Published in final edited form as:

J Environ Pathol Toxicol Oncol. 2013 ; 32(1): 29–39.

Chronic Chlorpyrifos Exposure Does Not Promote Prostate Cancer in Prostate Specific PTEN Mutant Mice

Robert U. Svensson¹, Nadine L. Bannick¹, Maximo J. Marin¹, Larry W. Robertson², Charles F. Lynch³, and Michael D. Henry^{1,4,*}

¹Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA 52242;

²Department of Occupational and Environmental Health, College of Public Health, University of Iowa, 100 Oakdale Campus, Coralville, IA 52241;

³Department of Epidemiology, College of Public Health, Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA 52242;

⁴Department of Pathology, Holden Comprehensive Cancer Center and Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242

Abstract

Environmental factors are likely to interact with genetic determinants to influence prostate cancer progression. The Agricultural Health Study has identified an association between exposure to organophosphorous pesticides including chlorpyrifos, and increased prostate cancer risk in pesticide applicators with a first-degree family history of this disease. Exploration of this potential gene-environment interaction would benefit from the development of a suitable animal model. Utilizing a previously described mouse model that is genetically predisposed to prostate cancer through a prostate-specific heterozygous PTEN deletion, termed C57/Luc/*Pten*^{p+/-}, we used bioluminescence imaging and histopathological analyses to test whether chronic exposure to chlorpyrifos in a grain-based diet for 32 weeks was able to promote prostate cancer development. Chronic exposure to chlorpyrifos in the diet did not promote prostate cancer development in C57/Luc/*Pten*^{p+/-} mice despite achieving sufficient levels to inhibit acetylcholinesterase activity in plasma. We found no significant differences in numbers of murine prostatic intraepithelial neoplasia lesions or disease progression in chlorpyrifos versus control treated animals up to 32 weeks. The mechanistic basis of pesticide-induced prostate cancer may be complex and may involve other genetic variants, multiple genes, or nongenetic factors that might alter prostate cancer risk during pesticide exposure in agricultural workers.

Keywords

organophosphate; pesticide; chlorpyrifos; prostate; cancer; PTEN

I. INTRODUCTION

Prostate cancer is the most commonly diagnosed visceral neoplasm and second leading cause of cancer death in U.S. males.¹ Intensive efforts are underway to identify both the genetic and environmental factors underlying its progression from an indolent, subclinical

disease to a life-threatening one. A number of studies have shown that farmers as a group have an elevated risk of prostate cancer possibly due to occupational exposures to various chemicals including pesticides and herbicides. The Agricultural Health Study (AHS), a very large prospective cohort study of farmers in Iowa and North Carolina, has reported an interesting association between exposure to a group of organophosphorous pesticides (OPs) and prostate cancer risk specifically in cases with a first-degree family history of this disease.² In that report, four of the six pesticides are chemically related OP insecticides: chlorpyrifos (CPF), coumaphos, fonofos, and phorate. Additional studies provide detailed analysis of exposure levels to these agents including CPF.³ Furthermore, these associations were not observed among those without a family history of the disease. Thus, these findings suggest a potential gene-environment interaction experienced by agricultural workers to alter prostate cancer risk.

A potential candidate for this interaction may involve the phosphatase and tensin homologue deleted on chromosome ten (PTEN), phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT) signaling pathway (PTEN-PI3K-AKT signaling pathway). Deregulation of the PI3K-AKT pathway is increasingly implicated in carcinogenesis (for review, see Ref. 4) and somatic mutations in PTEN, a tumor suppressor that acts as a brake on this pathway, have been frequently described in prostate cancer patients.⁵⁻⁷ However, whether OPs are able to cooperate with PTEN deletions and PI3K-AKT signaling to facilitate prostate carcinogenesis has not been investigated to date.

CPF, coumaphos, fonofos, and phorate are chemically related OPs that are widely used agricultural pesticides worldwide. CPF, for example, is used on major crops such as corn and soybeans that are abundant in the Midwestern states. Although discontinued for residential use in 2002 due to potential health concerns, the EPA estimates about three million kilograms of CPF is applied annually for agricultural purposes and is present in hundreds of commercial formulations (http://www.epa.gov/oppsrrd1/REDs/factsheets/chlorpyrifos_fs.htm). OPs are metabolically activated to an oxon form that is an irreversible inhibitor of cholinesterases and are thus potent neurotoxins.⁸ Extensive investigations have explored the potential for background exposure to CPF to cause adverse neurodevelopmental outcomes, but this remains unclear.⁹ Toxicology studies have generally shown that CPF is not genotoxic, mutagenic, or carcinogenic using standard assays (reviewed in Ref. 9). However, standard rodent cancer bioassays are not designed to detect prostate cancer, and it is likely these standard toxicologic assays do not account for interactions between these agents and genetic or epigenetic factors in humans that might combine to increase prostate cancer risk.

Going beyond associating an environmental exposure with a disease outcome to understanding the mechanistic basis for this association requires experimental approaches that may be difficult or impossible in humans. Therefore, the purpose of this study was to determine the effect of one of the most commonly used OPs worldwide, CPF, on prostate cancer development in a biologically relevant animal model. To this end we utilized a mouse model that is (i) genetically predisposed to prostate cancer based on a prostate-specific heterozygous deletion in the tumor suppressor *Pten* (C57/Luc/*Pten*^{p+/-}) and (ii) allows for bioluminescence imaging (BLI) of prostate tumorigenesis, facilitating the noninvasive detection of pathological changes in the prostate.¹⁰ In this study, we examined the effects of chronic dietary CPF exposure on prostate cancer development in C57/Luc/*Pten*^{p+/-} mice and we demonstrate the feasibility of using this mouse model for such a study.

II. MATERIALS AND METHODS

A. Animals

All animal procedures were performed with approval from the University of Iowa Animal Care and Use Committee and by the authors' Institutional Review Board. *C57/Luc/Pten^P* mice have been described previously.¹⁰ Age-matched cohorts of *C57/Luc/Pten^P* mice were generated using Jackson Laboratories JAX Speed Expansion Service. Mice were received from Jackson labs at five weeks old and genotyped for expression of *PbCre4+* as previously described.¹⁰ At eight weeks of age, animals underwent BLI and were randomized into two groups to be placed onto their respective diets (10 mice for CPF and eight mice for control chow).

B. Bioluminescence Imaging

All BLI was performed in an IVIS100 imaging system (Caliper Life Sciences, Hopkinton, Massachusetts) as described previously.¹⁰ Briefly, mice were anesthetized in a chamber of 3% isoflurane and maintained under 3% isoflurane on a 37°C heated stage for the duration of the imaging and imaged 8 min following D-luciferin injection using a 20 cm field of view and an exposure time varying from 10 to 30 s. Bioluminescence intensity values were calculated by measuring photon flux (photons/second) in a region of interest surrounding the bioluminescence signal with the region of interest edge defined as 5% maximum signal using Living Image software (version 3.2).

C. Diet Formulation

CPF and control diets were formulated by Harlan Laboratories Teklad Diet using an original NIH-31 open formula irradiated base diet 7017. The NIH-31 open formula autoclavable diet is formulated for maintenance, growth, reproduction, and lactation of rats and mice and is used at the NIH as the standard reference diet for biological and biomedical research. 7017 is supplemented with additional vitamins to ensure nutritional adequacy after autoclaving. CPF solubilized in corn oil (2% final concentration) was added to the 7017 base diet to achieve a final concentration of 0.025% CPF (250 ppm) and oven dried for 2 h at 60°C. A control diet containing no CPF was formulated under identical conditions. Formulated diets were analyzed for CPF concentration by high-pressure liquid chromatography with a reference standard purchased from Chem Service at the University of Iowa hygienic laboratory.

D. Histopathology

Prostates were fixed in 4% paraformaldehyde overnight, transferred to 30% ethanol, embedded in paraffin, and stained with Hematoxylin & Eosin (H&E). Specifically, 5µM prostate sections were prepared stepwise through the entire lobe and consecutively stained and analyzed in order to capture changes throughout the entire length of the ducts. Prostate sections were imaged using an Olympus BX-61 microscope (Olympus). Prostate pathology was assessed according to the criteria established by Shappell et al.¹¹

E. Acetylcholinesterase Activity Assay

Activity of acetylcholinesterase (AChE) in plasma harvested from *C57/Luc/Pten^P* mice was calculated using a QuantiChrom acetylcholinesterase assay kit (Bioassay Systems, Harvard) according to manufacturer's instructions. Briefly, plasma was isolated from whole blood via centrifugation (10 min at 13,000 rpm), following a submandibular cheek bleed of *C57/Luc/Pten^P* mice; 5 µl of plasma was used for subsequent AChE activity analysis.

F. Statistical Analyses

All statistical analyses were performed using Student's *t*-test.

III. RESULTS

A. Effects of Chronic Dietary Chlorpyrifos Exposure on Acetylcholinesterase Activity

In the *C57/Luc/Pten^P* mouse model, PTEN deletion is coupled to activation of firefly luciferase from the ROSA26 promoter in the same prostate epithelial cell, enabling BLI of prostate cancer tumorigenesis.¹⁰ Because bioluminescence intensity is tightly correlated with prostatic epithelial proliferation, BLI facilitates the noninvasive detection of pathological changes in the prostate. We have previously demonstrated that mice heterozygous for PTEN expression in the prostate (*C57/Luc/Pten^{P+/-}*) develop focal murine prostatic intraepithelial neoplasia (mPIN), which can be distinguished from normal prostate glands by small increases in bioluminescence signal intensity. Furthermore, in this model, mPIN lesions exhibit activation of PI3K-AKT signaling but never progress to invasive adenocarcinoma.¹⁰ We therefore reasoned that *Pten^{P+/-}* mice would provide a suitable model to determine whether chlorpyrifos exposure might accelerate the development of mPIN or promote the development of prostatic adenocarcinoma.

To determine the effect of chronic exposure of CPF on prostate cancer progression, we utilized a dietary route of administration of CPF. CPF has been evaluated in a number of chronic dietary exposure studies in mice at concentrations ranging from 0 to 250 ppm, corresponding to 31.7–55.1 mg/kg/d (reviewed in Ref. 9). In these studies, there was no increase in mortality associated with the diet at the highest exposure levels. Therefore, we formulated CPF chow at a target level of 250 ppm, which after testing by the University of Iowa hygienic laboratory was found to be ~220 ppm. We also formulated a control diet containing no CPF under identical conditions for consistency. Next, we generated cohorts of *C57/Luc/Pten^{P+/-}* mice and randomized them into two groups at eight weeks of age based on bioluminescence signal intensity. Mice were then placed on either a CPF diet or control diet ad libitum and monitored for general health status and prostate cancer progression via BLI (Fig. 1) and histology (Figs. 2 and 3). We found that mice fed CPF were not averse to the diet and consumed similar levels of food compared to control diet-fed mice (data not shown). However, we did detect an initial decrease in body weight of mice fed CPF at two weeks postdiet [Fig. 1(a)]. Following this initial decrease, CPF-fed mice became accustomed to the diet and consistently gained body weight for the duration of the study such that average body weights at the 32 week endpoint were almost identical [Fig. 1(a)]. CPF is metabolically converted by oxidative desulfuration into chlorpyrifos-oxon (CPO) and the insecticidal action of chlorpyrifos stems from inhibition of butyrylcholinesterase and acetylcholinesterase (AChE) by CPO, resulting in severe cholinergic toxicity.⁸ CPO is then inactivated by paraoxonase 1, and importantly, this enzyme exhibits relatively high activity in the C57Bl/6 strain.^{12,13} Therefore, to determine whether the exposure levels of CPF in *C57/Luc/Pten^P* mice is sufficient to elicit this response, we measured the activity of AChE in plasma collected from either CPF-fed or control diet-fed animals [Fig. 1(b)]. We found that the activity of AChE was reduced threefold in mice fed CPF compared to control diet-fed mice [Fig. 1(b)]. These data demonstrate that CPF is appropriately metabolized *in vivo* in *C57/Luc/Pten^P* mice and that exposure levels are sufficient to significantly inhibit AChE. We also monitored CPF-fed animals for clinical signs of toxicity throughout the duration of the study and found that 1 of 10 animals treated with CPF began to display signs of neurotoxicity at 28 weeks post-diet initiation.

B. Effects of Chronic Dietary Chlorpyrifos Exposure on Prostate Cancer Progression

To determine whether CPF was having an effect on prostate cancer progression, we performed BLI biweekly on CPF-fed and control diet-fed C57/Luc/*Pten*^{+/−} animals [Figs. 1(c), 4(c), and 4(d)]. In both treatment groups, we detected a continuous increase in bioluminescence signal intensity consistent with asynchronous expression of PbCre4+ and the development of focal mPIN as previously reported.¹⁰ However, we did not detect any significant difference in bioluminescence intensity between CPF-fed and control diet-fed mice throughout the duration of the study, indicating that chronic exposure to CPF did not have an effect on epithelial proliferation or mPIN development in C57/Luc/*Pten*^{+/−} animals for up to 32 weeks. To correlate our BLI findings to histopathology and to determine whether there were any underlying changes to microscopic prostate cancer progression such as invasion, we analyzed hematoxylin and eosin (H&E) stained prostate sections from CPF-fed and control diet-fed animals [Figs. 2(a) and 2(b)]. Specifically, prostate sections were prepared stepwise through the entire lobe and consecutively stained and analyzed in order to capture changes throughout the entire length of the ducts. The majority of prostate glands in both treatment groups appeared normal and were composed of a single layer of epithelial cells constrained within a basement membrane. However, we did detect discrete focal mPIN lesions and accompanying fibrosis in anterior prostate [Fig. 2(a)] and dorsal, lateral and to a lesser extent ventral prostate [Fig. 2(b)] in all of the animals analyzed, as previously reported.¹⁰ mPIN lesions were characterized by proliferation and stratification of epithelial cells displaying nuclear atypia according to the criteria established by Shappell et al.¹¹ Quantitation of mPIN lesions from serial prostate sections of CPF-fed and control diet-fed animals revealed no significant difference in the numbers of mPIN lesions in CPF-fed versus control diet-fed C57/Luc/*Pten*^{+/−} animals (Fig. 3). Furthermore, we did not detect any disease progression beyond mPIN in either of the treatment groups. Taken together, these data demonstrate that chronic exposure to CPF under the conditions used within this study is not able to promote the development of prostate cancer in a genetically predisposed animal model, C57/Luc/*Pten*^{+/−} mice, and highlight that the ability of CPF to alter prostate cancer risk may involve other genetic alterations either in combination or independent of PTEN status.

IV. DISCUSSION

How genetic determinants of prostate cancer may interact with pesticide exposure to alter prostate cancer risk is an important question in cancer epidemiology. In this study, we sought to determine the effect of chronic exposure to one the most commonly used insecticides worldwide, CPF, on prostate cancer development in mice genetically predisposed to prostate cancer via a prostate specific heterozygous PTEN deletion (C57/Luc/*Pten*^{+/−}). Using both BLI and histopathological analyses, we found that chronic exposure to CPF in the diet for 32 weeks did not promote prostate cancer development in C57/Luc/*Pten*^{+/−} mice despite being able to significantly inhibit AChE activity in plasma.

Initiated in 1993 and continuing to date, the AHS is the largest prospective cohort study undertaken to evaluate the relationship between agricultural exposures and disease.¹⁴ It involves 89,658 pesticide applicators and their spouses in Iowa and North Carolina enrolled between 1993 and 1997, representing 82% of the targeted population. Among the many findings in this study, prostate cancer was linked (significant interaction odds ratios) to exposure to six pesticides, among 50 evaluated, in patients with a first-degree family history of prostate cancer.² Following the initiation of our studies, Koutros et al. reported the interaction among pesticide use, 8q24 variants, and prostate cancer risk using a nested case control study and found that insecticides, particularly OPs, were significantly linked to increased prostate cancer risk (estimated odds ratio).¹⁵ This was the first study to describe

that the association between 8q24 SNPs and prostate cancer is modified by pesticide use. Several GWAS have identified genetic variants in the chromosomal region 8q24 in prostate cancer patients.¹⁶⁻¹⁹ Interestingly, the genetic variants of 8q24 are located in a region with no known protein coding gene but are located ~200 kb from the MYC gene, a well-established oncogene. Studies have demonstrated that pesticides can influence the expression of c-Myc,²⁰ however, whether c-Myc acts as a putative oncogene responsible for pesticide-induced prostate cancer remains unknown.

Although GWAS have not implicated PTEN as a germ line risk allele for prostate cancer, abundant evidence indicates that somatic mutation in PTEN is a common event in prostate cancer progression.^{7,21-23} PTEN functions primarily as a lipid phosphatase that negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate (PIP3) and thereby functions as a tumor suppressor by inhibiting the PI3-AKT signaling pathway.²⁴⁻²⁶ Recent studies have indicated that this signaling pathway is altered in as many as 42% of primary tumors and 100% of metastases.²⁷ Furthermore, prostate-specific deletion of PTEN in mice leads to prostate adenocarcinoma.²⁸ Thus, we reasoned that activation of the PI3K-Akt signaling pathway, via deletion of PTEN, may act as a surrogate for the genetic association between OP exposure and prostate cancer risk. Because we have recently shown that disease progression is slow when the prostate-specific PTEN mutation is expressed in the C57Bl/6 background, and PTEN heterozygotes only sporadically develop pre-neoplastic mPIN lesions, we thought that this would be an ideal background to evaluate the effects of CPF on disease progression.¹⁰ Moreover, subclinical, latent prostate cancer is highly prevalent and prostate progression is generally slow, so that clinical presentation may be a product of progression, rather than initiation.²⁹

We found that chronic exposure to CPF did not promote the development of prostate cancer in C57/Luc/*Pten*^{+/−} mice up to 32 weeks. We terminated the study at 32 weeks based on the fact that we had anticipated changes in the bioluminescence signal to be evident at this time if significant disease progression had occurred based on our previous studies.¹⁰ Quantitative histologic analyses determined that CPF was not able to promote an increase in the number of mPIN lesions in prostates nor was it able to promote the progression from mPIN to microinvasive cancer or prostate adenocarcinoma. Although this exploratory study was not sufficiently powered to detect weak effects, with the current sample size the study would have 67% power to detect a cancer risk of 5% in controls versus 55% in chlorpyrifos using a one-sided Fisher's exact test performed at the 5% level of significance, these data are the first to specifically evaluate the effect of CPF on prostate cancer in mice and provide initial evidence that the mechanistic basis of CPF-induced prostate cancer in humans may not solely involve PI3K-AKT activation. It is possible that the gene-environment interaction involving CPF is related to other genetic determinants such as *myc* as suggested by Koutros et al.¹⁵ Future experiments using relevant models such as the Hi-Myc mouse model of prostate cancer³⁰ may aid in identifying a role for *myc* in this process. Alternatively, since we have only evaluated the active agent CPF here, it is possible that other constituents within the farm-grade formulations of OPs are involved in the observed gene-environment interaction. Furthermore, effects of chronic exposure to CPF may develop after longer latency such that mice may need to be exposed for much longer durations, as we have only evaluated its effects up to 32 weeks in this study.

Although our studies here are negative, we do not conclude that CPF or other OPs do not have effects on prostate cancer initiation or progression. We developed C57/Luc/*Pten*^P mice to assess how genetic and environmental factors might affect prostate cancer progression and demonstrate the feasibility of doing so with this model in this study. Prostate cancer is a complex disease and one can postulate a number of mechanisms by which OPs might influence disease progression. Toxicology studies have generally shown that CPF is not

genotoxic or carcinogenic using standard assays (reviewed in Ref. 9). However, it should be noted that the mouse prostate is a difficult organ in which to observe carcinogenic effects since spontaneous prostate cancer is exceedingly rare and may exhibit long latency.³¹ However, CPF may have indirect effects on mutagenesis or carcinogenesis as they have been shown to induce reactive oxygen species by numerous studies (reviewed in Ref. 9). Other mechanisms by which organophosphorous chemicals may influence prostate cancer include endocrine disruption and interactions with the androgen receptor or potentially epigenetic effects.³²⁻³⁴

Acknowledgments

This work was supported by pilot grants from the University of Iowa Cancer and Aging Program (Grant No. 5 P20 CA103672), Center for Health Effects of Environmental Contamination, and Grants No. R21 CA137490, No. RC1 ES018097 (to MDH), No. P42 ES 013661(LWR), and No. P30 ES05605 (LWR). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the granting agencies. We thank members of the Henry lab for comments on the manuscript, and Michael R. Miller for assistance with the figures.

REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010; 60(5):277–300. [PubMed: 20610543]
2. Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, Knott C, Thomas K, Hoppin JA, Barker J, Coble J, Sandler DP, Blair A. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol.* 2003; 157(9):800–14. [PubMed: 12727674]
3. Thomas KW, Dosemeci M, Hoppin JA, Sheldon LS, Croghan CW, Gordon SM, Jones ML, Reynolds SJ, Raymer JH, Akland GG, Lynch CF, Knott CE, Sandler DP, Blair AE, Alavanja MC. Urinary biomarker, dermal, and air measurement results for 2,4-D and chlorpyrifos farm applicators in the Agricultural Health Study. *J Expo Sci Environ Epidemiol.* 2009; 20:119–134. [PubMed: 19240759]
4. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer.* 2002; 2(7):489–501. [PubMed: 12094235]
5. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science.* 1997; 275(5308):1943–7. [PubMed: 9072974]
6. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet.* 1997; 15(4):356–62. [PubMed: 9090379]
7. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res.* 1997; 57(22):4997–5000. [PubMed: 9371490]
8. Amitai G, Moorad D, Adani R, Doctor BP. Inhibition of acetylcholinesterase and butyrylcholinesterase by chlorpyrifos-oxon. *Biochem Pharmacol.* 1998; 56(3):293–9. [PubMed: 9744565]
9. Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, Coyle J, McKhann G, Mobley WC, Nadel L, Neubert D, Schulte-Hermann R, Spencer PS. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit Rev Toxicol.* 2008; 38(Suppl 2):1–125. [PubMed: 18726789]
10. Svensson RU, Haverkamp JM, Thedens DR, Cohen MB, Ratliff TL, Henry MD. Slow disease progression in a C57BL/6 pten-deficient mouse model of prostate cancer. *Am J Pathol.* 2011; 179(1):502–12. [PubMed: 21703427]
11. Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R, Ward JM, Cardiff RD. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting

- of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer Res.* 2004; 64(6):2270–305. [PubMed: 15026373]
12. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature.* 1998; 394(6690):284–7. [PubMed: 9685159]
 13. Wehner JM, Murphy-Erdosh C, Smolen A, Smolen TN. Genetic variation in paraoxonase activity and sensitivity to diisopropylphosphofluoridate in inbred mice. *Pharmacol Biochem Behav.* 1987; 28(2):317–20. [PubMed: 2825223]
 14. Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, Pennybacker M, Rothman N, Dosemeci M, Bond AE, Blair A. The Agricultural Health Study. *Environ Health Perspect.* 1996; 104(4):362–9. [PubMed: 8732939]
 15. Koutros S, Beane Freeman LE, Berndt SI, Andreotti G, Lubin JH, Sandler DP, Hoppin JA, Yu K, Li Q, Burdette LA, Yuenger J, Yeager M, Alavanja MC. Pesticide use modifies the association between genetic variants on chromosome 8q24 and prostate cancer. *Cancer Res.* 2010; 70(22):9224–33. [PubMed: 20978189]
 16. Witte JS. Multiple prostate cancer risk variants on 8q24. *Nat Genet.* 2007; 39(5):579–80. [PubMed: 17460686]
 17. Yeager M, Chatterjee N, Ciampa J, Jacobs KB, Gonzalez-Bosquet J, Hayes RB, Kraft P, Wacholder S, Orr N, Berndt S, Yu K, Hutchinson A, Wang Z, Amundadottir L, Feigelson HS, Thun MJ, Diver WR, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Cangel-Tassin G, Cussenot O, Valeri A, Andriole GL, Crawford ED, Haiman CA, Henderson B, Kolonel L, Le Marchand L, Siddiq A, Riboli E, Key TJ, Kaaks R, Isaacs W, Isaacs S, Wiley KE, Gronberg H, Wiklund F, Stattin P, Xu J, Zheng SL, Sun J, Vatten LJ, Hveem K, Kumle M, Tucker M, Gerhard DS, Hoover RN, Fraumeni JF Jr, Hunter DJ, Thomas G, Chanock SJ. Identification of a new prostate cancer susceptibility locus on chromosome 8q24. *Nat Genet.* 2009; 41(10):1055–7. [PubMed: 19767755]
 18. Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Xu J, Blondal T, Kostic J, Sun J, Ghosh S, Stacey SN, Mouy M, Saemundsdottir J, Backman VM, Kristjansson K, Tres A, Partin AW, Albers-Akkers MT, Godino-Ivan Marcos J, Walsh PC, Swinkels DW, Navarrete S, Isaacs SD, Aben KK, Graif T, Cashy J, Ruiz-Echarri M, Wiley KE, Suarez BK, Witjes JA, Frigge M, Ober C, Jonsson E, Einarsson GV, Mayordomo JI, Kiemeny LA, Isaacs WB, Catalona WJ, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet.* 2007; 39(5):631–7. [PubMed: 17401366]
 19. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearnhead P, Yu K, Chatterjee N, Wang Z, Welch R, Staats BJ, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cangel-Tassin G, Cussenot O, Valeri A, Andriole GL, Gelmann EP, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover R, Hunter DJ, Chanock SJ, Thomas G. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet.* 2007; 39(5):645–9. [PubMed: 17401363]
 20. Maire MA, Rast C, Landkocz Y, Vasseur P. 2,4-Dichlorophenoxyacetic acid: effects on Syrian hamster embryo (SHE) cell transformation, c-Myc expression, DNA damage and apoptosis. *Mutat Res.* 2007; 631(2):124–36. [PubMed: 17540612]
 21. Gray IC, Phillips SM, Lee SJ, Neoptolemos JP, Weissenbach J, Spurr NK. Loss of the chromosomal region 10q23–25 in prostate cancer. *Cancer Research.* 1995; 55(21):4800–3. [PubMed: 7585509]
 22. Trybus TM, Burgess AC, Wojno KJ, Glover TW, Macoska JA. Distinct areas of allelic loss on chromosomal regions 10p and 10q in human prostate cancer. *Cancer Res.* 1996; 56(10):2263–7. [PubMed: 8625295]
 23. Ittmann M. Allelic loss on chromosome 10 in prostate adenocarcinoma. *Cancer Res.* 1996; 56(9):2143–7. [PubMed: 8616863]
 24. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. *Proc Natl Acad Sci U S A.* 1998; 95(23):13513–8. [PubMed: 9811831]

25. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*. 1998; 95(1):29–39. [PubMed: 9778245]
26. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem*. 1998; 273(22):13375–8. [PubMed: 9593664]
27. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino PT, Sander C, Sawyers CL, Gerald WL. Integrative genomic profiling of human prostate cancer. *Cancer Cell*. 2010; 18(1):11–22. [PubMed: 20579941]
28. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS, Liu X, Wu H. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell*. 2003; 4(3):209–21. [PubMed: 14522255]
29. Delongchamps NB, Singh A, Haas GP. The role of prevalence in the diagnosis of prostate cancer. *Cancer Control*. 2006; 13(3):158–68. [PubMed: 16885911]
30. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R, Thomas GV, Sawyers CL. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. *Cancer Cell*. 2003; 4(3):223–38. [PubMed: 14522256]
31. Shirai T, Takahashi S, Cui L, Futakuchi M, Kato K, Tamano S, Imaida K. Experimental prostate carcinogenesis - rodent models. *Mutat Res*. 2000; 462(2-3):219–26. [PubMed: 10767633]
32. Hodgson E, Rose RL. Organophosphorus chemicals: potent inhibitors of the human metabolism of steroid hormones and xenobiotics. *Drug Metab Rev*. 2006; 38(1-2):149–62. [PubMed: 16684654]
33. Tamura H, Yoshikawa H, Gaido KW, Ross SM, DeLisle RK, Welsh WJ, Richard AM. Interaction of organophosphate pesticides and related compounds with the androgen receptor. *Env Health Perspect*. 2003; 111(4):545–52. [PubMed: 12676613]
34. Ray DE, Richards PG. The potential for toxic effects of chronic, low-dose exposure to organophosphates. *Toxicol Lett*. 2001; 120(1-3):343–51. [PubMed: 11323193]

FIGURE 1.

Assessing the effect of chronic CPF exposure on prostate tumorigenesis in *C57/Luc/Pten^{D+/-}* mice via bioluminescence imaging (BLI). **(a)** Body weights of mice fed CPF decreased at two weeks postdiet but then consistently increased for the duration of the study similar to controls. **(b)** Activity of AChE in plasma from mice fed CPF is significantly reduced at four weeks and 32 weeks. (***) $p < 0.001$; Student's t-test.) **(c)** Bioluminescent overlay images from the IVIS100 (top panel) and quantitation of bioluminescence signal (bottom) reveal similar increases in bioluminescence intensity in CPF- and control-treated mice up to 32 weeks. Y-axis represents bioluminescence signal as log photon flux (photons/sec) from the region of interest surrounding the prostate.

FIGURE 2.

Histopathology of prostates from CPF- and control-treated C57/Luc/*Pten*^{p+/-} mice. CPF- and control diet-fed mice were analyzed for pathological changes to the prostate via H&E staining. **(a)** Representative histopathology of anterior prostates from CPF-treated (left) and control-treated (right) animals. Arrows denote examples mPIN lesions. **(b)** Representative histopathology of dorsal (D), lateral (L), and ventral (V) prostates from CPF-treated (left) and control-treated (right) animals. Arrows denote examples of mPIN lesions. Degree of mPIN in all lobes is similar between CPF- and control-treated C57/Luc/*Pten*^{p+/-} mice. Bars: 200μm.

FIGURE 3.

Quantitation of mPIN lesions from CPF- and control-treated *C57/Luc/Pten^{p+/-}* mice. mPIN lesions were counted and expressed as a percentage of the total glands within dorsal, lateral, ventral, and anterior prostate. There were no significant differences in mPIN lesions between CPF- and control-treated animals.

FIGURE 4.

[Q4] Assessing the effect of chronic CPF exposure in C57/Luc/*Pten*^{p+/-} mice. Individual body weights of C57/Luc/*Pten*^{p+/-} mice fed either control chow (**a**) or CPF (**b**). Chronic CPF exposure caused varying degrees of net body weight gain in C57/Luc/*Pten*^{p+/-} mice. Individual BLI curves for mice fed either control chow (**c**) or CPF (**d**). Chronic CPF exposure did not promote prostate tumorigenesis in C57/Luc/*Pten*^{p+/-} mice.