

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPROVAL PACKAGE FOR:**

**APPLICATION NUMBER**

**21-387**

**ENVIRONMENTAL ASSESSMENT/FONSI**

**REVIEW  
OF  
ENVIRONMENTAL ASSESSMENT  
FOR**

**NDA 21-387**

**Provachol® 40 mg and Bufferin® Tablets 81 mg  
or 325 mg combination package**

**Division of Cardio-Renal Drug Products (HFD-110)  
Center for Drug Evaluation and Research**

## EXECUTIVE SUMMARY

### **A FONSI is recommended.**

NDA 21-387, Provachol® 40 mg and Bufferin® Tablets 81 mg or 325 mg combination package requests approval for sale of these two products in a combination blister package. Provachol® is approved as a cholesterol lowering agent and has been shown to reduce risk of heart attacks. Bufferin is approved for use in the treatment of pain and inflammation and also for its anti-platelet effects to decrease the risk of blood clot formation.

No information is provided for pravastatin because the EIC is — ppb based on a 5<sup>th</sup> year — production of — kg for their pravastatin production line.

In addition, the calculated value for the EIC is — ppb for aspirin and — ppb for salicylic acid using the 2002 peak production estimate of — kg for aspirin.

Both aspirin and its major active metabolite, salicylic acid, are expected to undergo considerable degradation in sewage treatment plants, as well as by hydrolysis (aspirin). Photolysis may also contribute to their degradation, although this is expected to be a slower process. These substances are not considered volatile and are expected to exist as ionized species at pHs normally found in surface and ground waters. They are not expected to partition to the atmospheric compartment. They are expected to be mobile in soils.

Aspirin is metabolized to both active (salicylic acid) and inactive metabolites that are excreted in the urine and partition to the aquatic compartment. Both aspirin and salicylic acid are expected to biodegrade and/or hydrolyze rapidly in the environment and are not expected to bioaccumulate. The environmental monitoring data (available through literature from other countries) for aspirin and salicylic acid shows values that approximate the calculated EIC, both before and after correction for environmental depletion mechanisms. The ratios of EC<sub>50</sub> from ecotoxicological studies to EIC for aspirin and salicylic acid are both greater than —

Based on this data, environmental effects are not expected.

## REVIEW ENVIRONMENTAL ASSESSMENT

1. **Date:** June 22, 2001

The firm explains that the EA is for pravastatin sodium tablets and buffered aspirin tablets co-packaged products. The relevant drug substance and active ingredient for this environmental assessment is aspirin. Additionally, its major active metabolite salicylic acid is considered. Pravastatin sodium qualifies for a categorical exclusion from submitting an environmental assessment.

ADEQUATE

2. **Name of applicant/petitioner:**

Bristol-Myers Squibb Company

ADEQUATE

3. **Address:**

P.O. Box 4000  
Princeton, New Jersey 08543-4000

ADEQUATE

4. **Description of the proposed action:**

- a. **Requested Approval:**

Bristol-Myers Squibb has filed a new drug application pursuant to section 505b of the FD&C Act for Provachol® 40 mg and Bufferin® Tablets 81 mg or 325 mg combination package. This Environmental Assessment (EA) has been submitted pursuant to 21 CFR part 25.

ADEQUATE

**b. Need for Action:**

Provachol® is approved as a cholesterol lowering agent and has been shown to reduce risk of heart attacks. Bufferin is approved for use in the treatment of pain and inflammation and also for its anti-platelet effects to decrease the risk of blood clot formation. This NDA requests approval for sale of these two products in a combination blister package.

ADEQUATE

**c. Expected Locations of Use (Drug Product):**

The tablets are sold to hospitals, clinics, and pharmacies throughout the USA for use by both in-patient and out-patient populations. There is no particular geographic region mentioned.

ADEQUATE

**d. Disposal Sites**

At U.S. hospitals, clinics, and pharmacies empty or partially empty containers will be disposed of according to the facility's procedures. Empty or partially empty containers from homes of patients will typically be disposed of by the community's solid waste management system that could include landfills, incineration and/or recycling. Minimal quantities of the unused drug could be disposed of directly into the sewer system.

ADEQUATE

5. **Identification of chemical substances that are the subject of the proposed action:**

The relevant drug substance and active ingredient for this environmental assessment filing is aspirin. Additionally, its major active metabolite is salicylic acid.

**Drug Substance:** Aspirin  
**Brand/Proprietary Name/Tradename:** Bufferin  
**Chemical Name:** Benzoic acid, 2-(acetyloxy)-(9CI) (CAS)  
**CAS #:** 50-78-2  
**Molecular Weight:** 180.15  
**Molecular Formula:** C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>  
**Structural Formula:** Provided on Page 259 of the EA

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**Active Metabolite:** Salicylic acid  
**Chemical Name:** Benzoic acid, 2-(hydroxy)-(9CI) (CAS)  
**CAS #:** 69-72-7  
**Molecular Weight:** 138.12  
**Molecular Formula:** C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>  
**Structural Formula:** Provided on Page 259 of the EA

ADEQUATE

6. **Environmental Issue:**

a. **Environmental Fate of Released Substances: Identification of Substances of Interest**

The firm explains that aspirin has been used as a medicinal for decades and has not been associated with significant environmental impacts. The data provided in the next few sections and in the data tables in the non-confidential appendix have been obtained from a literature review of the Hazardous Substances Database (reference 1), and other primary and secondary sources. In addition, aspirin and the major active metabolite, salicylic acid, have been monitored in the environment and data from these analyses are included in the relevant sections below.

ADEQUATE

i. **Identification of Substances of Interest**

The firm explains that aspirin is extensively and rapidly metabolized in humans. Approximately 1% of an oral dose is excreted as unchanged

aspirin. The major excreted metabolites are salicylic acid (10%) and salicyluric acid (75%) with approximately 15% of metabolites excreted as the ether and ester glucuronides of salicylic acid. Gentisic acid is an additional excreted metabolite accounting for less than 1% of the total metabolites. Salicylate is considered to be the active metabolite of aspirin, whereas the glycine and glucuronide conjugates are not expected to be as biologically active. There have recently been reports of cleavage of glucuronide metabolites of drugs by organisms in wastewater treatment plants to regenerate the active drug moiety, so it may be possible that the salicylic acid glucuronides can be cleaved to regenerate salicylate. Salicylic acid is therefore the subject of much of the following discussion as it is the major active substance that would enter the environment after use of this product. Information for aspirin is also included.

The firm notes that salicylic acid is a naturally-occurring substance in the environment. It is found in many microorganisms and in common fruits (apples, oranges, plums, and grapes) as well as various parts of plants such as tulips, hyacinths, violets, *Spiraea ulmaria*, and *Pinus radiata*.

ADEQUATE

#### ii. Physical and Chemical Characterization

Basic physicochemical properties of aspirin and salicylate are summarized in the data tables of the non-confidential appendix. Both aspirin and salicylic acid are somewhat water soluble (~0.2-0.5%) and ionizable compounds. At relatively neutral pH they would be expected to exist primarily as ionized species. They have relatively low octanol/water partition coefficients. They are solid substances at room temperature, and have negligible vapor pressure at ambient temperature and low Henry's law constants. Based on this information, these compounds are expected to distribute to the aqueous compartment. Additionally the Koc values suggest that they would have potential for moderate to high mobility in soils. Bioconcentration factors have also been estimated for these substances and they are not expected to bioaccumulate in aquatic organisms.

ADEQUATE

#### iii. Environmental Depletion Mechanisms

Aspirin is expected to degrade in water via both hydrolysis and biodegradation mechanisms. Photolysis may also contribute to the depletion of aspirin. Sewage treatment has been reported to remove 81% of aspirin. Concentrations measured in rivers and streams in Germany

were less than \_\_\_\_\_ with maximum concentrations in sewage effluents of \_\_\_\_\_ mcg/l.

Salicylic acid is rapidly biodegraded by aerobic and anaerobic mechanisms in water and sludge systems, especially after acclimation. Sewage treatment has been reported to remove 90% of salicylic acid. Concentrations ranging from \_\_\_\_\_ mcg have been reported in rivers and streams in Germany.

ADEQUATE

#### iv. Environmental Concentrations

Based on annual usage volumes for the US market, the Maximum Expected Environmental Concentration (MEEC) has been estimated for the aqueous compartment. This calculation was done in accordance with the July 1998 Environmental Assessment guidance. The firm explains that for the purpose of this assessment, the MEEC is considered to be equivalent to the Expected Introduction Concentration (EIC).

The EIC for pravastatin sodium is \_\_\_\_\_ ppb based on a 5<sup>th</sup> year production of \_\_\_\_\_ kg for their pravastatin production line. The firm notes that the Expected Environmental Concentration (ECC) for aspirin/salicylic acid is likely to be considerably lower due to both environmental depletion mechanisms (sewage treatment) and by dilution of water exiting publicly-owned treatment works (POTWs). The calculated value for the EIC is \_\_\_\_\_ ppb for aspirin and \_\_\_\_\_ ppb for salicylic acid using the 2002 production estimate of \_\_\_\_\_ kg for aspirin (confidential appendix 1).

ADEQUATE

#### v. Summary

Both aspirin and its major active metabolite, salicylic acid, are expected to undergo considerable degradation in sewage treatment plants, as well as by hydrolysis (aspirin). Photolysis may also contribute to their degradation, although this is expected to be a slower process. These substances are not considered volatile and are expected to exist as ionized species at pHs normally found in surface and ground waters. They are not expected to partition to the atmospheric compartment. They are expected to be mobile in soils.

ADEQUATE

**b. Environmental Effects of Release Substances**

**i. Tiered Approach to Environmental Effects Testing**

As this assessment is based on a review of existing literature on aspirin/salicylic acid, environmental fate and effects testing was not conducted by Bristol-Myers Squibb Company. The results of the environmental fate studies summarized above indicate that salicylic acid is expected to partition to the aquatic compartment. Most of the ecotoxicological studies that we have identified have focused on assessment of effects on aquatic organisms and are summarized in the following sections.

ADEQUATE

**ii. Microbial Inhibition**

The firm states that the potential for aspirin and salicylic acid to affect wastewater treatment microorganisms was not specifically described in the literature they reviewed. However, they have provided an assessment of inhibition of the biodegradation of a reference substance as part of the standard OECD 301 protocol. They explain that this study has been conducted with no mention of inhibition of biodegradation in the original published article, it is assumed that inhibition of sludge microorganisms did not occur. Salicylic acid has also been evaluated for inhibition of luminescence in bacteria. The EC50 value was ~ mg/L, a moderately high value.

ADEQUATE

**iii. Tier 1-2 Acute Toxicity to Aquatic Species**

The data summary tables (included in this review) contain a summary of the results of the aquatic toxicity studies conducted with aspirin and/or salicylic acid. Aspirin has been evaluated only in an acute toxicity study in *Daphnia* with a resulting EC50 of —mg/L. There is additional supporting information for this calculation in the non-confidential section of this EA in which results from literature searches are included.

The firm explains that there is considerably more data on salicylic acid. The substance has been evaluated in both standard and non-standard aquatic toxicity studies and has also been studied for its effects on plant species. The firm's assessment focuses on the effects on aquatic species. *Daphnia magna*, green alga, and a ciliate species. In addition, effects have

been evaluated on fish embryos and fish cells in culture. The cultured cells were quite resistant to salicylic acid. The fish embryos were the most sensitive receptor with an EC50 of  $\sim$  mg/L. Daphnia, algae, and ciliates were relatively resistant to salicylic acid. There is additional supporting information for this calculation in the non-confidential section of this EA in which results from literature searches are included.

ADEQUATE

**iv. Test Methods and Test Organisms**

The firm explains that the studies reported in the literature used both standard and non-standard methodologies for fate and effects assessment. In addition, there is actual environmental monitoring data available to support this assessment.

ADEQUATE

**c. Summary of Environmental Fate and Effects**

Aspirin is metabolized to both active (salicylic acid) and inactive metabolites that are excreted in the urine and partition to the aquatic compartment. Both aspirin and salicylic acid are expected to biodegrade and/or hydrolyze rapidly in the environment and are not expected to bioaccumulate. The environmental monitoring data that is available from other countries for aspirin and salicylic acid shows values that approximate the calculated EIC, both before and after correction for environmental depletion mechanisms. The ratios of EC50 from ecotoxicological studies to EIC for aspirin and salicylic acid are both greater than

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pages of trade

secret and/or

confidential

commercial

information

**7. Mitigation Measures**

As significant adverse environmental effects are not predicted, mitigation measures are not warranted.

ADEQUATE

**8. Alternatives to the proposed action**

Since significant environmental effects are not predicted to occur, and since mitigation measures are not proposed, consideration of alternatives to the proposed action is not necessary.

ADEQUATE

**9. List of Preparers**

The firm states that Eileen Hayes, Sc.D., DABT, Associate Director, Occupational & Environmental Toxicology, has been a practicing toxicologist since 1979 with experience in occupational and environmental toxicology and chemical metabolism. She received the B.S. in Pharmacy from Northeastern University, the Sc.D. in Toxicology from Harvard School of Public Health and post-doctoral training at Brigham & Women's Hospital/Harvard Medical School.

ADEQUATE

**10. References**

References are provided for the literature references and FDA Environmental Assessment guidance.

ADEQUATE

**11. Appendices**

The non-confidential appendices contain the data summary tables for aspirin and salicylic acid and selected abstracts from the literature search. The confidential appendix contains production volumes and expected environmental concentrations.

ADEQUATE

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this page is the manifestation of the electronic signature.  
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/s/

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Melissa Maust  
11/7/01 07:43:15 AM  
ENV ASSESSMENT

A FONSI is recommended.

Nancy Sager  
11/7/01 08:06:30 AM  
ENV ASSESSMENT

Yuan-Yuan Chiu  
11/14/01 02:26:16 PM  
CHEMIST  
concurrred

**ENVIRONMENTAL ASSESSMENT**  
**AND**  
**FINDING OF NO SIGNIFICANT IMPACT**  
**FOR**

**NDA 21-387**

**Provachol® 40 mg and Bufferin® Tablets 81 mg  
or 325 mg combination package**

**Division of Cardio-Renal Drug Products (HFD-110)  
Center for Drug Evaluation and Research**

## FINDING OF NO SIGNIFICANT IMPACT

NDA 21-387

(Provachol® 40 mg and Bufferin® Tablets 81 mg  
or 325 mg combination package)

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement, therefore, will not be prepared.

In support of their new drug application for Provachol® 40 mg and Bufferin® Tablets 81 mg or 325 mg combination package, Bristol-Meyers Squibb Company has prepared an environmental assessment (attached) in accordance with 21 CFR Part 25 which evaluates the potential environmental impacts of the use and disposal from use of the product. The relevant drug substance and active ingredient for this environmental assessment is aspirin (Bufferin®). Additionally, its major active metabolite salicylic acid is considered. Information on pravastatin sodium (Provachol®) is not provided because the expected environmental concentration is less than 1 ppb.

Provachol® is approved as a cholesterol lowering agent and has been shown to reduce risk of heart attacks. Bufferin® is approved for use in the treatment of pain and inflammation and also for its anti-platelet effects to decrease the risk of blood clot formation. This NDA requests approval for sale of these two products in a combination blister package.

Aspirin is metabolized to both active (salicylic acid) and inactive metabolites that are excreted in the urine and partition to the aquatic compartment. Both aspirin and salicylic acid are expected to biodegrade and/or hydrolyze rapidly in the environment and are not expected to bioaccumulate. Based on the ecotoxicity data, no environmental effects are anticipated at the expected environmental concentrations.

Provachol® 40 mg and Bufferin® Tablets 81 mg or 325 mg combination package will be primarily sold to hospitals, clinics, and pharmacies throughout the USA for use by both in-patient and out-patient populations. At U.S. hospitals, clinics, and pharmacies empty or partially empty containers will be disposed of according to the facility's procedures. Empty or partially empty containers from homes of patients will typically be disposed of by the community's solid waste management system that could include landfills, incineration and/or recycling. Minimal quantities of the unused drug could be disposed of directly into the sewer system.

*The Center for Drug Evaluation and Research has concluded that the product can be used and disposed of without any expected adverse environmental effects. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.*

**PREPARED BY**

Melissa J. Maust, Chemist  
Center for Drug Evaluation and Research

**CONCURRED BY**

Nancy B. Sager, Environmental Officer  
Center for Drug Evaluation and Research

**CONCURRED BY**

Yuan-yuan Chiu, Ph.D., Director, Office of New Drug Chemistry  
Center for Drug Evaluation and Research

Attachment: Environmental Assessment  
Appended Electronic Signature Page

## **PART IV. ENVIRONMENTAL ASSESSMENT**

### **1 ENVIRONMENTAL ASSESSMENT**

#### Pravastatin Sodium Tablets and Buffered Aspirin Tablets Co-packaged Products

The environmental assessment for the pravastatin sodium tablets and buffered aspirin tablets co-packaged products is provided on the following pages. The relevant drug substance and active ingredient for this environmental assessment is aspirin. Additionally, its major active metabolite salicylic acid is considered. Pravastatin sodium qualifies for a categorical exclusion from submitting an environmental assessment.

**Environmental Assessment**

**PRAVACHOL TABLETS AND BUFFERIN TABLETS IN BLISTER PACKAGE**

**New Drug Application**

**APPEARS THIS WAY  
ON ORIGINAL**

06/18/01

**Section 1. Date**

June, 2001

**Section 2. Name of Applicant/Petitioner**

Bristol-Myers Squibb Company

**Section 3. Address (Mailing)**

P.O. Box 4000  
Princeton, NJ 08543-4000

**Section 4. Description of Proposed Action**

**a. Requested Approval**

Bristol-Myers Squibb is filing a New Drug Application pursuant to Section 505 (b) of the Federal Food, Drug and Cosmetic Act for Pravachol® 40 mg and Bufferin® Tablets 81 mg or 325 mg combination package. This Environmental Assessment (EA) is being submitted pursuant to 21 CFR part 25.

**b. Need for Action**

Pravachol® is approved as a cholesterol lowering agent and has been shown to reduce risk of heart attacks. Bufferin is approved for use in the treatment of pain and inflammation and also for its anti-platelet effects to decrease the risk of blood clot formation. This NDA requests approval for sale of these two products in a combination blister package.

**c. Locations of Use**

The tablets are sold to hospitals, clinics and pharmacies throughout the USA for use by both in-patient and out-patient populations.

**d. Disposal Sites**

At U.S. hospitals, clinics and pharmacies empty, or partially empty, containers will be disposed of according to the facility's procedures. Empty or partially empty containers from homes of patients will typically be disposed of by a community's solid waste management system that could include landfills, incineration and/or recycling. Minimal quantities of unused drug could be disposed of in sewer systems.

06/18/01

**Section 5 Identification of Substances that Are the Subject of the Proposed Action**

This NDA is for Pravachol® Tablets and Bufferin Tablets. The relevant drug substance and active ingredient for this environmental assessment filing is aspirin. Additionally, its major active metabolite salicylic acid is considered.

With respect to pravastatin sodium (API in Pravachol®) the proposed action will not significantly affect the quality of the human environment and meets the requirements for a categorical exclusion from submitting an environmental assessment, 21 CFR 25.31(b). In addition, to Bristol-Myers Squibb Company's knowledge, no extraordinary circumstances exist [21 CFR 25.15 (d)]. This drug is manufactured using a fermentation process followed by a synthetic step and is not known to be derived from any wild-sourced plant and/or animal material. This action may increase the use of the active moiety, but the estimated concentration (EIC) of the substance at the point of entry into the aquatic environment, from this product and from existing Pravachol® products will be below 1 part per billion. (See also Confidential Appendix I.)

**a. Nomenclature**

**i. Established Name (U.S. Adopted Name - USAN)**

Aspirin

**ii. Brand/Proprietary Name/Tradename**

Bufferin

**iii. Chemical Names**

- Chemical Abstracts (CA) Index Name

Benzoic acid, 2-(acetyloxy)- (9CI)

**b. Chemical Abstracts Service (CAS) Registration Number**

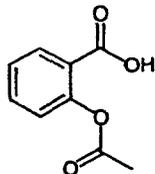
50-78-2

**c/d. Molecular Formulas and Molecular Weights**

Molecular Formula is:  $C_9H_8O_4$

Molecular Weight is: 180.15

e. Structural (graphic) Formula for aspirin



b. Nomenclature for salicylic acid

i. Established Name (U.S. Adopted Name - USAN)

Salicylic acid

ii. Brand/Proprietary Name/Tradename

Not applicable

iii. Chemical Names

• Chemical Abstracts (CA) Index Name

Benzoic acid, 2-hydroxy- (9CI)

b. Chemical Abstracts Service (CAS) Registration Number

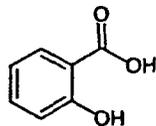
69-72-7

c/d. Molecular Formulas and Molecular Weights

Molecular Formula is: C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>

Molecular Weight is: 138.12

e. Structural (graphic) Formula for salicylic acid



**Section 6 Environmental Issues**

**a. Environmental Fate of Released Substances**

Aspirin has been used as a medicinal for decades and has not been associated with significant environmental impacts. The data discussed in the following sections and in the data tables in the non-confidential appendix have been obtained from a literature review of the Hazardous Substances Database (1), and other primary and secondary sources. As both aspirin and its major active metabolite salicylic acid have been monitored in the environment, data from these analyses is also noted here.

**i. Identification of Substances of Interest**

Aspirin is extensively and rapidly metabolized in humans. Approximately 1% of an oral dose is excreted as unchanged aspirin. The major excreted metabolites are salicylic acid (10%) and salicyluric acid (75%) with approximately 15% of metabolites excreted as the ether and ester glucuronides of salicylic acid. Gentisic acid is an additional excreted metabolite accounting for less than 1% of the total metabolites (2). Salicylate is considered to be the active metabolite of aspirin (2), whereas the glycine and glucuronide conjugates are not expected to be as biologically active. There have recently been reports of cleavage of glucuronide metabolites of drugs by organisms in wastewater treatment plants to regenerate the active drug moiety, so it may be possible that the salicylic acid glucuronides can be cleaved to regenerate salicylate. Salicylic acid is therefore the subject of much of the following discussion as it is the major active substance that would enter the environment after use of this product, however information is also included for aspirin.

It is noted that salicylic acid is a naturally-occurring substance in the environment. It is found in many microorganisms and in common fruits (apples, oranges, plums and grapes), as well as in various parts of plants such as tulips, hyacinths, violets, *Spiraea ulmaria* and *Pinus radiata* (1).

**ii. Physical and Chemical Characterization**

Basic physicochemical properties of aspirin and salicylate are summarized in the data tables of the Non-Confidential Appendix. Both aspirin and salicylic acid are somewhat water soluble and ionizable compounds. At relatively neutral pH they would be expected to exist primarily as ionized species. They have relatively low octanol/water partition coefficients. They are solid substances at room temperature, and have negligible vapor pressure at ambient temperature and low Henry's law constants. Based on this information, these compounds are expected to distribute to the aqueous compartment. Additionally the Koc values suggest that they would have potential for moderate to high mobility in soils.

Bioconcentration factors have also been estimated for these substances and they are not expected to bioaccumulate in aquatic organisms.

**iii. Environmental Depletion Mechanisms**

Aspirin is expected to degrade in water via both hydrolysis and biodegradation mechanisms. Photolysis may also contribute to the depletion of aspirin. Sewage treatment has been reported to remove 81% of aspirin. Concentrations measured in rivers and streams in Germany were less than 1 mcg/l, with maximum concentrations in sewage effluents of ~ mcg/l (3).

Salicylic acid is rapidly biodegraded by aerobic and anaerobic mechanisms in water and sludge systems, especially after acclimation. Sewage treatment has been reported to remove 90% of salicylic acid. Concentrations ranging from ~ ng to ~ mcg have been reported in rivers and streams in Germany (3).

**iv. Environmental Concentrations**

Based on annual usage volumes for the US market, the Maximum Expected Environmental Concentration (MEEC) has been estimated for the aqueous compartment, according to the guidance provided by the FDA in July, 1998 (Ref. 4). For the purpose of this assessment the MEEC is considered to be equivalent to the Expected Introduction Concentration (EIC). It should be noted that the Expected Environmental Concentration (ECC) is likely to be at considerably lower than this due to both environmental depletion mechanisms (sewage treatment) and by dilution of water exiting publicly-owned treatment works (POTWs). The calculation for the EIC is shown in the Confidential Appendix 1.

**v. Summary**

Both aspirin and its major active metabolite, salicylic acid, are expected to undergo considerable degradation in sewage treatment plants, as well as by hydrolysis (aspirin). Photolysis may also contribute to their degradation, although this is expected to be a slower process. These substances are not considered volatile and are expected to exist as ionized species at pHs normally found in surface and ground waters. They are not expected to partition to the atmospheric compartment. They are expected to be mobile in soils. The maximum expected environmental concentration (MEEC) is found in Confidential Appendix 1.

**b. Environmental Effects of Released Substances**

**i. Tiered Approach to Environmental Effects Testing**

As this assessment is based on a review of existing literature on aspirin and salicylic acid, environmental fate and effects testing was not conducted by Bristol-Myers Squibb Company. The results of the environmental fate studies

summarized above indicate that salicylic acid is expected to partition to the aquatic compartment. Most of the ecotoxicological studies that we have identified have focused on assessment of effects on aquatic organisms and are summarized below.

**ii. Microbial Inhibition**

The potential for aspirin and salicylic acid to affect wastewater treatment microorganisms was not specifically described in the literature that we have reviewed. However, an assessment of inhibition of the biodegradation of a reference substance is part of the standard OECD 301 protocols. As this study has been conducted with no mention of inhibition of biodegradation in the original published article, it is assumed that inhibition of sludge microorganisms did not occur. Salicylic acid has also been evaluated for inhibition of luminescence in bacteria. The EC50 value was 90 mg/l, a moderately high value.

**iii. Tier 1-2 Acute Toxicity to Aquatic Species**

The data tables of the Non-confidential Appendix also contains a summary of the results of the aquatic toxicity studies conducted with aspirin and/or salicylic acid. Aspirin has been evaluated only in an acute toxicity study in *Daphnia* with a resulting EC50 of 61 mg/l. See Non-confidential Appendix for further discussion of this.

There is considerably more data on salicylic acid. This substance has been evaluated in both standard and non-standard aquatic toxicity studies and has also been studied for its effects on plant species. This assessment focuses on the effects on aquatic species. *Daphnia magna*, green alga, and a ciliate species have been evaluated as well as toxicity to fish embryos and fish cells in culture. The cultured cells were quite resistant to salicylic acid. The fish embryos were the most sensitive receptor with an EC50 of 37 mg/l. *Daphnia*, algae and ciliates were relatively resistant to salicylic acid. See Non-confidential Appendix for further discussion of this.

**iv. Test Methods and Test Organisms**

As noted above, the studies reported in the literature utilized both standard and non-standard methodologies for environmental fate and effects assessment. In addition, there is actual environmental monitoring data available to support this assessment. In a separate literature search we have noted a number of reports of studies of salicylic acid effects on plant species. Selected abstracts from this literature search are attached as Non-Confidential Appendix 2.

**c. Summary of Environmental Fate and Effects**

Aspirin is metabolized to both active (salicylic acid) and inactive metabolites that are excreted in the urine and partition to the aquatic compartment. Both aspirin and salicylic acid are expected to biodegrade and/or hydrolyze rapidly in the environment and are not expected to bioaccumulate. The environmental monitoring data that is available from other countries for aspirin and salicylic acid shows values that approximate the calculated EIC, both before and after correction for environmental depletion mechanisms. The ratios of the EC50 from ecotoxicological studies to EIC for aspirin and salicylic acid are both greater than

**Section 7 Mitigation Measures**

As significant adverse environmental effects are not predicted, mitigation measures are not warranted.

**Section 8 Alternatives to the Proposed Action**

Since significant environmental effects are not predicted to occur, and since mitigation measures are not proposed, consideration of alternatives to the proposed action is not necessary.

**Section 9 List of Preparers**

Eileen Hayes, Sc.D., DABT, Associate Director, Occupational & Environmental Toxicology, has been a practicing toxicologist since 1979 with experience in occupational and environmental toxicology and chemical metabolism. She received the B.S. in Pharmacy from Northeastern University, the Sc.D. in Toxicology from Harvard School of Public Health and post-doctoral training at Brigham & Women's Hospital/ Harvard Medical School.

**Section 10 References**

- (1) Hazardous Substance Database Search,
- (2) Eds, *Goodman and Gilman's Pharmacological Basis of Therapeutics*, Ninth ed. 19xx, pp.
- (3) Stuer-Lauridsen F., Birkved, M., Hansen, L.P., Holten Lutzhoft and Halling-Sørensen, B., Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere* 40, 783-793, 2000.
- (4) Food and Drug Administration, Guidance for Industry: Environmental Assessment of Human Drugs and Biologics Applications, July 1998

06/18/01

- (5) Ayscough, N.J., Fawell, J., Franklin, G., and Young, W., Review of Human Pharmaceuticals in the Environment, R&D Technical Report P390, Research Contractor WRc-NSF Ltd.; Commissioned by the UK Environment Agency, 2000.
- (6) Henschel, K.-P., Wenzel, A., Diedrich, M. and Fliedner, A., Environmental Hazard Assessment of Pharmaceuticals, Reg. Toxicol. Pharmacol. 25:220-225, 1997.

#### Section 11 Appendices

Two non-confidential appendices and one confidential appendix follow.

**NON-CONFIDENTIAL APPENDIX 1**  
**Data Summary Tables**

06/18/01

NON-CONFIDENTIAL APPENDIX 1 - TABLE 1

DATA SUMMARY TABLE - ASPIRIN	
<b>PHYSICAL/CHEMICAL CHARACTERIZATION</b>	
Water solubility (ref. 1)	4600 mg/L at 25 degrees C
Ionization constant (ref. 1)	pKa = 3.49 at 25 degrees C
Partition coefficient - Kow (octanol/water); [log Kow] (ref. 1)	Kow = 15.49; [log Kow = 1.19]
Adsorption to soils - Koc (soil/water); [log Koc] (ref. 1)	Koc = 42 and 106 (calc); [log Koc = 1.62 and 2.03 (calc)]
Bioconcentration Factor - BCF; [log BCF] (ref. 1)	BCF = 4.68 and 5.37 (calc); [log BCF = 0.67 and 0.73 (calc)]
Vapor Pressure (ref. 1)	$2.52 \times 10^{-5}$ mm Hg at 25 degrees C (calc)
Henry's Law constant (ref. 1)	$1.3 \times 10^{-9}$
<b>Depletion Mechanisms</b>	
Biodegradation - anaerobic (ref. 1)	After acclimation with sludge, aspirin is almost completely mineralized. Over a 56 day period, starting at 50 ppm, 96% of the theoretical methane was generated.
Photolysis (ref. 1)	Estimated half-life (in air) = 19.8 days
Hydrolysis (ref. 1)	T <sub>1/2</sub> at pH 3.5 = 12.5 days T <sub>1/2</sub> at pH 5 = 5.4 days T <sub>1/2</sub> at pH 7.4 = 6.3 days T <sub>1/2</sub> at pH 9.5 = 2.2 days T <sub>1/2</sub> at pH 11.3 = 1.2 hours
Sewage Treatment	Reported to remove 81% of aspirin
Environmental concentrations (3)	0.34 mcg/liter was reported in rivers and streams in Germany
<b>Environmental Effects</b>	
Daphnia magna reproduction (21 days) (3,5)	EC50 = 61-68 mg/l

Abbreviations used in the Table: Calc = Calculated

06/18/01

NON-CONFIDENTIAL APPENDIX 1 - TABLE 2

DATA SUMMARY TABLE - SALICYLIC ACID	
PHYSICAL/CHEMICAL CHARACTERIZATION	
Water solubility (ref. 1)	2059 mg/L at 25 degrees C
Ionization constants (ref. 1)	pKa1 = 2.97; pKa2 = 13.4
Partition coefficients - Kow (octanol/water); [log Kow] (ref. 1)	Kow = 181.97; [log Kow = 2.26]
Adsorption to soils - Koc (soil/water); [log Koc] (ref. 1)	Koc = 65 and 104 (calc); [log Koc = 1.81 and 2.02 (calc)]
Bioconcentration Factor - BCF; [log BCF] (ref. 1)	BCF = 8.32 - 30.90 (calc); [log BCF = 0.92 - 1.49 (calc)]
Vapor Pressure (ref. 1)	$8.2 \times 10^{-5}$ mm Hg at 25 degrees C (calc)
Henry's Law constant (ref. 1)	$7.3 \times 10^{-9}$
Depletion Mechanisms	
Biodegradation - soil (ref. 1)	Rapid biodegradation has been reported in soil grab samples
Biodegradation - water (ref. 1)	Rapid biodegradation has been reported under both aerobic and anaerobic conditions. When incubated in sludge systems, degradation is more rapid after an initial acclimation phase. For instance, in 14 hours 87% of 100 ppm salicylic acid was degraded by an activated sludge.
Photolysis (ref. 1)	Estimated half-life (in air) = 1.2 days Estimated half-life (in water) = 30 to 47 and 40 to 142 days for salicylic acid and the salicylate ion, respectively;
Sewage Treatment	Reported to remove 90% of salicylic acid
Reported Environmental Concentrations (ref. 1,3,5)	Concentrations ranging up to 0.1 mcg/l have been reported in rivers in Japan. Concentrations up to 4.1 mcg have been reported in rivers and streams in Germany.
Environmental Effects	
Scenedesmus subspicatus (algae) - 72 hr EC50 (growth inhibition) (ref. 3)	> 100 mg/l
Tetrahymena pyriformis (ciliate) - 48 hr EC50 (growth inhibition) (ref. 3)	>100 mg/l
Daphnia magna (water flea) - EC50 (acute, immobilisation) (ref. 3)	118 mg/l

06/18/01

DATA SUMMARY TABLE - SALICYLIC ACID	
Vibrio fischeri (bacteria) - 30 minute EC50 (luminescence) (ref. 3)	90 mg/l
Bluegill sunfish cells in vitro - EC50 (acute, cytotoxicity) (ref. 3)	>500 mg/l
Brachydanio rerio (zebra fish) embryos - (mortality, pulse rate) (ref. 3)	37 mg/l, 50 mg/l

Abbreviations used in the Table: Calc = Calculated

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**NON-CONFIDENTIAL APPENDIX 2**  
**Selected Abstracts from Literature Search**

06/18/01

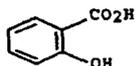
=> file reg  
FILE 'REGISTRY' ENTERED AT 14:11:44 ON 30 MAR 2001

Please note that search-term pricing does apply when  
conducting SmartSELECT searches.

=> s salicylic acid/cn  
L1 1 SALICYLIC ACID/CN

=> d 11 1

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2001 ACS  
RN 69-72-7 REGISTRY  
CN Benzoic acid, 2-hydroxy- (9CI) (CA INDEX NAME)  
OTHER CA INDEX NAMES:  
CN Salicylic acid (6CI, 8CI)  
OTHER NAMES:  
CN 2-Carboxyphenol  
CN 2-Hydroxybenzenecarboxylic acid  
CN 2-Hydroxybenzoic acid  
CN o-Carboxyphenol  
CN o-Hydroxybenzoic acid  
CN Phenol-2-carboxylic acid  
CN Psoriacid-S-Stift  
CN Retarder W  
CN Rutranex  
CN Salicylic acid collodion  
CN Salonil  
FS 3D CONCORD  
DR 7681-06-3, 8052-31-1  
MF C7 H6 O3  
CI COM  
LC STN Files: AGRICOLA, AIDSLINE, ANABSTR, APILIT, APILIT2, APIPAT,  
APIPAT2, BEILSTEIN\*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA,  
CANCERLIT, CAOLD, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMINFORMRX,  
CHEMLIST, CIN, CSCHEM, CSNB, DDFU, DETHERM\*, DIOGENES, DIPPR\*, DRUGU,  
EMBASE, GMELIN\*, HODOC\*, HSDB\*, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE,  
MRCK\*, MSDS-OHS, NAPRALERT, NIOSHTIC, PDLCOM\*, PIRA, PROMT, RTECS\*,  
SPECINFO, SYNTHLINE, TOXLINE, TOXLIT, TULSA, ULIDAT, USAN, USPATFULL,  
VETU, VTB  
(\*File contains numerically searchable property data)  
Other Sources: DSL\*\*, EINECS\*\*, TSCA\*\*  
(\*\*Enter CHEMLIST File for up-to-date regulatory information)



15315 REFERENCES IN FILE CA (1967 TO DATE)  
1988 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA  
15339 REFERENCES IN FILE CAPLUS (1967 TO DATE)  
7 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

06/18/01

=> file agricola, biosis, toxlit, ulidat  
FILE 'AGRICOLA' ENTERED AT 14:12:15 ON 30 MAR 2001

FILE 'BIOSIS' ENTERED AT 14:12:15 ON 30 MAR 2001  
COPYRIGHT (C) 2001 BIOSIS(R)

FILE 'TOXLIT' ENTERED AT 14:12:15 ON 30 MAR 2001

FILE 'ULIDAT' ENTERED AT 14:12:15 ON 30 MAR 2001  
COPYRIGHT (C) 2001 Umweltbundesamt, D-14191 Berlin (UBA)

=> s soil? or sludge? or sediment or sediments or daphnia? or fish? or  
earthworm?

~~L2~~ 985840 SOIL? OR SLUDGE? OR SEDIMENT OR SEDIMENTS OR DAPHNIA? OR FISH?  
OR EARTHWORM?

=> s alga? or flora? or fauna? or lake? or river? or ocean? or wildlife  
L3 554861 ALGA? OR FLORA? OR FAUNA? OR LAKE? OR RIVER? OR OCEAN? OR  
WILDLI

FE

=> s 12 or 13  
L4 1398634 L2 OR L3

=> s 11  
L5 10304 L1

=> s 14 and 15  
L6 275 L4 AND L5

=> dup remove 16  
PROCESSING COMPLETED FOR L6  
L7 255 DUP REMOVE L6 (20 DUPLICATES REMOVED)

=> s 17 and py>1995  
3 FILES SEARCHED...  
L8 84 L7 AND PY>1995

=> d 18 1 bib ab

L8 ANSWER 7 OF 84 AGRICOLA  
AN 2000:31375 AGRICOLA  
DN IND22034017  
TI Pseudomonas aeruginosa 7NSK2-induced systemic resistance in tobacco  
depends on in planta salicylic acid accumulation but is not associated  
with PR1a expression.  
AU Meyer, G. de; Audenaert, K.; Hofte, M.  
CS University Gent, Gent, Belgium.  
AV DNAL (SB599.E97)  
SO European journal of plant pathology, Aug 1999. Vol. 105, No. 5.  
p. 513-517  
Publisher: Dordrecht ; Boston : Kluwer Academic Publishers, c1994-  
CODEN: EPLPEH; ISSN: 0929-1873  
NTE Includes references

06/18/01

CY Netherlands  
DT Article  
FS Non-U.S. Imprint other than FAO  
LA English  
AB Root colonization by rhizobacteria can induce a systemic resistance in plants that is phenotypically similar to systemic acquired resistance induced by a localized pathogen infection. We used the tobacco-tobacco mosaic virus model to investigate whether the systemic resistance induced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 is mediated by the systemic acquired resistance signal transduction pathway. Experiments with nahG-transformed tobacco revealed that *Pseudomonas aeruginosa* 7NSK2-induced resistance depended on in planta salicylic acid accumulation for its expression but not for its induction and is, in this respect, similar to systemic acquired resistance. However, *Pseudomonas aeruginosa* 7NSK2-induced resistance was, unlike systemic acquired resistance, not associated with PRL1 expression at the time of challenge with tobacco mosaic virus. This suggests that *Pseudomonas aeruginosa* 7NSK2 treatment would only potentiate defense gene expression in systemic tissue, which would also explain why its level of resistance is lower than in case of systemic acquired resistance. Because we demonstrated that induced resistance by *Pseudomonas aeruginosa* 7NSK2 exclusively depends on the production of salicylic acid by this strain our conclusions might also account for other salicylic acid-producing and resistance-inducing rhizobacteria.

L8 ANSWER 8 OF 84 AGRICOLA  
AN 2000:31370 AGRICOLA  
DN IND22033995  
TI Role of salicylic acid in systemic resistance induced by pseudomonas spp.

against *Pythium aphanidermatum* in cucumber roots.  
AU Chen, C.; Belanger, R.R.; Benhamou, N.; Paulitz, T.C.  
CS Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec, Canada.  
AV DNAL (SB599.E97)  
SO European journal of plant pathology, Aug 1999. Vol. 105, No. 5. p. 477-486  
Publisher: Dordrecht ; Boston : Kluwer Academic Publishers, c1994-  
CODEN: EPLPEH; ISSN: 0929-1873

NTE Includes references  
CY Netherlands  
DT Article  
FS Non-U.S. Imprint other than FAO  
LA English  
AB *Pseudomonas corrugata* strain 13 and *P. aureofaciens* strain 63-28, applied to roots, induced systemic resistance against *Pythium aphanidermatum* in cucumber roots. Salicylic acid (SA) from bacterial culture or plant tissues was quantified by high performance liquid chromatography. Both strains produced SA in King's B broth and also induced cucumber roots to accumulate endogenous SA one day after bacterial inoculation. Using a split root system, more SA accumulated in roots treated with bacteria than in distant roots on the opposite side of the root system in the first two

06/18/01

days, but this difference disappeared after 3-4 days. SA levels were significantly higher in plants treated with bacteria compared to the split control, from one to five days after bacterization. SA did not inhibit mycelial growth of *Pythium aphanidermatum* at 100-200 microgram ml<sup>-1</sup> in vitro, but higher levels inhibited mycelial growth. Zoospore germination increased at concentrations of 10-500 microgram ml<sup>-1</sup>, but decreased at 1000 microgram ml<sup>-1</sup> compared to lower concentrations. Exogenously applied SA failed to induce local or systemic resistance against a challenge infection by the pathogen in planta. The results of this study show that exogenous applied SA does not induce systemic resistance to cucumber root rot caused by *P. aphanidermatum*, but endogenous SA accumulation in cucumber roots may be involved in induced systemic resistance.

L8 ANSWER 13 OF 84 AGRICOLA  
AN 2000:9137 AGRICOLA  
DN IND22019534  
TI Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean.  
AU Meyer, G. de; Capieau, K.; Audenaert, K.; Buchala, A.; Metraux, J.P.; Hofte, M.  
CS University Gent, Belgium.  
SO Molecular plant-microbe interactions : MPMI, May 1999. Vol. 12, No. 5. p. 450-458  
Publisher: St. Paul, MN : APS Press, [c1987-  
CODEN: MPMIEL; ISSN: 0894-0282  
NTE Includes references  
CY Minnesota; United States  
DT Article  
FS U.S. Imprints not USDA, Experiment or Extension  
LA English  
AB Root colonization by specific nonpathogenic bacteria can induce a systemic resistance in plants to pathogen infections. In bean, this kind of systemic resistance can be induced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 and depends on the production of salicylic acid by this strain. In a model with plants grown in perlite we demonstrated that *Pseudomonas aeruginosa* 7NSK2-induced resistance is equivalent to the inclusion of 1 nM salicylic acid in the nutrient solution and used the latter treatment to analyze the molecular basis of this phenomenon. Hydroponic feeding of 1 nM salicylic acid solutions induced phenylalanine ammonia-lyase activity in roots and increased free salicylic acid levels in leaves. Because pathogen-induced systemic acquired resistance involves similar changes it was concluded that 7NSK2-induced resistance is mediated by the systemic acquired resistance pathway. This conclusion was validated by analysis of phenylalanine ammonia-lyase activity in roots and of salicylic acid levels in leaves of soil-grown plants treated with *Pseudomonas aeruginosa*. The induction of systemic acquired resistance

06/18/01

by nanogram amounts of salicylic acid is discussed with respect to long-distance signaling in systemic acquired resistance.

L8 ANSWER 28 OF 84 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 2000:513076 BIOSIS  
DN PREV200000513076  
TI Effects of salicylic acid on the development and root nodulation of soybean seedlings.  
AU Lian, B.; Zhou, X.; Miransari, M.; Smith, D. L. (1)  
CS (1) Department of Plant Science, McGill University, Macdonald Campus, Sainte-Anne-de-Bellevue, PQ, H9X 3V9 Canada  
SO Journal of Agronomy and Crop Science, (October, 2000) Vol. 185, No. 3, pp. 187-192. print.  
ISSN: 0931-2250.  
DT Article  
LA English  
SL English; German  
AB Salicylic acid (SA) is recognized as an endogenous regulator of plant metabolism, mainly involved in induction of systemic acquired resistance (SAR). Exogenous SA can also induce a SAR reaction and SAR gene expression. Excessive SAR-related activity can be an overall cost to the plant in terms of energy and materials expended unnecessarily.

Elicitation

of plant defence responses might also block beneficial plant-microbe interactions and result in negative effects on plant growth. The objective of this study was to investigate the effects of SA concentration (5, 1, 0.5, 0.1 and 0 mM) on soybean seedling growth and nodulation by watering of soybean seedling roots or soaking of seedling leaves with SA solutions.

It was found that 5 mM SA had negative effects on soybean seedling development, but other concentrations of SA did not affect the development of soybean seedlings. In addition, there were no negative effects on seedling development due to SA soaking of seedling leaves. Soybean seedling growth in sterile soil was reduced due to repressed nitrogen uptake following addition of 5 mM SA, indicating that some concentrations of SA can alter the N nutrition of seedlings. A model is presented that ties SA to nodule formation and plant growth.

L8 ANSWER 29 OF 84 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 2000:297512 BIOSIS  
DN PREV200000297512  
TI Effect of salicylic acid on growth, development and some biochemical aspects of soybean (Glycine max L. Merrill).  
AU Kumar, Pramod (1); Dube, S. D. (1); Chauhan, V. S. (1)  
CS (1) Crop Improvement Division, Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, 263601 India  
SO Indian Journal of Plant Physiology, (Oct. Dec., 1999) Vol. 4, No. 4, pp. 327-330. print.  
ISSN: 0019-5502.  
DT Article  
LA English  
SL English  
AB Salicylic acid (SA) viz., (0, 25, 50, 75, 100, 125 and 150 ppm) sprayed

06/18/01

at  
12, 24 and 36 days after sowing accelerated nitrate reductase activity  
and  
enhanced the content of total soluble proteins. Foliar sprays of SA  
hastened the floral bud and pod formation by 2-5 days. Number of  
flowers and pods per plant, grain yield and other attributes were  
maximum  
when 50 ppm SA was sprayed at 24 DAS. Grain yield was found to be  
correlated with NR activity, total soluble proteins, flowers and pods  
per  
plant, pod weight per plant, test weight and harvest index.

L8 ANSWER 30 OF 84 BIOSIS COPYRIGHT 2001 BIOSIS

AN 2000:154860 BIOSIS

DN PREV200000154860

TF Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple  
stress

tolerance in bean and tomato plants.

AU Senaratna, Tissa (1); Touchell, Darren; Bunn, Eric; Dixon, Kingsley

CS (1) Kings Park and Botanic Garden, West Perth, WA, 6005 Australia

SO Plant Growth Regulation., (Feb., 2000) Vol. 30, No. 2, pp.

157-161.

ISSN: 0167-6903.

DT Article

LA English

SL English

AB The hypothesis that physiologically active concentrations of salicylic  
acid (SA) and its derivatives can confer stress tolerance in plants was  
evaluated using bean (*Phaseolus vulgaris* L.) and tomato (*Lycopersicon  
esculentum* L.). Plants grown from seeds imbibed in aqueous solutions  
(0.1-0.5 mM) of salicylic acid or acetyl salicylic acid (ASA) displayed  
enhanced tolerance to heat, chilling and drought stresses. Seedlings  
acquired similar stress tolerance when SA or ASA treatments were applied  
as soil drenches. The fact that seed inhibition with SA or ASA  
confers stress tolerance in plants is more consistent with a signaling  
role of these molecules, leading to the expression of tolerance rather  
than a direct effect. Induction of multiple stress tolerance in plants

by

exogenous application of SA and its derivatives may have a significant  
practical application in agriculture, horticulture and forestry.

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Nancy Sager  
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Yuan-Yuan Chiu  
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