domain (PMC5584442). In parallel, the spike trimers or the receptor binding domain can be incorporated into alphavirus vectored or nanoparticle vaccines for delivery, either as aerosols, in baits, or as large droplet delivery vehicles (PMC4058772, PMC5423355, PMC2883479, PMC5578707, PMC3014161). Initially, we will test various delivery vehicles in controlled conditions in bats in a laboratory setting, taking the best candidate forward for testing in the field.
The Baric laboratory has built recombinant S pike glycoproteins harboring structurally defined domains from SARS epidemic strains, pre-epidemic strains like SCH014 and zoonotic strains like HKU3. It is anticipated that recombinant S glycoprotein based vaccines harboring immunogenic blocks across the group 2B coronaviruses will induce broad based immune responses that simultaneously reduce genetically heterogeneous virus burdens in bats, thereby reducing disease risk (and transmission risk to people) in these animals for multiple years (PMC3977350, PMC2588415).

While there are clear advantages to working with fixed populations of cave-dwelling bats, molecule or vaccine delivery is technically challenging. Dr. Tonie Rocke, who developed, trialed, field-tested and rolled out the prairie dog plague vaccine (*9*), and is currently working on vaccines to bat rabies (*10, 11*) and white-nose syndrome, will manage a series of experiments in the lab and field to perfect a delivery system for both arms of TA2.

We have found that the immune dampening features are highly conserved in all bat species tested so far. Duke-NUS has established a breeding colony of cave nectar bats for experimental use (one of very few experimental bat breeding colonies in the world and the only one in SE Asia!). So our initial proof of concept test can be done in this experimental colony. We will then extend the test to a small group of wild-caught *Rhinolophus sinicus* bats at Wuhan Institute of Zoology. We (Prof. Wang) have previous experience conducting SARS-CoV infection experiments with bat species from the same genus in the BSL4 facility at the Australian Animal Health Laboratory in Australia (L.Wang, unpublished results). First, we will use our recently proven technology to design LIPS assays to the specific high zoonotic-risk SARSr-CoVs (*12*). We will conduct serological analysis on bats captured for infection experiments, to assess prior exposure to specific strains. These LIPS assays will be made available for use in people to assess exposure of the general population around bat caves in China, and for potential use by the warfighter to assess exposure to SARSr-CoVs during combat missions.

Finally, work on a delivery method for immunological countermeasures will be overseen by Dr. Tonie Rocke at the National Wildlife Health Center who has proven capacity to develop and take animal vaccines through to licensure (*9*). Using her captive Jamaican fruitbat colony (*10, 11*), Dr. Rocke will trial out the following strategies for delivery of the molecules, inocula proposed above: 1) aerosolization; 2) transdermally applied nanoparticles; 3) sticky edible spray that bats will groom from each other; 4) automated spray triggered by timers and movement detectors at critical cave entry points.. (Details and ideas please Tonie!). These approaches will then be tested on wild bats in our three cave sites in Yunnan Province. Fieldwork will be conducted under the auspices of Dr. Rocke, EHA field staff, and Dr. Yunzhi Zhang (Yunnan CDC, Consultant with EcoHealth Alliance). Sections of bat caves will be cordoned off and different application methods trialed out. A small number of bats will be captured and assayed for viral load and immune function after treatment, but so as not to disturb the colony, most viral load work will be conducted on fresh fecal pellets collected daily on the cave floor. EHA has unique access to these sites in Yunnan Province, with our field teams conducting surveillance there for around 10 years, under the guidance of Drs. Shi and Zhang. In year 1 of project DEFUSE, we will seek permission for these experimental inoculations in cave sites in Yunnan from the Provincial Forestry Department. We do

**Commented [J30]:** Eonycterus and Pteropus are evolutionarily related to Rhinolophus – we may want to have some language asserting our confidence that what we know about bat immunity so far will apply to SARS CoV reservoir species.

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**Commented [J31]:** We should be clear as to whether we're deploying a vaccine or an immune-modulator that promotes innate immunity. When we mention Tonie's experience with vaccine deployment it looks like that what we're planning.

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**Commented [J32]:** This probably won't work as bats may move throughout the cave – mixing application techniques. It would be more practical to use a different technique on each cave.

not envisage problems getting permission, as we have worked with the Forestry Department collaboratively for the last few years, we have the support of the Yunnan CDC, and we are releasing molecules that are not dangerous to people or wildlife.

E. Capabilities:

*A brief summary of expertise of the team, including subcontractors and key personnel. A principal investigator for the project must be identified, and a description of the team's organization. Include a description of the team's organization including roles and responsibilities. Describe the organizational experience in this area, existing intellectual property required to complete the project, and any specialized facilities to be used as part of the project. List Government furnished materials or data assumed to be available.*

****Note: While only the proposal requires an organization chart, it may be helpful to include in the abstract if we have the space.**

- This organization chart would include (as applicable): (1) the programmatic relationship of team members; (2) the unique capabilities of team members; (3) the task responsibilities of team members; (4) the teaming strategy among the team members; (5) key personnel with the amount of effort to be expended by each person during each year.

The lead institution for Project DEFUSE is EcoHealth Alliance, New York, an international research non-profit focused on emerging zoonotic diseases. The project will be led by PI Dr. Peter Daszak, who has 20+ years' experience managing lab, field and modeling research projects on emerging zoonoses, including as EHA institutional lead, Head of Modeling and Analytics, and member of the Executive Committee for the $130 million USAID EPT/PREDICT. Dr. Daszak will oversee and coordinate all project activities, as well as lead the modeling and analytic work for TA1. Dr. Billy Karesh, who has 40+ years' experience managing wildlife disease and zoonotic disease projects, will manage partnership activities and relationships and outreach. Dr. Jon Epstein, who has 15 years' experience working with bats and emerging zoonoses, including SARSr-CoVs and MERS-CoV, will coordinate work on bat immune priming and boosting trials. Dr. Kevin Olival and Dr. Noam Ross will manage and conduct the modeling and analytical approaches for this project. The EHA team has extensive experience working with the other team members on previous and current research including Dr. Wang (15+ years); Dr. Shi (15+ years); Dr. Baric (5+ years) and Dr. Rocke (15+ years)

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*Team:*

*Lead Organization: EcoHealth Alliance, New York*
*PI:* Peter Daszak Ph.D., President & Chief Scientist, EcoHealth Alliance, 3 months/year
*Key Personnel:*
Billy Karesh DVM, Executive VP for Policy & Health, 1 month/year
Kevin J. Olival Ph.D, VP for Scientific Research, 1 month/year
Jonathan H. Epstein DVM Ph.D., VP for Science & Outreach, 0.5 months/year
Carlos Zambrana-Torrelio Ph.D., Assoc. VP for Conservation & Health, 1 month/year
Noam Ross Ph.D., Senior Research Scientist, 2 months/year
Evan Eskew, Research Scientist, 2 months/year
Hongying Li, Program Coordinator, China Programs, 3 months/year
TBD Postdoctoral Researcher modeling and analysis, 12 months/year
TBD Research Assistant, 12 months/year
TBD Program Assistant, 12 months/year
Guangjian Zhu Ph.D., Consultant Field Lead, China Programs, 6 months/year
Yunzhi Zhang Ph.D., Consultant, Yunnan CDC, China, 2 months/year

*Subcontract #1: University of North Carolina Medical School*
*Organizational Lead:* Prof. Ralph Baric Ph.D., 2 months/year
Dr. Tim Sheahan (6 months/yr)
Dr. Amy Sims (4 months/yr)
Sarah Leist, Postdoctoral fellow (4 months/yr)
Boyd Yount, Research Analyst, 12 months/year
Trevor Scobey, Research Technician, 6 months/yr

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**Deleted:** ssistant

*Subcontract #2: USGS National Wildlife Health Center*
*Organizational Lead:* Tonie Rocke Ph.D., 2 months/year, no salary requested
TBD Research Technician, 9 months/year

*Subcontract #3: Duke NUS, Singapore*
*Organizational Lead:* Prof. Linfa Wang Ph.D., 2 months/year
XXX
TBD Research Assistant, 12 months/year
XXX

*Subcontract #4: Wuhan Institute of Virology, China*
*Organizational Lead:* Prof Zhengli Shi Ph.D., 2 months/year
Peng Zhou Ph.D., 2 months/year
TBD Research Assistant, 12 months/year

F. If desired, include a brief bibliography

*Links to relevant papers, reports, or resumes of key performers.*
Do not include more than two resumes as part of the abstract.
****Resumes count against the abstract page limit.**

**Dr. Peter Daszak** is President and Chief Scientist of EcoHealth Alliance, a US-based organization that conducts research and outreach programs on emerging zoonotic diseases. He has published over 300 scientific papers, including the first global map of EID hotspots, strategies to estimate unknown viral diversity in wildlife, predictive models of virus-host relationships, and evidence of the bat origin of SARS-CoV and other emerging viruses. Dr Daszak is Chair of the National Academy of Sciences, Engineering and Medicine's Forum on Microbial Threats and is a member of the Executive Committee and the EHA institutional lead for USAID-EPT-PREDICT. He serves on the NRC Advisory Committee to the USGCRP, the DHS CEEZAD External Advisory Board, and the WHO R&D Blueprint Pathogen Prioritization expert group, and has advised the Director for Medical Preparedness Policy on the White House National Security Staff on global health issues. Dr Daszak won the 2000 CSIRO medal for collaborative research.

**Prof. Ralph Baric** is a UNC Lineberger Comprehensive Cancer Center member and Professor in the UNC-Chapel Hill Department of Epidemiology and Department of Microbiology and Immunology. His work focuses on coronaviruses as models to study the genetics of RNA virus transcription, replication, persistence, and cross species transmission and pathogenesis. Dr. Baric and his group have developed a platform strategy to access the potential "preepidemic" risk associated with zoonotic virus cross species transmission potential and evaluation of countermeasure potential to control future outbreaks of disease (PMC5798318, PMC5567817, PMC5380844, PMC5578707, PMC4801244, PMC4797993). His work crosses the boundaries of microbiology, virology, immunology and epidemiology, looking especially at the population genetics of viruses to find the molecular building blocks for more effective vaccines.

****General Notes**:

- DARPA will evaluate proposals using the following criteria, listed in descending order of importance:

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1) **5.1.1. Overall Scientific and Technical Merit**

The proposed technical approach is innovative, feasible, achievable, and complete. Task descriptions and associated technical elements provided are complete and in a logical sequence with all proposed deliverables clearly defined such that a final outcome that achieves the goal can be expected as a result of award. The proposal identifies major technical risks and planned mitigation efforts are clearly defined and feasible. The proposed PREEMPT Risk Mitigation Plan effectively provides the following: an assessment of potential risks; proposed guidelines to ensure maximal biosafety and biosecurity; a risk management plan for responsible communications; and a plan to address how input from the Government and community stakeholders will be considered regarding communication and publication of potentially sensitive dual-use information.

2) **5.1.2. Potential Contribution and Relevance to the DARPA Mission**

The potential contributions of the proposed effort are relevant to the national technology base. Specifically, DARPA's mission is to make pivotal early technology investments that create or prevent strategic surprise for U.S. National Security. The proposer clearly demonstrates its capability to transition the technology to the research, industrial, and/or operational military communities in such a way as to enhance U.S. defense. In addition, the evaluation will take into consideration the extent to which the proposed intellectual property (IP) rights will potentially impact the Government's ability to transition the technology.

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3) **5.1.3. Cost Realism**

The proposed costs are realistic for the technical and management approach and accurately reflect the technical goals and objectives of the solicitation. The proposed costs are consistent with the proposer's Statement of Work and reflect a sufficient understanding of the costs and level of effort needed to successfully accomplish the proposed technical approach. The costs for the prime proposer and proposed subawardees are substantiated by the details provided in the proposal (e.g., the type and number of labor hours proposed per task, the types and quantities of materials, equipment and fabrication costs, travel and any other applicable costs and the basis for the estimates).

It is expected that the effort will leverage all available relevant prior research in order to obtain the maximum benefit from the available funding. For efforts with a likelihood of commercial application, appropriate direct cost sharing may be a positive factor in the evaluation. **DARPA recognizes that undue emphasis on cost may motivate proposers to offer low-risk ideas with minimum uncertainty and to staff the effort with junior**

**personnel in order to be in a more competitive posture. DARPA discourages such cost strategies.**

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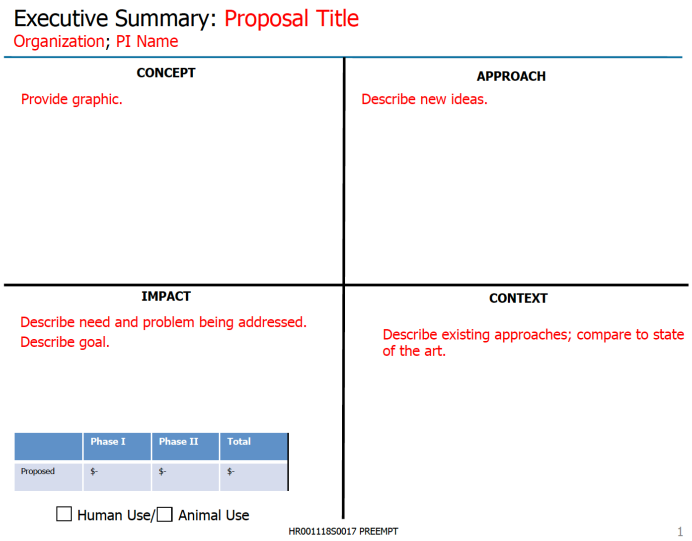


**Attachment 1: Executive Summary Slide template**

**Citations**


1. K. J. Olival *et al.*, Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646-650 (2017).
2. G. Zhang *et al.*, Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460 (2013).
3. J. Xie *et al.*, Dampened STING-Dependent Interferon Activation in Bats. *Cell host & microbe*, (2018).
4. P. Zhou *et al.*, Contraction of the type I IFN locus and unusual constitutive expression of IFN-αin bats. *Proceedings of the National Academy of Sciences of the United States of America*, 201518240-201518246 (2016).
5. M. Ahn, J. Cui, A. T. Irving, L.-F. Wang, Unique Loss of the PYHIN Gene Family in Bats Amongst Mammals: Implications for Inflammasome Sensing. *Scientific Reports* **6**, (2016).
6. J. Zhao *et al.*, Intranasal Treatment with Poly(I.C) Protects Aged Mice from Lethal Respiratory Virus Infections. *Journal of Virology* **86**, 11416-11424 (2012).

7. J. Wu *et al.*, Poly(I:C) Treatment Leads to Interferon-Dependent Clearance of Hepatitis B Virus in a Hydrodynamic Injection Mouse Model. *Journal of Virology* **88**, 10421-10431 (2014).
8. X. F. Deng *et al.*, A Chimeric Virus-Mouse Model System for Evaluating the Function and Inhibition of Papain-Like Proteases of Emerging Coronaviruses. *Journal of Virology* **88**, 11825-11833 (2014).
9. T. E. Rocke *et al.*, Sylvatic Plague Vaccine Partially Protects Prairie Dogs (Cynomys spp.) in Field Trials. *Ecohealth* **14**, 438-450 (2017).
10. B. Stading *et al.*, Protection of bats (Eptesicus fuscus) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. *Plos Neglect. Trop. Dis.* **11**, (2017).
11. B. R. Stading *et al.*, Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Free-tailed bat (Tadarida brasiliensis). *Vaccine* **34**, 5352-5358 (2016).
12. P. Zhou *et al.*, Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. *Nature* **In press**, (2018
).

**Commented [J36]:** Is this reference correct? I couldn't find it online.