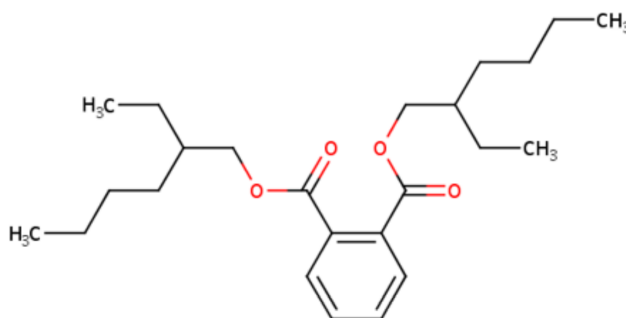

**Data Quality Evaluation and Data Extraction Information for
Dermal Absorption for
Diethylhexyl Phthalate (DEHP)
(1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester)**

Systematic Review Support Document for the Risk Evaluation

CASRN: 117-81-7



December 2025

This supplemental file contains information regarding the data evaluation results for data sources that met the PECO screening criteria for the *Risk Evaluation for Diethylhexyl Phthalate (DEHP)* and were used to characterize dermal absorption. EPA conducted data quality evaluations based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration for the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. Key parameters and corresponding data for each condition were extracted from the reference. EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as the '2021 Draft Systematic Review Protocol'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Systematic Review Protocol for Diethylhexyl Phthalate (DEHP)*.

To evaluate dermal absorption references, EPA consulted several OECD documents when considering quality rankings for individual metrics. Each condition (*e.g.*, individual concentrations tested or different experimental designs) is evaluated independently within a given reference. Therefore each reference may have more than one overall quality determination (OQD) to more appropriately reflect the quality of each condition. No OQD is determined for each reference as a whole, if it contains data from more than one condition. A single reference may evaluate only a limited number of conditions (*e.g.*, use of only the neat compound). If all other methods and results are adequate, the study may be considered acceptable for certain conditions of use. However, the study may still be limited for use in the risk evaluation because it may not address other uses (*e.g.*, lower concentrations, certain solvents/diluents).

Table of Contents

HERO ID	Reference	Page
In vitro		
679215	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. <i>Fundamental and Applied Toxicology</i> 19(4):493-497.	4
1335593	Eastman Kodak, (1989). The in vito percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.	10
2215406	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). <i>Toxicology Letters</i> 224(1):47-53.	16
67185	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. <i>Toxicology and Applied Pharmacology</i> 115(2):216-223.	23
2219803	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. <i>Food and Chemical Toxicology</i> 65:105-114.	45
1333950	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. <i>Toxicology In Vitro</i> 12(1):47-55.	56
674473	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. <i>Environmental Health Perspectives</i> 74(0):223-227.	72
3859042	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. <i>Toxicology and Applied Pharmacology</i> 328(Elsevier):10-17.	79
In vivo - Animal		
1335670	Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.	91
673605	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. <i>Food and Chemical Toxicology</i> 34(3):267-276.	97
675074	Elsisi, A. E., Carter, D. E., Sipes, I. G. (1989). Dermal absorption of phthalate diesters in rats. <i>Fundamental and Applied Toxicology</i> 12(1):70-77.	113
682050	Melnick, R. L., Morrissey, R. E., Tomaszewski, K. E. (1987). Studies by the national toxicology program on di-2-ethylhexylphthalate. <i>Toxicology and Industrial Health</i> 3(2):99-118.	118
67185	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. <i>Toxicology and Applied Pharmacology</i> 115(2):216-223.	121

Study Citation:	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology 19(4):493-497.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	679215			
Unique ID:	F-344 rat			
Domain	Metric		Rating	Comments
Domain 1: Test Substance	Metric 1:	Test substance identity	Medium	The radiolabeled test substance was identified as [14C] di(2-ethylhexyl) phthalate (DEHP). No CASRN, structure, position of the radiolabel, or description of physical properties were included. Unlabeled DEHP was also used.
	Metric 2:	Test substance source	High	The source of the radiolabeled test substance was Sigma Chemical Co., (St. Louis, MO). Lot or batch number was not provided. The source of the non-radiolabeled DEHP was Eastman Chemical Co (Kingsport, TN). The identity of the test substances was verified by the performing laboratory.
	Metric 3:	Test substance purity	High	The purities were greater than 97% for the non-radiolabeled and radiolabeled chemicals; determined by gas chromatography/mass spectrometry or high-performance liquid chromatography.
Domain 2: Test Design	Metric 4:	Reference compounds	Medium	A concurrent reference compound was not tested along with the test substance; however, the lab has performed dermal absorption studies in the past. The study also provides comparisons of the values they obtained with other studies.
	Metric 5:	Assay procedures	Low	The assay procedure was partially described. The study used a vertical static diffusion set-up. The temperature of the skin surface was maintained at is not reported; however, the study states the diffusion cells were incubated at a constant temperature of 30oC for 32 hours. This is lower than the OECD 28 recommended temperature of 32+/- 1oC. Humidity was not reported. 300 uL of the test chemical was added to the donor compartment. The volume of the receptor compartment is not reported and it was not specified whether the sample volumes were replaced. The receptor solution chamber was stirred continuously with a magnetic stirring bar. The receptor solution consisted of Dulbecco's phosphate-buffered isotonic saline containing Volpo-20 (based on a previous study showing different absorption rates when different receptor fluids were used for hydrophobic compounds). The exposed skin surface area was 1.02 or 0.636 cm2. It is unclear why two different areas are reported. It is suspected that one may be for human skin and one may be for rat skin, but this is not clear. The donor compartment was not covered. The integrity of the skin was verified using tritiated water. The study does not report information regarding the quantification of receptor fluid signal:noise ratio.
Continued on next page ...				

...continued from previous page

Study Citation:	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology 19(4):493-497.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	679215			
Unique ID:	F-344 rat			
Domain	Metric	Rating	Comments	
	Metric 6:	Standards for tests	Low	The skin integrity was determined using tritiated water. Integrity was determined before the application of the test substance. The study states samples from the initial integrity test that were deemed outliers were not included in subsequent calculations. These data are not provided, and it is not reported what was considered an outlier. A damage ratio was reported (pre/post application of the test substance was reported). Without the raw data on the tritiated water or information as to what the study considered acceptable measurements of integrity; it is difficult to determine if the skin samples used had good integrity. The mean ± SD permeation coefficient was 2.97 ± 1.26 x 10^-3 (see Metric 17 for more details on integrity). CV values were not reported but could be determined using the data provided. Percent recoveries were not determined, but this is not an important endpoint for infinite exposure scenarios.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	Storage of test substance was not adequately reported; however, this is not likely to substantially impact results. The substance in non-volatile. Test substance was used neat; therefore, discussion of preparation was not necessary.
	Metric 8:	Consistency of exposure administration	Low	The thickness of the skin was reported as full thickness (rat). OECD guidelines recommend avoiding skin thicker than >1 mm for this type of study. The actual thickness was not specified. Two contact areas were described in the report: 1.02 or 0.636 cm2 . It is unclear if the areas were consistent across replicates. The application volume was 300 uL.
	Metric 9:	Reporting of concentrations	Low	The test substance was studied neat. The specific activity of the test substance (mixture of radiolabeled and non-radiolabeled) was 500 uCi/g. The dose (mg/cm2) was not reported, only a volume of 300 uL. It is possible the density of DEHP could be used to calculate an approximate dose (mg); however, the actual application area used is unclear (see Metric 8).
	Metric 10:	Exposure frequency	Low	The duration of exposure was 32 hours. Durations that exceed 24 hours may be necessary for lipophilic test substances; however, there may be concerns about skin integrity. This study did not assess integrity or conduct visual examinations after dosing.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study only tested a single dose (neat test substance).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Skin was obtained from the abdominal area of male Fischer 344 rats. Skin was used as full thickness immediately after harvest. The thickness was not reported. The integrity of the skin was evaluated by measuring the permeability of tritiated water at the beginning of the experiment.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate (n=11).
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology 19(4):493-497.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	679215			
Unique ID:	F-344 rat			
Domain		Metric	Rating	Comments
	Metric 14:	Outcome assessment methodology	High	The outcome assessment methodology addresses the intended outcomes. An infinite dose was added to the donor compartment; 300uL per 1.02 or 0.636 cm ² . According to OECD 28 guidelines, "at least 100 uL/cm ² of pure substance should be used to establish an undepletable dose". Kp was determined from an infinite dose scenario.
	Metric 15:	Consistency of outcome assessment	Medium	Most information was reported and the same protocol was used across replicates. The volume of receptor fluid removed was not reported at each collection was not reported.
	Metric 16:	Sampling adequacy and sensitivity	Low	Duplicate samples were collected every hour and assayed for radioactivity by liquid scintillation spectrometry. No additional details were provided, including how samples were handled after collection, whether measurements were adjusted for background, number of counts, or what the limit of detection was.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	The study did not report the thickness of the skin studied; therefore, it is unclear how much variation existed between the samples. Skin integrity was evaluated using tritiated water, and the resulting permeability constants ranged from 1.09 to 5.13 x 10 ⁻³ cm/hr with a mean ± SD of 2.97 ± 1.26 x 10 ⁻³ . This is higher than guideline recommendations (WHO, 2006 specifies coefficients above 2.5 x 10 ⁻³ cm/h should be rejected), but below the limit value of 4.5 x 10 ⁻³ cm/h. The authors did not specify what they considered to be "an extreme value" but noted that samples that were "established to be unrepresentative" were not included in calculations.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility in the receptor fluid was not demonstrated. OECD guidelines recommend that with very lipophilic substances, such as DEHP, that BSA should be added to the receptor fluid to overcome solubility restrictions. The study used Volpo-20 (polyethylene glycol monooleyl ether) to improve solubility to the receptor fluid, although it did not report or test the solubility of the test substance in this solution.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	Methods for the calculation of the permeability constant, absorption rate and damage ratio of samples are clearly and properly reported. The coefficients of variation were 31% for the absorption rate and 30% for Kp. Sufficient information is available for EPA to calculate an upper-end value to account for variability in the results.
	Metric 20:	Data interpretation	High	Data were properly interpreted. Permeability (Kp) was calculated using infinite concentration. Recovery was not reported, but this is not an important endpoint for infinite exposures.
	Metric 21:	Reporting of data	Medium	Calculated absorption rate and permeability constant are reported. The study does not graphically show the permeation curves.
Overall Quality Determination			Medium	

Study Citation:	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology 19(4):493-497.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	679215			
Unique ID:	Human			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	Medium	The radiolabeled test substance was identified as [14C] di(2-ethylhexyl) phthalate (DEHP). No CASRN, structure, position of the radiolabel, or description of physical properties were included. Unlabeled DEHP was also used.	
Metric 2:	Test substance source	High	The source of the radiolabeled test substance was Sigma Chemical Co., (St. Louis, MO). Lot or batch number was not provided. The source of the non-radiolabeled DEHP was Eastman Chemical Co (Kingsport, TN). The identity of the test substances was verified by the performing laboratory.	
Metric 3:	Test substance purity	High	The purities were greater than 97% for the non-radiolabeled and radiolabeled chemicals; determined by gas chromatography/mass spectrometry or high-performance liquid chromatography.	
Domain 2: Test Design				
Metric 4:	Reference compounds	Medium	A concurrent reference compound was not tested along with the test substance; however, the lab has performed dermal absorption studies in the past. The study also provides comparisons of the values they obtained with other studies.	
Metric 5:	Assay procedures	Low	The assay procedure was partially described. The study used a vertical static diffusion set-up. The temperature of the skin surface was maintained at is not reported; however, the study states the diffusion cells were incubated at a constant temperature of 30oC for 32 hours. This is lower than the OECD 28 recommended temperature of 32+/- 1oC. Humidity was not reported. 300 uL of the test chemical was added to the donor compartment. The volume of the receptor compartment is not reported and it was not specified whether the sample volumes were replaced. The receptor solution chamber was stirred continuously with a magnetic stirring bar. The receptor solution consisted of Dulbecco's phosphate-buffered isotonic saline containing Volpo-20 (based on a previous study showing different absorption rates when different receptor fluids were used for hydrophobic compounds). The exposed skin surface area was 1.02 or 0.636 cm2. It is unclear why two different areas are reported. It is suspected that one may be for human skin and one may be for rat skin, but this is not clear. The donor compartment was not covered. The integrity of the skin was verified using tritiated water. The study does not report information regarding the quantification of receptor fluid signal:noise ratio.	
Metric 6:	Standards for tests	Low	The skin integrity was determined using tritiated water. Integrity was determined before the application of the test substance. The study states samples from the initial integrity test that were deemed outliers were not included in subsequent calculations. These data are not provided, and it is not reported what was considered an outlier. A damage ratio was reported (pre/post application of the test substance was reported). Without the raw data on the tritiated water or information as to what the study considered acceptable measurements of integrity; it is difficult to determine if the skin samples used had good integrity. The mean ± SD permeation coefficient was 1.56 ± 0.83 x 10^-3 (see Metric 17 for more details on integrity). CV values were not reported but could be determined using the data provided. Percent recoveries were not determined, but this is not an important endpoint for infinite exposure scenarios.	

Continued on next page ...

...continued from previous page

Study Citation:	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology 19(4):493-497.		
Chemical:	Diethylhexyl Phthalate		
Exposure Type:	Parent compound		
HERO ID:	679215		
Unique ID:	Human		
Domain	Metric	Rating	Comments
Domain 3: Exposure Characterization			
Metric 7:	Preparation and storage of test substance (chemical)	Medium	Storage of test substance was not adequately reported; however, this is not likely to substantially impact results. The substance in non-volatile. Test substance was used neat; therefore, discussion of preparation was not necessary.
Metric 8:	Consistency of exposure administration	Low	The thickness of the stratum corneum (human) was not reported. Two contact areas were described in the report: 1.02 or 0.636 cm ² . It is unclear if the areas were consistent across replicates. The application volume was 300 uL.
Metric 9:	Reporting of concentrations	Low	The test substance was studied neat. The specific activity of the test substance (mixture of radiolabeled and non-radiolabeled) was 500 uCi/g. The dose (mg/cm ²) was not reported, only a volume of 300 uL. It is possible the density of DEHP could be used to calculate an approximate dose (mg); however, the actual application area used is unclear (see Metric 8).
Metric 10:	Exposure frequency	Low	The duration of exposure was 32 hours. Durations that exceed 24 hours may be necessary for lipophilic test substances; however, there may be concerns about skin integrity. This study did not assess integrity or conduct visual examinations after dosing.
Metric 11:	Number of exposure groups and concentration spacing	Low	The study only tested a single dose (neat test substance).
Domain 4: Test Model			
Metric 12:	Test model (skin)	Low	Whole human skin samples from cadaver (black and white males and females) were obtained from the National Disease Research Interchange in Philadelphia, PA and were frozen at -70oC. Stratum corneum was separated by immersing the skin in warm (60oC) water and separating the outermost layer. The thickness was not reported. Stratum corneum were then refrozen at -70oC or stored at 4oC if used within 24-48 hours. OECD 28 guidelines state "Skin should not be stored at very low temperatures since it has been shown that storage of skin at -80oC can enhance permeability ". The guidelines also state "it is inadvisable to refreeze and thaw skin specimens as this can increase the permeability". However, the integrity of the human skin was tested at the beginning of the experiment and was appropriate.
Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate (n=4). This is the minimum recommended sample size according to OECD TG 428.
Domain 5: Outcome Assessment			
Metric 14:	Outcome assessment methodology	High	The outcome assessment methodology addresses the intended outcomes. An infinite dose was added to the donor compartment; 300uL per 1.02 or 0.636 cm ² . According to OECD 28 guidelines, "at least 100 uL/cm ² of pure substance should be used to establish an undepletable dose". Kp was determined from an infinite dose scenario.
Metric 15:	Consistency of outcome assessment	Medium	Most information was reported and the same protocol was used across replicates. The volume of receptor fluid removed was not reported at each collection was not reported.
Continued on next page ...			

...continued from previous page

Study Citation:	Barber, E. D., Teetsel, N. M., Kolberg, K. F., Guest, D. (1992). A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundamental and Applied Toxicology 19(4):493-497.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	679215			
Unique ID:	Human			
Domain	Metric		Rating	Comments
	Metric 16:	Sampling adequacy and sensitivity	Low	Duplicate samples were collected every hour and assayed for radioactivity by liquid scintillation spectrometry. No additional details were provided, including how samples were handled after collection, whether measurements were adjusted for background, number of counts, or what the limit of detection was.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Low	The study did not report the thickness of the skin studied; therefore, it is unclear how much variation existed between the samples. Skin integrity was evaluated using tritiated water, the permeability constants varied from 0.74 to 2.96 x 10 ⁻³ with a mean of 1.56 ± 0.83 x 10 ⁻³ . This is close to the accepted median of 1.5 x 10 ⁻³ .
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility in the receptor fluid was not demonstrated. OECD guidelines recommends with very lipophilic substances, such as DEHP, that BSA should be added to the receptor fluid to overcome solubility restrictions. The study used Volpo-20 (polyethylene glycol monooleyl ether) to improve solubility to the receptor fluid, although did not report or test the solubility of the test substance in this solution.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	High	Methods for the calculation of the permeability constant, absorption rate and damage ratio of samples are clearly and properly reported. The coefficients of variation were 20% for the absorption rate and for the Kp.
	Metric 20:	Data interpretation	High	Data were properly interpreted. Permeability (Kp) was calculated using infinite concentration. Recovery was not reported, but this is not an important endpoint for infinite exposures.
	Metric 21:	Reporting of data	Medium	Calculated absorption rate and permeability constant are reported. The study does not graphically show the permeation curves.
Overall Quality Determination			Medium	

Study Citation:	Eastman Kodak, (1989). The in vitro percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335593			
Unique ID:	Human skin			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester; CASRN 117-81-7 (DEHP). Both unlabeled and 14C radiolabeled test substances were used. The position of the radiolabel was not specified. Unlabeled DEHP was sourced from Tennessee Eastman Company. Radiolabeled DEHP was sourced from Arthur D. Little, Inc., but it had originally been purchased from Sigma Chemical Company. The test substance was analytically verified by the performing laboratory. The unlabeled test material was 99.3% pure as determined by GC. GC/MS was also done to analytically verify the test material to be DEHP. The radiolabeled chemical was also analyzed by GC and was >99% pure with a specific activity of 3.7 mCi/mmole.
	Metric 2:	Test substance source	High	
	Metric 3:	Test substance purity	High	
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported. Exposures were conducted under presumably static conditions in Franz-type glass diffusion cells. The permeability of the skin samples was tested with tritiated H2O prior to exposure to the test substance. The skin samples were rinsed, the chambers were filled with saline and stirred overnight and then rinsed again prior to exposure to the test substance. The surface area of the skin samples was not specified and a dose in mg/cm2 or volume/cm2 was not reported. What is presumed to be an infinite dose of the test substance was added to the donor chambers; the volume added was (200-300 uL). It is unclear why a range rather than a singular volume was given. The receptor fluid was Dulbecco's PBS containing 6% Volpo-20 (a non-ionic detergent) and antibiotics. A magnetic stir bar was used and receptor fluid temperature was held at 30 degrees C. Humidity was not specified. It was not stated whether the chambers were closed or left open. Receptor fluid was sampled hourly for a total of 32 hours, which is longer than typically recommended. The receptor fluid was refilled after each sampling. Additional time was required in order to "obtain statistically significant numbers of DPM in the samples of receptor fluid." No details on the sensitivity of scintillation counting were provided. Radioactivity was tested in receptor fluid only. After the 32-hour exposure, the donor chambers were rinsed with 50% aqueous ethanol and receptor chambers were rinsed with saline. They were re-filled with receptor fluid, stirred overnight, and then integrity was tested again using tritiated H2O. The study did not report % recovery. The variance across replicates is low (SD <25% for all endpoints). Skin integrity was determined by measuring the permeability to tritiated H2O both before and after exposure to the test substance. Two outlier data points were identified and eliminated. In the remaining samples, the initial Kp was acceptable (0.900 x 10^-3 cm/h)
	Metric 5:	Assay procedures	Low	
	Metric 6:	Standards for tests	Low	

Continued on next page ...

...continued from previous page

Study Citation:	Eastman Kodak, (1989). The in vitro percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335593			
Unique ID:	Human skin			
Domain	Metric	Rating	Comments	
Domain 3: Exposure Characterization				
Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details of test substance preparation were included in the appendix. Radiolabeled test substance was diluted with unlabeled test substance. Nominal specific radioactivity (509.68 uCi/g) and concentration (0.981 g/mL) were reported. The measured specific radioactivity was 506.121 uCi/g. No details on stability or mixing to assure homogeneity, mixing temperature, stirring methods, or storage conditions were reported. The solubility of the test substance was evaluated in PBS receptor fluid alone, or in PBS with 6% Volpo-20.	
Metric 8:	Consistency of exposure administration	Low	Details of exposure administration were insufficiently reported. There were potentially moderate differences in the volume applied (reported as 200 to 300 uL), and the skin thickness and surface area were not reported.	
Metric 9:	Reporting of concentrations	Medium	The study reported a concentration of 0.981 g/mL	
Metric 10:	Exposure frequency	High	The initial duration was set to 8 hours but was extended an additional 8 hours, and then to a total of 32 hours. This is longer than current guidelines which indicate a preference of not more than 24 hours. However, longer durations are sometimes required for lipophilic compounds. This study noted a 2-3 hour lag time before DEHP was detected in the receptor solution, and at 8 hours, the radioactivity was not significantly above background levels. The damage ratio for human skin showed minimal to no differences in the amount of damage compared with controls after 32 hours.	
Metric 11:	Number of exposure groups and concentration spacing	Low	The study used a single exposure group, of undiluted DEHP at 0.981 g/mL. This is less than recommended by OECD TG 428.	
Domain 4: Test Model				
Metric 12:	Test model (skin)	Low	Human abdominal skin from three donors (age/sex not specified) was obtained from the National Disease Research Institute. It was noted that samples were selected that had been stored properly to ensure no degradation, but the methods of storage were not specified. The stratum corneum was separated from whole skin using a 60-degree water bath and then was re-frozen (temperature not specified) until the start of the experiment. The skin thickness and surface area were not reported. These reporting limitations may have a substantial impact on the study results. Typically, split-thickness human skin samples are preferred.	
Metric 13:	Number/Replicates per group	Medium	From each of the three donors, there were two test cells and 1 control cell that contained saline only. In total, there were 6 test replicates and three control replicates for the single exposure group. The number of replicates was appropriate as per OECD 428.	
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:	Eastman Kodak, (1989). The in vitro percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335593			
Unique ID:	Human skin			
Domain	Metric	Rating	Comments	
	Metric 14:	Outcome assessment methodology	Medium	What was presumed to be an infinite dose scenario was used for Kp determinations; there are some uncertainties because the mass or volume per surface area was not specified. The outcome assessment methodology addressed the outcome of interest. It is not clear whether infinite exposure at the concentration tested was appropriate for the conditions of use.
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The duration of exposure and sampling period was consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Medium	The study indicated that radioactivity levels were not significantly above background after 8 hours. The scintillation counts/sample and/or duration of radioactivity detection were not specified. The sampling allowed for graphical presentations of receptor fluid contents vs. time.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Skin integrity was measured by a less desirable method (tritiated water); there were two test samples from different donors that were outliers in the initial permeability test; the results for other samples were acceptable ($K_p < 4.5 \times 10^{-3}$ cm/h). In the post-exposure permeability tests, two samples (the same two that were outliers in the initial tests) had Kp values above the acceptable range. A damage ratio of 2.6 was determined for this study in the DEHP samples. The damage ratio for saline controls was 1.81 indicating DEHP did not cause significant damage to the human epidermal membranes.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	High	With the addition of 6% Volpo to the receptor fluid, the solubility of the test material was acceptable. The solubility of DEHP in the receptor solution did not appear to influence the rate of skin penetration.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	High	The calculation methods were clearly described and were appropriate. Kp measurements were based on linear regression. Radioactivity in the receptor fluid was presented across time. Outliers were excluded from calculations. The standard deviations relative to the mean were <25%.
	Metric 20:	Data interpretation	High	Recovery was not determined for this study. The study appropriately determined Kp and absorption rates. The percent absorption is not relevant in an infinite dose study. Raw data are provided in the appendices.
	Metric 21:	Reporting of data	High	Data were adequately reported for the intended outcomes of interest. Values were provided as means \pm SD. There are no concerns about data presentation.
Overall Quality Determination		Medium		

Study Citation:	Eastman Kodak, (1989). The in vivo percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335593			
Unique ID:	Full thickness rat skin			
Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester; CASRN 117-81-7 (DEHP). Both unlabeled and 14C radiolabeled test substances were used. The position of the radiolabel was not specified.
	Metric 2:	Test substance source	High	Unlabeled DEHP was sourced from Tennessee Eastman Company. Radiolabeled DEHP was sourced from Arthur D. Little, Inc., but it had originally been purchased from Sigma Chemical Company. The test substance was analytically verified by the performing laboratory.
	Metric 3:	Test substance purity	High	The unlabeled test material was 99.3% pure as determined by GC. GC/MS was also done to analytically verify the test material to be DEHP. The radiolabeled chemical was also analyzed by GC and was >99% pure with a specific activity of 3.7 mCi/mmol.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Low	Exposures were conducted under presumably static conditions in Franz-type glass diffusion cells. The permeability of the skin samples was tested with tritiated H2O prior to exposure to the test substance. The skin samples were rinsed, the chambers were filled with saline and stirred overnight and then rinsed again prior to exposure to the test substance. The surface area of the skin samples was not specified and a dose in mg/cm2 or volume/cm2 was not reported. What is presumed to be an infinite dose of the test substance was added to the donor chambers; the volume added was (200-300 uL). It is unclear why a range rather than a singular volume was given. The receptor fluid was Dulbecco's PBS containing 6% Volpo-20 (a non-ionic detergent) and antibiotics. A magnetic stir bar was used and receptor fluid temperature was held at 30 degrees C. Humidity was not specified. It was not stated whether the chambers were closed or left open. Receptor fluid was sampled hourly for a total of 32 hours, which is longer than typically recommended. The receptor fluid was refilled after each sampling. Additional time was required in order to "obtain statistically significant numbers of DPM in the samples of receptor fluid." No details on the sensitivity of scintillation counting were provided. Radioactivity was tested in receptor fluid only. After the 32-hour exposure, the donor chambers were rinsed with 50% aqueous ethanol and receptor chambers were rinsed with saline. They were re-filled with receptor fluid, stirred overnight, and then integrity was tested again using tritiated H2O.
	Metric 6:	Standards for tests	Low	The study did not report % recovery. The variance across replicates can be determined using the data provided (see Metric 19 for more details). Skin integrity was determined by measuring the permeability to tritiated H2O both before and after exposure to the test substance. Two outlier data points were identified and eliminated. In the remaining samples, the initial Kp was acceptable (2.18 x 10^-3 cm/h).
Domain 3: Exposure Characterization				
Continued on next page ...				

...continued from previous page

Study Citation:		Eastman Kodak, (1989). The in vito percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.		
Chemical:		Diethylhexyl Phthalate		
Exposure Type:		Parent compound		
HERO ID:		1335593		
Unique ID:		Full thickness rat skin		
Domain	Metric	Rating	Comments	
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details of test substance preparation were included in the appendix. Radiolabeled test substance was diluted with unlabeled test substance. Nominal specific radioactivity (509.68 uCi/g) and concentration (0.981 g/mL) were reported. The measured specific radioactivity was 506.121 uCi/g. No details on stability or mixing to assure homogeneity, mixing temperature, stirring methods, or storage conditions were reported. The solubility of the test substance was evaluated in PBS receptor fluid alone, or in PBS with 6% Volpo-20.
	Metric 8:	Consistency of exposure administration	Low	Details of exposure administration were insufficiently reported. There were potentially moderate differences in the volume applied (reported as 200 to 300 uL), and the skin thickness and surface area were not reported.
	Metric 9:	Reporting of concentrations	Medium	The study reported a concentration of 0.981 g/mL
	Metric 10:	Exposure frequency	Low	The initial duration was set to 8 hours but was extended an additional 8 hours, and then to a total of 32 hours. This is longer than current guidelines which indicate a preference of not more than 24 hours. However, longer durations are sometimes required for lipophilic compounds. This study noted a 2-3 hour lag time before DEHP was detected in the receptor solution, and at 8 hours, the radioactivity was not significantly above background levels. The damage ratio for rat skin treated with DEHP showed some damage after 32 hours, compared with controls. The study did not indicate why exposures were not stopped at 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study used a single exposure group, of undiluted DEHP at 0.981 g/mL. This is less than recommended by OECD TG 428.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Rat abdominal skin was excised from 9-11 week-old Fischer-344 rats (3/experiment). Excess fat and connective tissues were removed. Full-thickness skin pieces were cut to the size of the diffusion cells and were used within 45 min or less. The skin thickness and surface area were not reported. These reporting limitations may have a substantial impact on the study results. OECD TG 156 specify that full-thickness skin should not be used to calculate fluxes. It is unclear how appropriate this model was for an infinite dose study.
	Metric 13:	Number/Replicates per group	Medium	From each of the three donors, there were two test cells and 1 control cell that contained saline only. In total, there were 6 test replicates and three control replicates for the single exposure group. The number of replicates was appropriate as per OECD 428.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Medium	What was presumed to be an infinite dose scenario was used for Kp determinations; there are some uncertainties because the mass or volume per surface area was not specified. The outcome assessment methodology addressed the outcome of interest. It is not clear whether infinite exposure at the concentration tested was appropriate for the conditions of use.

Continued on next page ...

...continued from previous page

Study Citation:	Eastman Kodak, (1989). The in vivo percutaneous absorption of di(2-ethylhexyl) phthalate through human stratum corneum and full thickness rat (F-344) skin with attached appendix and cover letter dated 062789.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335593			
Unique ID:	Full thickness rat skin			
Domain	Metric	Rating	Comments	
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The duration of exposure and sampling period was consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Medium	The study indicated that radioactivity levels were not significantly above background after 8 hours. The scintillation counts/sample and/or duration of radioactivity detection were not specified. The sampling allowed for graphical presentations of receptor fluid contents vs. time.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Low	Skin integrity was tested in a pre-test using tritiated H2O. There were two outliers and variability was high even with the exclusion of those outliers from the data analysis. The entire study was repeated a second time due to high variability in the pre-exposure skin permeability tests. In the repeated study, the pre-exposure permeability tests were acceptable ($K_p < 4.5 \times 10^{-3}$ cm/h), and variation was low. In the post-exposure permeation test, tritiated H2O was inadvertently omitted from the three control samples and those data were not available. The K_p values of the test groups were higher (6.1 to 18.4). Damage ratios were determined; in the repeated study, the damage ratio of treated rat skin was 6.90 ± 1.34 compared to a damage ratio of 1.81 ± 0.08 for the saline controls, indicating DEHP caused slight damage to the rat skin samples after 32 hours of exposure, which likely had an impact on the study results.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	High	With the addition of 6% Volpo to the receptor fluid, the solubility of the test material was acceptable. The solubility of DEHP in the receptor solution did not appear to influence the rate of skin penetration.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	The calculation methods were clearly described and were appropriate. K_p measurements were based on linear regression. Radioactivity in the receptor fluid was presented across time. Outliers were excluded from calculations. The coefficient of variation for both the permeability constant and the absorption rate was 32%. (which is greater than the recommended <25%); however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results.
	Metric 20:	Data interpretation	High	Recovery was not determined for this study. The study appropriately determined K_p and absorption rates. The percent absorption is not relevant in an infinite dose study. Raw data are provided in the appendices.
	Metric 21:	Reporting of data	High	Data were adequately reported for the intended outcomes of interest. Values were provided as means \pm SD. There are no concerns about data presentation.
Overall Quality Determination			Medium	

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2215406			
Unique ID:	Hopf et al. 2014			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	High	The test substance was identified as DEHP (Cas No 117-81-7) . The test substance was unlabeled. Limited physical/chemical properties were listed.	
Metric 2:	Test substance source	High	The source of the test substance was Sigma-Aldrich (Buchs, Switzerland). The lot or Batch no. was not reported. Certificates of analysis are typically available from the supplier's website but were not included in the study report. The test substance was not analytically verified by the performing laboratory.	
Metric 3:	Test substance purity	High	The purity of the test substance was reported to be 99.5%.	
Domain 2: Test Design				
Metric 4:	Reference compounds	Low	A concurrent reference compound was not tested along with the test substance.	
Metric 5:	Assay procedures	Medium	Neat DEHP was applied to metabolically active human skin samples under flow-through conditions. A rack of six jacketed flow-through diffusion cells was used. The temperature was 32oC, humidity was not reported. RPMI-1640 was used as the receptor fluid (volume 8.5 ml). A peristaltic pump automatically removed and replaced 40ul/min of receptor fluid throughout the study. The receptor compartment was not reported to be constantly stirred. The test substance was not volatile; however, the authors did not state if the donor compartment was covered (with parafilm or some other material). D4 was applied at a rate of 1114.1 mg/cm2 or 554 mg/cm2 (see Metric 9), either concentration is appropriate for an infinite dose scenario. It was not reported whether the system was open, occluded or semi-occluded. The frequency of sampling from the receptor compartment was not specified in the methods, but the information can be extracted from the figures shown. Receptor fluid was analyzed for the DEHP metabolite MEHP. The results stated that no DEHP was detected in the reservoir fluid. Although the authors provided some justification for the measurement of metabolites, this is atypical for this study type. The limited of quantitation of the metabolites was reported.	
Metric 6:	Standards for tests	Low	Human skin was obtained from patients undergoing abdominoplasty. The skin was used for these studies within 2 hours from the time the surgery ended. The integrity of the skin was determined by stable trans epidermal water loss (TEWL) readings. If the readings were above 11 g/m2/hr, the skin was considered damaged and replaced. CV values for precision of analyte quantification were reported to be <5%.	
Domain 3: Exposure Characterization				
Metric 7:	Preparation and storage of test substance (chemical)	Medium	Storage of test substance was not adequately reported; however, this is not likely to substantially impact results. The substance is non-volatile. Test substance was used neat; therefore, discussion of preparation was not necessary.	

Continued on next page ...

...continued from previous page

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2215406			
Unique ID:	Hopf et al. 2014			
Domain	Metric	Rating	Comments	
	Metric 8:	Consistency of exposure administration	Medium	Details of exposure administration were reported; some inconsistencies were identified, but exposures were consistent across replicates. The dermatomed skin thickness was 800 um. A 1 or 2 ml volume (see Metric 9) of neat DEHP was applied to a skin area of 1.77 cm2.
	Metric 9:	Reporting of concentrations	Low	The test substance was applied to the skin neat; however, there are discrepancies in the dose applied. The methods state that 2mL was applied at a dose per cm2 of 1,114.1 mg/cm2. The footnotes in Table 2 suggest that 1mL was applied and reported a dose of 980 mg (based on density), which with a skin area of 1.77 cm2, would be 554 mg/cm2.
	Metric 10:	Exposure frequency	Low	The duration of exposure is not clear. The methods state that the duration of sampling was "24 or 72 hours"; however, figures show data collection for 48 hours. OECD 428 guidelines indicate that skin may start to deteriorate beyond 24 hours, but for substances that penetrate the skin slowly, longer times may be required. This study did not assess skin integrity at the end of the experiment to determine whether deterioration had occurred. There was a significant lag in the detection of metabolites following application (e.g., detection began more than 30 hours post-dosing and had not leveled off by 48 hours, suggesting slow penetration.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study tested neat (this form) and emulsified DEHP. These were selected to represent exposures among workers manufacturing DEHP.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Human skin was obtained from patients undergoing abdominoplasty. The skin was dermatomed to 800um thickness which is thicker than guideline specifications for split-thickness samples of 200-400um). The skin samples were used within 2 hours from the end of surgery. Integrity was tested by TEWL test. To be used, the skin had a TEWL of less than 11 g/m2/h. Details of the donors (age, sex, ethnicity, and BMI) were not specified, which could have an impact on the study results.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates/group (n=4) and concentration studies (infinite) were appropriate according to OECD guidelines. It is not clear if each donor was studied more than once. The study only reports n=4 and does not report any variance within each donor.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Medium	An infinite dose scenario was used for Kp determinations. This study used a non-radioactive test substance, and the parent compound and main metabolite MEHP were detected using HPLC and MS/MS. The outcome assessment methodology was sensitive for the outcomes of interest.
	Metric 15:	Consistency of outcome assessment	High	The outcome assessment was carried out consistently. The same protocol was applied to all replicates and the same receptor fluid was used. The assessment of concentrations (of metabolites) in receptor fluid was analyzed consistently using HPLC followed by MS/MS.

Continued on next page ...

...continued from previous page

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2215406			
Unique ID:	Hopf et al. 2014			
Domain	Metric	Rating	Comments	
	Metric 16:	Sampling adequacy and sensitivity	High	The number of samples analyzed was appropriate. Samples were analyzed via HPLC followed by MS/MS. Quantification limits were reported. The cumulative concentration of the metabolite MEHP was determined at sixteen time points within the 48 hours.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Low	Skin integrity was measured by TEWL. If the readings were above 11 g/m2/hr, the skin was considered damaged and replaced. This is a slight deviation from guidelines which suggest viable skin have a TEWL reading of less than 10 grams/m2/hour to be used. The recorded TEWL readings were not reported. Information (such as gender, age, ethnicity, or BMI) on the four donors was not provided. Differences in these parameters may impact results. It is not clear how many times replicates of one donor were tested. No indication of variance within a donor is given. Skin integrity at the end of the experiment was not assessed.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility in the receptor fluid was not demonstrated. OECD guidelines recommend with very lipophilic substances, such as DEHP, that BSA should be added to the receptor fluid to overcome solubility restrictions. However, the authors state that “5% BSA has been shown to inhibit skin metabolism, and therefore interfere with permeation (Haberland et al., 2006; Zhang et al. 2009). Alcohol is also not an option as it will not allow for the metabolism in the skin to remain viable. “Cell culture buffers (RPMI 1640 solution) may be good alternatives as they allow for skin metabolism, which has been shown in a recent study with di-butyl phthalate (DBP) (Beydon et al., 2010)”. This study therefore used RPMI 1640 without BSA as to not interfere with the metabolism of DEHP in the skin.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	Calculations of Kp/flux and lag times were reported. “Permeability coefficients (Kp) were estimated from the slopes of the cumulative absorption plots over time. Lag times were estimated as the intercept of the steady state portion of the permeability rate (J) curves with the time axis. s. Individual Js were calculated from each diffusion cell and the average and standard deviations were calculated for the group.” The CV value for the slope of the linear portion of the permeation curve (J) = 130%. Sufficient information (e.g., arithmetic mean and standard deviation) are provided to for alternative calculations. No variance was reported for Kp, and CV values cannot be determined. Individual replicate data were not reported precluding the ability to conduct independent analysis for Kp.
	Metric 20:	Data interpretation	High	Permeability (Kp) was calculated using infinite concentration and was based on the linear portion of a permeation curve. These data were based on the quantification of MEHP (a metabolite) in the receptor fluid vs. DEHP.
Continued on next page ...				

...continued from previous page

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.
Chemical:	Diethylhexyl Phthalate
Exposure Type:	Parent compound
HERO ID:	2215406
Unique ID:	Hopf et al. 2014

Domain	Metric	Rating	Comments
	Metric 21: Reporting of data	Low	Individual data were not provided in this published report. Cumulative absorption curves show concentrations of the MEHP metabolite over time. The results qualitatively state that no DEHP or d4-DEHP was detected; but it is unclear how many of the neat samples were tested (an n=6 for this measurement was specified for a total of 4 DEHP and 4 D4-DEHP samples), and the LOQ for DEHP was not specified. There are discrepancies in the results text. The text reports that Figure 1 is for "neat d4-DEHP" and Fig. 2 is for "DEHP (aq)". However, Fig. 1 is for DEHP (neat) and Fig. 2 is for D4-DEHP (aq).

Overall Quality Determination**Medium**

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2215406			
Unique ID:	Hopf et al. 2014- d4-DEHP emulsion			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	Low	The test substance was identified as deuterated DEHP (d4-DEHP). No details on how many/or position of hydrogens that were replaced with deuterium. No physical chemical properties were provided, there could be significant physical differences from the parent DEHP compound that could impact the study results.	
Metric 2:	Test substance source	Low	The source of the test substance was Cambridge Isotope Laboratories (ReseaChem GmbH, Burgdorf, Switzerland). No Lot or Batch no. was reported and a certificate of analysis was not included in the study report. The test substance was not analytically verified by the performing laboratory.	
Metric 3:	Test substance purity	Low	The purity of the test substance was not reported.	
Domain 2: Test Design				
Metric 4:	Reference compounds	Low	A concurrent reference compound was not tested along with the test substance.	
Metric 5:	Assay procedures	Medium	An aqueous emulsion of D4-DEHP was applied to metabolically active human skin samples under flow-through conditions. A rack of six jacketed flow-through diffusion cells was used. The temperature was 32oC, humidity was not reported. RPMI-1640 was used as the receptor fluid (volume 8.5 ml). A peristaltic pump automatically removed and replaced 40ul/min of receptor fluid throughout the study. The receptor compartment was not reported to be constantly stirred. The test substance was not volatile; however, the authors did not state if the donor compartment was covered (with parafilm or some other material). D4 was applied at a reported rate of 140.7 mg/cm2; however, this may be in error (see Metric 9). The desire was presumably to achieve infinite exposure. It was not reported whether the system was open, occluded or semi-occluded. The frequency of sampling from the receptor compartment was not specified in the methods, but the information can be extracted from the figures shown. Receptor fluid was analyzed for the DEHP metabolite MEHP. The results stated that no D4- DEHP was detected in the reservoir fluid. Although the authors provided some justification for the measurement of metabolites, this is atypical for this study type. The limited of quantitation of the metabolites was reported.	
Metric 6:	Standards for tests	Low	Human skin was obtained from patients undergoing abdominoplasty. The skin was used for these studies within 2 hours from the time the surgery ended. The integrity of the skin was determined by stable trans epidermal water loss (TEWL) readings. If the readings were above 11 g/m2/hr, the skin was considered damaged and replaced. CV values for precision of analyte quantification were reported to be <5%. No other CV values were reported. See Metric 19 for additional discussion on CV values.	
Domain 3: Exposure Characterization				
Metric 7:	Preparation and storage of test substance (chemical)	Low	Preparation details were not adequately reported. D4-DEHP was emulsified in an RPMI buffer solution. No further details or tests of homogeneity were described. The timing of preparation and storage details, if relevant, were not reported. The test substance is non-volatile. The concentration of test substance in the emulsion was 166 ug/ml (nominal).	
Continued on next page ...				

...continued from previous page

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2215406			
Unique ID:	Hopf et al. 2014- d4-DEHP emulsion			
Domain	Metric	Rating	Comments	
	Metric 8:	Consistency of exposure administration	Medium	Exposure was consistent. The skin thickness was 800 um. The volume applied was 1.5 ml of 166 ug/ml d4-DEHP. The skin surface area was 1.77 cm2
	Metric 9:	Reporting of concentrations	Low	The reported applied dose per cm2 was 140.7 mg/cm2. This may be in error. Based on the other information reported (i.e., a concentration of 166 ug/mL, application of 1.5 mL, and a skin surface area of 1.77 cm2), the applied dose would be 140.7 ug/cm2 (1,000x less). In Table 2, the study reports a Dose (mg) of 50. It is unclear where this value comes from. Based on the information provided, a dose of 229 ug was used (166 ug/mL x 1.5 mL). No analytical measurements were conducted.
	Metric 10:	Exposure frequency	High	The exposure duration was 24 hours which is consistent with OECD Guidelines and appropriate for the outcome of interest (Kp/flux and lag time). A linear portion of the absorption curve was attained.
	Metric 11:	Number of exposure groups and concentration spacing	Low	One dose of D4-DEHP was tested. The dose tested was chosen to represent aerosol exposures among workers (system does not allow for the generation of aerosols depositing directly on the skin disks).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Human skin was obtained from patients undergoing abdominoplasty. The skin was dermatomed to 800um thickness which is thicker than guideline specifications for split-thickness samples of 200-400um). The skin samples were used within 2 hours from the end of surgery. Integrity was tested by TEWL test. To be used, the skin had a TEWL of less than 11 g/m2/h. Details of the donors (age, sex, ethnicity, and BMI) were not specified, which could have an impact on the study results.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates/group is unclear. Figure 2 shows a total of 5 replicates, but Table 2 reports results from 4 replicates. The study reports that 4 donors were used for the experiments, it is unclear if one donor was studied more than once. OECD guidelines specify a minimum of 4 replicates.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	High	An infinite dose (847 uL/cm2) scenario was used for Kp determinations. This study used a non-radioactive test substance, and the parent compound and main metabolite D4-MEHP were detected using HPLC and MS/MS. The outcome assessment methodology was sensitive for the outcomes of interest.
	Metric 15:	Consistency of outcome assessment	High	The outcome assessment was carried out consistently. The same protocol was applied to all replicates and the same receptor fluid was used. The assessment of concentrations (of metabolites) in receptor fluid was analyzed consistently using HPLC followed by MS/MS.
	Metric 16:	Sampling adequacy and sensitivity	High	The number of samples analyzed was appropriate. Samples were analyzed via HPLC. The cumulative concentration of the metabolite MEHP was determined at 8 time points within 24 hours. A linear portion of the absorption curve with at least three data points was attained.
Continued on next page ...				

...continued from previous page

Study Citation:	Hopf, N. B., Berthet, A., Vernez, D., Langard, E., Spring, P., Gaudin, R. (2014). Skin permeation and metabolism of di(2-ethylhexyl) phthalate (DEHP). Toxicology Letters 224(1):47-53.		
Chemical:	Diethylhexyl Phthalate		
Exposure Type:	Parent compound		
HERO ID:	2215406		
Unique ID:	Hopf et al. 2014- d4-DEHP emulsion		
Domain	Metric	Rating	Comments
Domain 6: Confounding/Variable Control			
Metric 17:	Confounding variables in test design and procedures	Medium	Skin integrity was measured by TEWL. If the readings were above 11 g/m ² /hr, the skin was considered damaged and replaced. This is a slight deviation from guidelines which suggested viable skin have a TEWL reading of less than 10 grams/m ² /hour in order to be used. The recorded TEWL readings were not reported. Information (such as gender, age, ethnicity, or BMI) on the four donors was not provided. Differences in these parameters may impact results. It is not clear how many times replicates of one donor was tested.
Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility in the receptor fluid was not demonstrated. OECD guidelines recommends with very lipophilic substances, such as DEHP, that BSA should be added to the receptor fluid to overcome solubility restrictions. However, the authors state that "5% BSA have shown to inhibit skin metabolism, and therefore interfere with permeation (Haberland et al., 2006; Zhang et al. 2009). Alcohol is also not an option as it will not allow for the metabolism in the skin to remain viable. "Cell culture buffers (RPMI 1640 solution) may be good alternatives as they allow for skin metabolism, which has been shown in a recent study with di-butyl phthalate (DBP) (Beydon et al., 2010)". This study therefore used RPMI 1640 without BSA as to not interfere with metabolism of DEHP in the skin.
Domain 7: Data Presentation and Analysis			
Metric 19:	Data analysis	Low	Calculations of Kp/flux and lag times were reported. "Permeability coefficients (Kp) were estimated from the slopes of the cumulative absorption plots over time. Lag times were estimated as the intercept of the steady state portion of the permeability rate (J) curves with the time axis. Individual Js were calculated from each diffusion cell and the average and standard deviations were calculated for the group." No CV values for these endpoints were provided. Only "J" the slope of the linear portion of the permeation curve reported both an arithmetic mean and standard deviation. A CV value of 84 was calculated for this review. There is sufficient information to re-calculate using a higher absorption value. A CV value for the the Kp could not be determined.
Metric 20:	Data interpretation	High	Data were interpreted correctly. Permeability (Kp) was calculated using infinite concentration.
Metric 21:	Reporting of data	Low	In Table 2, the study reports an n=4; however, in Figure 2 there is data for 5 cells (up to 8 hours). The discrepancy is not discussed. There were only 4 donors, so it is not known if the authors may have repeated one donor. Kp was reported without measures of variance. Only a qualitative statement was made stating that no parent D4-DEHP was detected. Individual data were not reported. There are discrepancies in the results text. The text reports that Figure 1 is for "neat d4-DEHP" and Fig. 2 is for "DEHP (aq)". However, Fig. 1 is for DEHP (neat) and Fig. 2 is for D4-DEHP (aq).
Overall Quality Determination		Low	

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Low dose- viable skin			
Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted.
	Metric 2:	Test substance source	High	The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not included certificates of analyses. Lot numbers were not provided.
	Metric 3:	Test substance purity	High	The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	A reference compound was not used and there is no indication in the text regarding the performing laboratory’s history of performing these tests.
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Low dose- viable skin			
Domain	Metric	Rating	Comments	
	Metric 5:	Assay procedures	Low	The assay procedures specified in the report were partially described. A flow-through diffusion set-up was used. Details of the set-up were found in cited reference Bronaugh and Stewart, 1985 (HERO 2776481). The study does not report if there was an equilibrium period after the skin was placed into the chamber (guidelines recommend a 30-minute equilibrium period). Finite doses of DEHP (33.6, 153, or 313 nmol/cm2) were applied to freshly excised guinea pig skin (from 5 or more animals; location on the animal not reported). The skin discs punched were 2.0 cm2 which were mounted onto the diffusion cells. The inner surface area of the skin was not reported; however, in the cited reference describing the set-up, the surface area exposed to the chemical was 0.64 cm2. It is assumed this is the same for this experiment. The test substance was applied to the skin in 10 ul acetone solution. This volume is slightly more than OECD 428 guidelines of up to 10 ul/cm2 be applied to the skin. The cells were occluded with a screw cap. The thickness of the skin samples was 200 um. The receptor solution was Hepes-buffered Hanks' balanced salt solution (HHBSS) containing gentamicin and 4% bovine serum albumin (BSA). OECD 156 guidelines recommend that receptor fluid contain 5% BSA for lipophilic compounds; this slight variation is unlikely to have a substantial impact on results. The solubility of DEHP in the receptor fluid was not tested or reported; it is unclear if this would be rate-limiting. The receptor fluid temperature was maintained at 37oC; however, the temperature and humidity of the skin were not reported. The flow rate of receptor fluid was 1.5 mL/hour. Receptor fluid samples (volume not reported) were collected every 6 hours during the 24-hour exposure. After 24 hours, the skin was washed three times with 0.3 ml of 1% soap solution and three times with water. The skin was not tape-stripped. The skin wash, skin disc, and receptor fluids were analyzed for radioactivity. Radioactivity was measured using a Tri-Car liquid scintillation counter "equipped with automatic luminescence and color quench correction by the use of external standards." The sensitivity of quantification was not reported (signal-to-noise ratio), or the number of scintillations detected.
	Metric 6:	Standards for tests	Medium	The integrity of the skin was determined by applying tritiated water (0.33 µCi) to skin preparations. Perfusates were collected for 1 hour and deemed acceptable if leakage was <1% of the dose. This is slightly greater than the recommended value of ≤0.6% of the applied dose. The data are not shown. Percent recovery was reported. Coefficients of variation were not reported, but sufficient data were available for CV determination.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	The preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 10ul acetone solution to the skin at dose rates of 35.6, 153, or 313 nmol/cm2. No details are provided on the volume of radiolabeled material used or if lower doses were dilutions of a stock solution. Homogeneity or methods used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 8:	Consistency of exposure administration	Medium	The application volume (10 uL) and presumed skin surface area of 0.64 cm2 were consistent across groups. The skin thickness was 200 uM.
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Low dose- viable skin			
Domain	Metric	Rating	Comments	
	Metric 9:	Reporting of concentrations	Medium	The applied dose is reported as nmol/cm ² . Only nominal doses are reported.
	Metric 10:	Exposure frequency	High	Exposure duration was reported and appropriate for determining absorption. Test substance was in contact with the skin for 24 hours prior to washing, this is appropriate for more lipophilic compounds. Samples of receptor fluid were collected every 6 hours for a total of 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	High	There were 3 dose groups tested in a wide range of concentrations (35.6, 153, and 313 nmol/cm ²). Justification for dose selection was not provided by authors but was similar to the dose used for in vivo experiments in this paper (34 nmol/cm ²).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	High	This skin was obtained from female hairless guinea pigs aged 20-30 weeks (as noted for the in vivo portion of the study). This is not a preferred species, but the authors cited other studies also using the hairless guinea pig. The study authors do not specify the anatomical site where skin was collected from; it can reasonably be assumed to be skin from the dorsal area, as that was the site used for the in vivo portion of the study. Skin was dermatomed to a thickness of 200 um (actual range not provided). Membrane integrity was determined by measuring tritiated water loss prior to the experiment. Data were not reported, but acceptable levels were stated to be <1% of the initial dose.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate as per OECD 428. Guidelines recommend a minimum of 4 replicated per test preparation. This study examined at least 5 replicates/dose.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Medium	The outcome assessment methodology partially addressed the intended outcome of interest and was sensitive for the outcome. A finite dose was used to determine absorption. An application rate of 10 uL/0.64cm ² of diluted test substance differed slightly from the recommended 10 ul/cm ² for finite conditions. Timing and techniques of measurements were appropriate.
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The same duration of exposure, receptor fluid used, and sampling period were consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Low	The study reported adequate sampling size for the outcomes of interest. Scintillation counts/sample or duration of radioactivity detection were not reported. Ratio for detection (signal to noise ratio) was not reported. Scintillation counts were not shown. Graphical representation of absorption over time is shown.
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation: Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 67185 Unique ID: Low dose- viable skin				
Domain	Metric		Rating	Comments
	Metric 17:	Confounding variables in test design and procedures	Medium	Skin was obtained from female hairless guinea pigs. The split-thickness was reported to be 200 um. Skin integrity was measured by examining tritiated water leakage for one hour prior to application of the test substance. Leakage of <1% of the dose was considered acceptable. This differs slightly from recommendations of ≤0.6% of the applied dose. Results from the skin integrity test were not shown.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility of DEHP was not demonstrated in the receptor fluid, but solubility is not expected to be an issue based on expected concentrations.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	High	Statistical methods and calculations were appropriate. Absorption estimates were based on appropriate measurements and presented across time series. Absorption CVs were ≤25%.
	Metric 20:	Data interpretation	Medium	Absorption estimates were calculated appropriately from exposed skin and receptor fluid. Dislodgeable doses from skin washes were reported. Tape stripping was not performed but can be difficult for in vitro situations and when the duration of exposure is 24 hours. Recovery of the applied test substance was lower than recommended (78.4-84.7%) justification was not provided, but no cell wash was done, and the study did not assess whether any volatilization occurred.
	Metric 21:	Reporting of data	High	Data for all relevant endpoints (24-hour receptor fluid, skin disc, skin wash and total recovery) were reported quantitatively as means ± SD. Individual replicate data were not provided. Mean dermal penetration into the receptor fluid was shown graphically at each timepoint with SD.
Overall Quality Determination			Medium	

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	High dose- viable skin			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted.	
	Metric 2: Test substance source	High	The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not include certificates of analyses. Lot numbers were not provided.	
	Metric 3: Test substance purity	High	The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.	
Domain 2: Test Design				
	Metric 4: Reference compounds	Low	A reference compound was not used and there is no indication in the text regarding the performing laboratory’s history of performing these tests.	
	Metric 5: Assay procedures	Low	The assay procedures specified in the report were partially described. A flow-through diffusion set-up was used. Details of the set-up were found in cited reference Bronaugh and Stewart, 1985 (HERO 2776481). The study does not report if there was an equilibration period after the skin was placed into the chamber (guidelines recommend a 30-minute equilibrium period). Finite doses of DEHP (33.6, 153, or 313 nmol/cm2) were applied to freshly excised guinea pig skin (from 5 or more animals; location on the animal not reported). The skin discs punched were 2.0 cm2 which were mounted onto the diffusion cells. The inner surface area of the skin was not reported; however, in the cited reference describing the set-up, the surface area exposed to the chemical was 0.64 cm2. It is assumed this is the same for this experiment. The test substance was applied to the skin in 10 ul acetone solution. This volume is slightly more than OECD 428 guidelines of up to 10 ul/cm2 be applied to the skin. The cells were occluded with a screw cap. The thickness of the skin samples was 200 um. The receptor solution was Hepes-buffered Hanks’ balanced salt solution (HHBSS) containing gentamicin and 4% bovine serum albumin (BSA). OECD 156 guidelines recommend that receptor fluid contain 5% BSA for lipophilic compounds; this slight variation is unlikely to have a substantial impact on results. The solubility of DEHP in the receptor fluid was not tested or reported; it is unclear if this would be rate-limiting. The receptor fluid temperature was maintained at 37oC; however, the temperature and humidity of the skin were not reported. The flow rate of receptor fluid was 1.5 mL/hour. Receptor fluid samples (volume not reported) were collected every 6 hours during the 24-hour exposure. After 24 hours, the skin was washed three times with 0.3 ml of 1% soap solution and three times with water. The skin was not tape-stripped. The skin wash, skin disc, and receptor fluids were analyzed for radioactivity. Radioactivity was measured using a Tri-Car liquid scintillation counter “equipped with automatic luminescence and color quench correction by the use of external standards.” The sensitivity of quantification was not reported (signal-to-noise ratio), or the number of scintillations detected.	

Continued on next page ...

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	High dose- viable skin			
Domain	Metric	Rating	Comments	
	Metric 6:	Standards for tests	Medium	The integrity of the skin was determined by applying tritiated water (0.33 μ Ci) to skin preparations. Perfusates were collected for 1 hour and deemed acceptable if leakage was <1% of the dose. This is slightly greater than the recommended value of $\leq 0.6\%$ of the applied dose. The data are not shown. Percent recovery was reported. Coefficients of variation were not reported, but sufficient data were available for CV determination.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	The preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 10ul acetone solution to the skin at dose rates of 35.6, 153, or 313 nmol/cm2. No details are provided on the volume of radiolabeled material used or if lower doses were dilutions of a stock solution. Homogeneity or methods used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 8:	Consistency of exposure administration	Medium	The application volume (10 uL) and presumed skin surface area of 0.64 cm2 were consistent across groups. The skin thickness was 200 uM.
	Metric 9:	Reporting of concentrations	Medium	The applied dose is reported as nmol/cm2. Only nominal doses are reported.
	Metric 10:	Exposure frequency	High	Exposure duration was reported and appropriate for determining absorption. Test substance was in contact with the skin for 24 hours prior to washing, this is appropriate for more lipophilic compounds. Samples of receptor fluid were collected every 6 hours for a total of 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	High	There were 3 dose groups tested in a wide range of concentrations (35.6, 153, and 313 nmol/cm2). Justification for dose selection was not provided by authors but was similar to the dose used for in vivo experiments in this paper (34 nmol/cm2).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	High	This skin was obtained from female hairless guinea pigs aged 20-30 weeks (as noted for the in vivo portion of the study). This is not a preferred species, but the authors cited other studies also using the hairless guinea pig. The study authors do not specify the anatomical site where skin was collected from; it can reasonably be assumed to be skin from the dorsal area, as that was the site used for the in vivo portion of the study. Skin was dermatomed to a thickness of 200 um (actual range not provided). Membrane integrity was determined by measuring tritiated water loss prior to the experiment. Data were not reported, but acceptable levels were stated to be <1% of the initial dose.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate as per OECD 428. Guidelines recommend a minimum of 4 replicated per test preparation. This study examined at least 5 replicates/dose.
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	High dose- viable skin			
Domain	Metric	Rating	Comments	
	Metric 14:	Outcome assessment methodology	Medium	The outcome assessment methodology partially addressed the intended outcome of interest and was sensitive for the outcome. A finite dose was used to determine absorption. An application rate of 10 uL/0.64cm2 of diluted test substance differed slightly from the recommended 10 ul/cm2 for finite conditions. Timing and techniques of measurements were appropriate.
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The same duration of exposure, receptor fluid used, and sampling period were consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Low	The study reported adequate sampling size for the outcomes of interest. Scintillation counts/sample or duration of radioactivity detection were not reported. Ratio for detection (signal to noise ratio) was not reported. Scintillation counts were not shown. Graphical representation of absorption over time is shown.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Skin was obtained from female hairless guinea pigs. The split-thickness was reported to be 200 um. Skin integrity was measured by examining tritiated water leakage for one hour prior to application of the test substance. Leakage of <1% of the dose was considered acceptable. This differs slightly from recommendations of ≤0.6% of the applied dose. Results from the skin integrity test were not shown.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility of DEHP was not demonstrated in the receptor fluid, but solubility is not expected to be an issue based on expected concentrations.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	High	Statistical methods and calculations were appropriate. Absorption estimates were based on appropriate measurements and presented across time series.Absorption CVs were ≤25%.
	Metric 20:	Data interpretation	Medium	Absorption estimates were calculated appropriately from exposed skin and receptor fluid. Dislodgeable doses from skin washes were reported. Tape stripping was not performed but can be difficult for in vitro situations and when the duration of exposure is 24 hours. Recovery of the applied test substance was lower than recommended (78.4-84.7%) justification was not provided, but no cell wash was done, and the study did not assess whether any volatilization occurred.
	Metric 21:	Reporting of data	High	Data for all relevant endpoints (24-hour receptor fluid, skin disc, skin wash and total recovery) were reported quantitatively as means ± SD. Individual replicate data were not provided. Mean dermal penetration into the receptor fluid was shown graphically at each timepoint with SD.
Overall Quality Determination			Medium	

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	non- viable skin			
Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted.
	Metric 2:	Test substance source	High	The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not included certificates of analyses. Lot numbers were not provided
	Metric 3:	Test substance purity	High	The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	A reference compound was not used and there is no indication in the text regarding the performing laboratory’s history of performing these tests.
	Metric 5:	Assay procedures	Low	The assay procedures specified in the report were partially described. A flow-through diffusion set-up was used. Details of the set-up were found in cited reference Bronaugh and Stewart, 1985 (HERO 2776481). The study does not report if there was an equilibration period after the skin was placed into the chamber (guidelines recommend a 30-minute equilibrium period). Finite doses of DEHP (33.6, 153, or 313 nmol/cm2) were applied to freshly excised guinea pig skin (from 5 or more animals; location on the animal not reported). The punched skin discs were 2.0 cm2 and were mounted onto the diffusion cell. The inner surface area of the skin was not reported; however, in the cited reference describing the set-up, the surface area exposed to the chemical was 0.64 cm2. It is assumed this is the same for this experiment. The test substance was applied to the skin in 10 ul acetone solution. This volume is slightly more than OECD 428 guidelines of up to 10 ul/cm2 be applied to the skin. The cells were occluded with a screw cap. The thickness of the skin samples was 200 um. The receptor solution was Hepes-buffered Hanks’ balanced salt solution (HHBSS) containing gentamicin and 4% bovine serum albumin (BSA). OECD 156 guidelines recommend that receptor fluid contain 5% BSA for lipophilic compounds; this slight variation is unlikely to have a substantial impact on results. The solubility of DEHP in the receptor fluid was not tested or reported; it is unclear if this would be rate-limiting. The receptor fluid temperature was maintained at 37oC; however, the temperature and humidity of the skin were not reported. The flow rate of receptor fluid was 1.5 mL/hour. Receptor fluid samples (volume not reported) were collected every 6 hours during the 24-hour exposure. After 24 hours, the skin was washed three times with 0.3 ml of 1% soap solution and three times with water. The skin was not tape-stripped. The skin wash, skin disc, and receptor fluids were analyzed for radioactivity. Radioactivity was measured using a Tri-Car liquid scintillation counter “equipped with automatic luminescence and color quench correction by the use of external standards.” The sensitivity of quantification was not reported (signal-to-noise ratio), or the number of scintillations detected.

Continued on next page ...

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	non- viable skin			
Domain	Metric	Rating	Comments	
	Metric 6:	Standards for tests	Medium	The integrity of the skin was determined by applying tritiated water (0.33 μCi) to skin preparations. Perfusates were collected for 1 hour and deemed acceptable if leakage was <1% of the dose. This is slightly greater than the recommended value of ≤0.6% of the applied dose. The data are not shown. Percent recovery was reported (see Metric 20). CV values were not specified, but sufficient information is available for CV determination.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	The preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 10ul acetone solution to the skin at dose rates of 35.6 nmol/cm2. No details are provided on the volume of radiolabeled material used or if lower doses were a dilution of a stock solution. Homogeneity or method used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 8:	Consistency of exposure administration	Medium	The application volume (10 uL) and skin surface area of 0.64 cm2 were consistent across groups. The skin thickness was 200 uM.
	Metric 9:	Reporting of concentrations	Medium	The applied dose is reported as nmol/cm2. Only nominal doses are reported.
	Metric 10:	Exposure frequency	High	Exposure duration was reported and appropriate for determining absorption. The test substance was in contact with the skin for 24 hours prior to washing, this is appropriate for more lipophilic compounds. Samples of receptor fluid were collected every 6 hours for a total of 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	Low	Only one dose was selected. Justification for dose selection was not provided by authors but it was similar to the dose used for in vivo experiments in this paper (34 nmol/cm2).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	This skin was obtained from female hairless guinea pigs aged 20-30 weeks (presumed based on the descriptions provided for the in vivo portions of the study). This is not a preferred species for skin absorption studies; however, the authors cited other studies conducted on hairless guinea pigs. The study authors do not specify the anatomical site where skin was collected from; it can reasonably be assumed to be skin from the dorsal area, as that was the site used for the in vivo portion of the study. The skin was dermatomed to a thickness of 200 um. Membrane integrity was determined by measuring tritiated water loss prior to the experiment. Data were not reported, but acceptable levels were stated to be <1% of the initial dose.To study nonviable skin, Hepes-buffered Hanks' balanced salt solution used in the receptor fluid was replaced with distilled water containing 4% BSA. This method to study nonviable skin was used in another cited study (Collier et al. 1989). The premise is that the perfusate will not have the necessary nutrients to maintain metabolic activity when distilled water is used. The study authors state in the Methods section that a lactate dehydrogenase kit was used to determine skin viability; however, results on skin viability were not reported or mentioned.
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	non- viable skin			
Domain	Metric	Rating	Comments	
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate as per OECD 428. Guidelines recommend a minimum of 4 replicated per test preparation. This study examined at least 5 repli- cates/dose.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Medium	The outcome assessment methodology partially addressed the intended outcome of in- terest and was sensitive for the outcome. A finite dose was used to determine absorption. An application rate of 10 uL/0.64cm2 of diluted test substance differed slightly from the recommended 10 ul/cm2 for finite conditions. The timing and techniques of measure- ments were appropriate.
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The same duration of exposure, receptor fluid used, and sampling period were consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Low	The study reported adequate sampling sizes for the outcomes of interest. Scintillation counts/sample or duration of radioactivity detection were not reported. The ratio for detection (signal-to-noise ratio) was not reported. Scintillation counts were not shown. A graphical representation of absorption over time is shown.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Skin was obtained from female hairless guinea pigs. The split-thickness was reported to be 200 um. Skin integrity was measured by examining tritiated water leakage for one hour prior to the application of the test substance. Leakage of <1% of the dose was considered acceptable. This differs slightly from recommendations of ≤0.6% of the applied dose. Results from the skin integrity test were not shown.
	Metric 18:	Confounding variables in outcomes un- related to exposure	Medium	Solubility of DEHP was not demonstrated in the receptor fluid, but solubility is not expected to be an issue based on expected concentrations.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	High	Statistical methods and calculations were appropriate. Absorption estimates were based on appropriate measurements and presented across a time series. Absorption CVs were ≤25%.
	Metric 20:	Data interpretation	Medium	Absorption estimates were calculated appropriately from exposed skin and receptor fluid. Dislodgeable doses from skin washes were reported. Tape stripping was not per- formed but can be difficult for in vitro situations and when the duration of exposure is 24 hours. Recovery of the applied test substance was lower than recommended (86.5%) justification was not provided, but no cell wash was done, and the study did not assess whether any volatilization occurred.
	Metric 21:	Reporting of data	High	Data for all relevant endpoints (24-hour receptor fluid, skin disc, skin wash and total recovery) were reported quantitatively as means ± SD. Individual replicate data were not provided. Mean dermal penetration into the receptor fluid was shown graphically at each timepoint with SD.

Continued on next page ...

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.
Chemical:	Diethylhexyl Phthalate
Exposure Type:	Parent compound
HERO ID:	67185
Unique ID:	non- viable skin

Domain	Metric	Rating	Comments
--------	--------	--------	----------

Overall Quality Determination	Medium
--------------------------------------	---------------

Study Citation: Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 67185 Unique ID: Control for esterase inhibitor experiment				
Domain	Metric		Rating	Comments
Domain 1: Test Substance	Metric 1:	Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted.
	Metric 2:	Test substance source	High	The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not include certificates of analysis. Lot numbers were not provided
	Metric 3:	Test substance purity	High	The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.
Domain 2: Test Design	Metric 4:	Reference compounds	Low	A reference compound was not used and there is no indication in the text regarding the performing laboratory's history of performing these tests.
Continued on next page ...				

...continued from previous page

Study Citation: Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.				
Chemical: Diethylhexyl Phthalate				
Exposure Type: Parent compound				
HERO ID: 67185				
Unique ID: Control for esterase inhibitor experiment				
Domain	Metric		Rating	Comments
	Metric 5:	Assay procedures	Low	The assay procedures specified in the report were partially described. This was a control sample that was included with an additional esterase inhibitor test. A flow-through diffusion set-up was used. Details of the set-up can be found in the cited reference Bronaugh and Stewart, 1985 (HERO 2776481). The study does not report if there was an equilibration period after the skin was placed into the chamber (guidelines recommend a 30-minute equilibrium period). Finite doses of DEHP (not reported) were applied to freshly excised guinea pig skin (from 5 or more animals; location on the animal not reported). The punched skin discs were 2.0 cm ² , which were mounted onto the diffusion cell. The inner surface area of the skin was not reported; however, in the cited reference describing the set-up, the surface area exposed to the chemical is 0.64 cm ² . It is assumed this is the same for this experiment. The test substance was applied to the skin in 10 ul acetone solution. This volume is slightly more than OECD 428 guidelines of up to 10 ul/cm ² . The cells were occluded with a screw cap. The thickness of the skin samples was 200 um. The receptor solution was Hepes-buffered Hanks' balanced salt solution (HHBSS) containing gentamicin, 4% bovine serum albumin (BSA). OECD 156 guidelines recommend the receptor fluid contains 5% BSA for lipophilic compounds; this slight variation is unlikely to have a substantial impact on results. The solubility of DEHP in the receptor fluid was not tested or reported; it is unclear if this would be rate-limiting. The receptor fluid temperature was maintained at 37oC; however, the temperature and humidity of the skin were not reported. The flow rate of receptor fluid was 1.5 mL/hour. Receptor fluid samples (volume not reported) were collected every 6 hours during the 24-hour exposure. After 24 hours skin was washed three times with 0.3 ml of 1% soap solution and three times with water. The skin was not tape-stripped. The skin wash, skin disc, and receptor fluids were analyzed for radioactivity. Radioactivity was measured using a Tri-Car liquid scintillation counter "equipped with automatic luminescence and color quench correction by the use of external standards." The sensitivity of quantification is not reported (signal-to-noise ratio), or the number of scintillations detected.
	Metric 6:	Standards for tests	Medium	The integrity of the skin was determined by applying tritiated water (0.33 µCi) to skin preparation. Perfusates were collected for 1 hour and deemed acceptable if leakage was <1% of the dose. This is slightly greater than the recommended value of ≤0.6% of the applied dose. The data are not shown. Recovery was reported (87.2%). CV values were not specified, but sufficient data were provided to allow for CV determinations.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	The preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 10ul acetone solution to the skin. No details are provided on the volume of radiolabeled material used. Homogeneity or method used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 8:	Consistency of exposure administration	Medium	The application volume (10 uL) and presumed skin surface area of 0.64 cm ² were consistent across groups. The skin thickness was 200 uM.
Continued on next page ...				

...continued from previous page

Study Citation: Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 67185 Unique ID: Control for esterase inhibitor experiment				
Domain	Metric		Rating	Comments
	Metric 9:	Reporting of concentrations	Uninformative	The applied dose was not reported.
	Metric 10:	Exposure frequency	High	Exposure duration was reported and appropriate for determining absorption. The test substance was in contact with the skin for 24 hours prior to washing, this is appropriate for more lipophilic compounds. Samples of receptor fluid were collected every 6 hours for a total of 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	Low	It appears from the results, only one dose was studied in the esterase inhibitor experiment; this dose wasn't reported and the authors do not provide any reasoning for the dose chosen.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	High	This skin was obtained from female hairless guinea pigs aged 20-30 weeks (assumed based on information for the in vivo portion of the study). This is not a preferred species for skin absorption studies; however, the authors cited other studies conducted on hairless guinea pigs. The study authors do not specify the anatomical site where the skin was collected; it can reasonably be assumed to be skin from the dorsal area, as that was the site used for the in vivo portion of the study. The skin was dermatomed to a thickness of 200 um. Membrane integrity was determined by measuring tritiated water loss prior to the experiment. Data were not reported, but acceptable levels were stated to be <1% of the initial dose.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate as per OECD 428. Guidelines recommend a minimum of 4 replicates per test preparation. This study examined at least 5 replicates/dose.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Low	Minimal details were provided for the experiment including the esterase inhibitor. The goal was to determine the effects of the inhibitor on DEHP metabolism. It is presumed a finite dose was used, based on that was used in other portions of the study, but the dose for the esterase experiment was not reported so it cannot be determined if all of the conditions were appropriate. Percentages of the total dose in each receptor fluid collection time, skin, and skin wash were reported, along with skin + receptor fluid (% absorbed).
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The same duration of exposure, receptor fluid used, and sampling period were consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Low	The study reported an adequate sampling size for the outcomes of interest. Scintillation counts/sample or duration of radioactivity detection were not reported. The ratio for detection (signal-to-noise ratio) was not reported. Scintillation counts were not shown.
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation: Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.				
Chemical: Diethylhexyl Phthalate				
Exposure Type: Parent compound				
HERO ID: 67185				
Unique ID: Control for esterase inhibitor experiment				
Domain	Metric		Rating	Comments
	Metric 17:	Confounding variables in test design and procedures	Medium	Skin was obtained from female hairless guinea pigs. The split-thickness was reported to be 200 um. Skin integrity was measured by examining tritiated water leakage for one hour before the application of the test substance. Leakage of <1% of the dose was considered acceptable. This differs slightly from recommendations of $\leq 0.6\%$ of the applied dose. Results from the skin integrity test were not shown.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility of DEHP was not demonstrated in the receptor fluid, but solubility is not expected to be an issue based on expected concentrations.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	No statistical calculations were conducted. The study reported percentages of the dose measured in various compartments, as well as percentages of the MEHP metabolite. No percent absorption estimate was provided. Statistical methods and calculations were appropriate. Coefficients of variation could be calculated for various compartments (e.g., receptor fluid, skin, skin wash), and the total recovery reported; these CVs were $\leq 25\%$.
	Metric 20:	Data interpretation	Medium	Absorption estimates were calculated appropriately from exposed skin and receptor fluid. Dislodgeable doses from skin washes were reported. Tape stripping was not performed but can be difficult for in vitro situations and when the duration of exposure is 24 hours. Recovery of the applied test substance was 87.2% (for the control sample). This is less than the guideline specifications of $100 \pm 10\%$ for non-volatile test materials.
	Metric 21:	Reporting of data	Medium	Data for all relevant endpoints (24-hour receptor fluid, skin disc, skin wash and total recovery) were reported quantitatively as means \pm SD. Individual replicate data were not provided.
Overall Quality Determination			Uninformative	

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Mid dose- viable skin			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted.
	Metric 2:	Test substance source	High	The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not included certificates of analyses. Lot numbers were not provided.
	Metric 3:	Test substance purity	High	The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	A reference compound was not used and there is no indication in the text regarding the performing laboratory’s history of performing these tests.
	Metric 5:	Assay procedures	Low	The assay procedures specified in the report were partially described. A flow-through diffusion set-up was used. Details of the set-up were found in cited reference Bronaugh and Stewart, 1985 (HERO 2776481). The study does not report if there was an equilibration period after the skin was placed into the chamber (guidelines recommend a 30-minute equilibrium period). Finite doses of DEHP (33.6, 153, or 313 nmol/cm2) were applied to freshly excised guinea pig skin (from 5 or more animals; location on the animal not reported). The skin discs punched were 2.0 cm2 which were mounted onto the diffusion cells. The inner surface area of the skin was not reported; however, in the cited reference describing the set-up, the surface area exposed to the chemical was 0.64 cm2. It is assumed this is the same for this experiment. The test substance was applied to the skin in 10 ul acetone solution. This volume is slightly more than OECD 428 guidelines of up to 10 ul/cm2 be applied to the skin. The cells were occluded with a screw cap. The thickness of the skin samples was 200 um. The receptor solution was Hepes-buffered Hanks’ balanced salt solution (HHBSS) containing gentamicin and 4% bovine serum albumin (BSA). OECD 156 guidelines recommend that receptor fluid contain 5% BSA for lipophilic compounds; this slight variation is unlikely to have a substantial impact on results. The solubility of DEHP in the receptor fluid was not tested or reported; it is unclear if this would be rate-limiting. The receptor fluid temperature was maintained at 37oC; however, the temperature and humidity of the skin were not reported. The flow rate of receptor fluid was 1.5 mL/hour. Receptor fluid samples (volume not reported) were collected every 6 hours during the 24-hour exposure. After 24 hours, the skin was washed three times with 0.3 ml of 1% soap solution and three times with water. The skin was not tape-stripped. The skin wash, skin disc, and receptor fluids were analyzed for radioactivity. Radioactivity was measured using a Tri-Car liquid scintillation counter “equipped with automatic luminescence and color quench correction by the use of external standards.” The sensitivity of quantification was not reported (signal-to-noise ratio), or the number of scintillations detected.

Continued on next page ...

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Mid dose- viable skin			
Domain	Metric	Rating	Comments	
	Metric 6:	Standards for tests	Medium	The integrity of the skin was determined by applying tritiated water (0.33 μ Ci) to skin preparations. Perfusates were collected for 1 hour and deemed acceptable if leakage was <1% of the dose. This is slightly greater than the recommended value of \leq 0.6% of the applied dose. The data are not shown. Percent recovery was reported. Coefficients of variation were not reported, but sufficient data were available for CV determination.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	The preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 10ul acetone solution to the skin at dose rates of 35.6, 153, or 313 nmol/cm2. No details are provided on the volume of radiolabeled material used or if lower doses were dilutions of a stock solution. Homogeneity or methods used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 8:	Consistency of exposure administration	Medium	The application volume (10 uL) and presumed skin surface area of 0.64 cm2 were consistent across groups. The skin thickness was 200 uM.
	Metric 9:	Reporting of concentrations	Medium	The applied dose is reported as nmol/cm2. Only nominal doses are reported.
	Metric 10:	Exposure frequency	High	Exposure duration was reported and appropriate for determining absorption. Test substance was in contact with the skin for 24 hours prior to washing, this is appropriate for more lipophilic compounds. Samples of receptor fluid were collected every 6 hours for a total of 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	High	There were 3 dose groups tested in a wide range of concentrations (35.6, 153, and 313 nmol/cm2). Justification for dose selection was not provided by authors but was similar to the dose used for in vivo experiments in this paper (34 nmol/cm2).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	High	This skin was obtained from female hairless guinea pigs aged 20-30 weeks (as noted for the in vivo portion of the study). This is not a preferred species for skin absorption studies; however, the authors cited other studies conducted on hairless guinea pigs. The study authors do not specify the anatomical site where skin was collected from; it can reasonably be assumed to be skin from the dorsal area, as that was the site used for the in vivo portion of the study. Skin was dermatomed to a thickness of 200 um (actual range not provided). Membrane integrity was determined by measuring tritiated water loss prior to the experiment. Data were not reported, but acceptable levels were stated to be <1% of the initial dose.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate as per OECD 428. Guidelines recommend a minimum of 4 replicated per test preparation. This study examined at least 5 replicates/dose.
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Mid dose- viable skin			
Domain	Metric	Rating	Comments	
	Metric 14:	Outcome assessment methodology	Medium	The outcome assessment methodology partially addressed the intended outcome of interest and was sensitive for the outcome. A finite dose was used to determine absorption. An application rate of 10 uL/0.64cm2 of diluted test substance differed slightly from the recommended 10 ul/cm2 for finite conditions. Timing and techniques of measurements were appropriate.
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The same duration of exposure, receptor fluid used, and sampling period were consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Low	The study reported adequate sampling size for the outcomes of interest. Scintillation counts/sample or duration of radioactivity detection were not reported. Ratio for detection (signal to noise ratio) was not reported. Scintillation counts were not shown. Graphical representation of absorption over time is shown.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Skin was obtained from female hairless guinea pigs. The split-thickness was reported to be 200 um. Skin integrity was measured by examining tritiated water leakage for one hour prior to application of the test substance. Leakage of <1% of the dose was considered acceptable. This differs slightly from recommendations of ≤0.6% of the applied dose. Results from the skin integrity test were not shown.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility of DEHP was not demonstrated in the receptor fluid, but solubility is not expected to be an issue based on expected concentrations.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	High	Statistical methods and calculations were appropriate. Absorption estimates were based on appropriate measurements and presented across time series.Absorption CVs were ≤25%.
	Metric 20:	Data interpretation	High	Absorption estimates were calculated appropriately from exposed skin and receptor fluid. Dislodgeable doses from skin washes were reported. Tape stripping was not performed but can be difficult for in vitro situations and when the duration of exposure is 24 hours. Recovery of the applied test substance was 90.4% which is acceptable for a non-volatile test substance.
	Metric 21:	Reporting of data	High	Data for all relevant endpoints (24-hour receptor fluid, skin disc, skin wash and total recovery) were reported quantitatively as means ± SD. Individual replicate data were not provided. Mean dermal penetration into the receptor fluid was shown graphically at each timepoint with SD.
Overall Quality Determination			Medium	

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Control for esterase inhibitor experiment			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted. The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not include certificates of analysis. Lot numbers were not provided The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.
	Metric 2:	Test substance source	High	
	Metric 3:	Test substance purity	High	
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	A reference compound was not used and there is no indication in the text regarding the performing laboratory’s history of performing these tests.
Continued on next page ...				

...continued from previous page

Study Citation: Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.				
Chemical: Diethylhexyl Phthalate				
Exposure Type: Parent compound				
HERO ID: 67185				
Unique ID: Control for esterase inhibitor experiment				
Domain	Metric		Rating	Comments
	Metric 5:	Assay procedures	Low	The assay procedures specified in the report were partially described. This was an additional test that included an esterase inhibitor in the perfusate. A flow-through diffusion set-up was used. Details of the set-up can be found in the cited reference Bronaugh and Stewart, 1985 (HERO 2776481). The study does not report if there was an equilibration period after the skin was placed into the chamber (guidelines recommend a 30-minute equilibrium period). Finite doses of DEHP (not reported) were applied to freshly excised guinea pig skin (from 5 or more animals; location on the animal not reported). The punched skin discs were 2.0 cm ² , which were mounted onto the diffusion cell. The inner surface area of the skin was not reported; however, in the cited reference describing the set-up, the surface area exposed to the chemical is 0.64 cm ² . It is assumed this is the same for this experiment. The test substance was applied to the skin in 10 µl acetone solution. This volume is slightly more than OECD 428 guidelines of up to 10 µl/cm ² . The cells were occluded with a screw cap. The thickness of the skin samples was 200 µm. The receptor solution was Hepes-buffered Hanks' balanced salt solution (HHBSS) containing gentamicin, 4% bovine serum albumin (BSA), and 174 mg/L phenylmethylsulfonyl fluoride (esterase inhibitor). OECD 156 guidelines recommend the receptor fluid contains 5% BSA for lipophilic compounds; this slight variation is unlikely to have a substantial impact on results. The solubility of DEHP in the receptor fluid was not tested or reported; it is unclear if this would be rate-limiting. The receptor fluid temperature was maintained at 37°C; however, the temperature and humidity of the skin were not reported. The flow rate of receptor fluid was 1.5 mL/hour. Receptor fluid samples (volume not reported) were collected every 6 hours during the 24-hour exposure. After 24 hours skin was washed three times with 0.3 ml of 1% soap solution and three times with water. The skin was not tape-stripped. The skin wash, skin disc, and receptor fluids were analyzed for radioactivity. Radioactivity was measured using a Tri-Car liquid scintillation counter "equipped with automatic luminescence and color quench correction by the use of external standards." The sensitivity of quantification is not reported (signal-to-noise ratio), or the number of scintillations detected.
	Metric 6:	Standards for tests	Medium	The integrity of the skin was determined by applying tritiated water (0.33 µCi) to skin preparation. Perfusates were collected for 1 hour and deemed acceptable if leakage was <1% of the dose. This is slightly greater than the recommended value of ≤0.6% of the applied dose. The data are not shown. Recovery was reported (91.1%). CV values were not specified, but sufficient data were provided to allow for CV determinations.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	The preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 10 µl acetone solution to the skin. No details are provided on the volume of radiolabeled material used. Homogeneity or method used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 8:	Consistency of exposure administration	Medium	The application volume (10 µL) and presumed skin surface area of 0.64 cm ² were consistent across groups. The skin thickness was 200 µm.
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Control for esterase inhibitor experiment			
Domain	Metric	Rating	Comments	
	Metric 9:	Reporting of concentrations	Uninformative	The applied dose was not reported.
	Metric 10:	Exposure frequency	High	Exposure duration was reported and appropriate for determining absorption. The test substance was in contact with the skin for 24 hours prior to washing, this is appropriate for more lipophilic compounds. Samples of receptor fluid were collected every 6 hours for a total of 24 hours.
	Metric 11:	Number of exposure groups and concentration spacing	Low	It appears from the results, only one dose was studied in the esterase inhibitor experiment; this dose wasn't reported and the authors do not provide any reasoning for the dose chosen.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	High	This skin was obtained from female hairless guinea pigs aged 20-30 weeks (assumed based on information for the in vivo portion of the study). This is not a preferred species for skin absorption studies; however, the authors cited other studies conducted on hairless guinea pigs. The study authors do not specify the anatomical site where the skin was collected; it can reasonably be assumed to be skin from the dorsal area, as that was the site used for the in vivo portion of the study. The skin was dermatomed to a thickness of 200 um. Membrane integrity was determined by measuring tritiated water loss prior to the experiment. Data were not reported, but acceptable levels were stated to be <1% of the initial dose.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was appropriate as per OECD 428. Guidelines recommend a minimum of 4 replicates per test preparation. This study examined at least 5 replicates/dose.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Low	Minimal details were provided for the experiment including the esterase inhibitor. The goal was to determine the effects of the inhibitor on DEHP metabolism. It is presumed a finite dose was used, based on that was used in other portions of the study, but the dose for the esterase experiment was not reported so it cannot be determined if all of the conditions were appropriate. Percentages of the total dose in each receptor fluid collection time, skin, and skin wash were reported, along with skin + receptor fluid (% absorbed).
	Metric 15:	Consistency of outcome assessment	High	Details of outcome assessment protocol were reported and outcomes were assessed consistently across study replicates. The same duration of exposure, receptor fluid used, and sampling period were consistent across replicates.
	Metric 16:	Sampling adequacy and sensitivity	Low	The study reported an adequate sampling size for the outcomes of interest. Scintillation counts/sample or duration of radioactivity detection were not reported. The ratio for detection (signal-to-noise ratio) was not reported. Scintillation counts were not shown.
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Control for esterase inhibitor experiment			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Medium	Skin was obtained from female hairless guinea pigs. The split-thickness was reported to be 200 um. Skin integrity was measured by examining tritiated water leakage for one hour before the application of the test substance. Leakage of <1% of the dose was considered acceptable. This differs slightly from recommendations of ≤0.6% of the applied dose. Results from the skin integrity test were not shown.	
	Metric 18: Confounding variables in outcomes un-related to exposure	Medium	Solubility of DEHP was not demonstrated in the receptor fluid, but solubility is not expected to be an issue based on expected concentrations.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	No statistical calculations were conducted. The study reported percentages of the dose measured in various compartments, as well as percentages of the MEHP metabolite. No percent absorption estimate was provided. Statistical methods and calculations were appropriate. Coefficients of variation could be calculated for various compartments (e.g., receptor fluid, skin, skin wash), and the total recovery reported; these CVs were ≤25%.	
	Metric 20: Data interpretation	High	Absorption estimates were calculated appropriately from exposed skin and receptor fluid. Dislodgeable doses from skin washes were reported. Tape stripping was not performed but can be difficult for in vitro situations and when the duration of exposure is 24 hours. Recovery of the applied test substance was 91.1% (for the sample with the inhibitor).	
	Metric 21: Reporting of data	Medium	Data for all relevant endpoints (24-hour receptor fluid, skin disc, skin wash and total recovery) were reported quantitatively as means ± SD. Individual replicate data were not provided.	
Overall Quality Determination		Uninformative		

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Mouse-DEHP			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test substance identity	High	The test substance was identified as DEHP. A CASRN was not provided. The test substance was not radiolabeled.	
	Metric 2: Test substance source	High	The test substance was obtained from Sigma-Aldrich (St. Louis, MO). The batch/lot number was not provided. Test substance identity was not certified by the in the publication but could be verified on manufacturer’s website.	
	Metric 3: Test substance purity	High	The reported purity was >99%	
Domain 2: Test Design				
	Metric 4: Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.	
	Metric 5: Assay procedures	Medium	Tests were conducted in a Franz diffusion cell, presumably under static conditions. Freshly excised skin samples were mounted onto the cells (n = 4) leaving a diffusion area of 0.785 cm2. The exposure solution in the donor chamber contained a 5.4mM concentration of the test substance in 40% ethanol, and the receptor fluid was 40% ethanol. It is unclear if this was intended to be an infinite or finite exposure. A magnetic stir bar was used and the receptor was maintained at 37 degrees C. The pH was 7.4. Humidity was not reported, and it was not stated whether the chambers were left open or closed. The exposure duration was 12 hours. During exposure, receptor fluid aliquots were taken every 3 hours, and the volume was replaced with fresh fluid. HPLC was used to analyze the test substance in the receptor fluid and also in homogenized skin at the end of the exposure period. The limits of detection were reported. Tape stripping was mentioned, but may be part of a separate experiment in which concentrations in hair follicles were measured.	
	Metric 6: Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use, and percent recovery was not determined. The variation across replicates for the reported endpoints can be determined from the data available see metric 19 for more details. There is no text indicating the test met pre-established criteria. Inadequate data were provided in the results to demonstrate that the test conformed to current standards or guidelines.	
Domain 3: Exposure Characterization				
	Metric 7: Preparation and storage of test substance (chemical)	Low	Limited details on the test substance preparation were provided. No details on stability, homogeneity, mixing, or storage conditions were reported. The test substance was delivered in 40% ethanol, which is often used, and is appropriate as a receptor fluid for lipophilic test substances, but it is unclear if it is also appropriate in the donor chamber. Solubility was not confirmed. Generally, the use of radiolabeled test substances is preferred for penetration studies. This study only used unlabeled test substances. The lack of details could substantially impact the study results.	

Continued on next page ...

...continued from previous page

Study Citation: Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 2219803 Unique ID: Mouse-DEHP				
Domain		Metric	Rating	Comments
	Metric 8:	Consistency of exposure administration	Low	The diffusion area was reported (0.785 cm ²) and consistent across replicates. Each replicate was exposed to a 5.4 mM concentration of the test substance; however, the volume added to each donor chamber and the skin thicknesses were not reported. These missing details could have a substantial impact on the study results.
	Metric 9:	Reporting of concentrations	Low	Insufficient information on dosing was provided. The reported concentration was 5.4 mM, which is presumed to be nominal. There is no indication that the exposure concentration was analytically verified. The mass per skin area (mg/cm ²) or volume per area (mL/cm ²) were not reported. It is unclear if conditions were met for an infinite exposure.
	Metric 10:	Exposure frequency	Low	The exposure duration of 12 hours was not justified by the study authors. It may reflect an appropriate 'in-use' practice; however, no test substance was measured in the receptor fluid indicating that the exposure duration may have been too short, especially when full-thickness skin is used. Longer times (sometimes >24 hrs) are often required for highly lipophilic substances.
	Metric 11:	Number of exposure groups and concentration spacing	Low	Fewer than three concentrations were tested. This study only tested one concentration and it was not justified by the study authors.
Domain 4: Test Model	Metric 12:	Test model (skin)	Low	Full-thickness skin was excised from the dorsal regions of 8-week-old mice and 1-week-old pigs. It was not specified how many animals were used to obtain the samples. Mice are not a typical species for dermal absorption studies, and it is unclear whether this species is appropriate for this study type. However, the authors justified the use of nude mouse skin by indicating that it has a similar number of layers (3-4 cell layers) as the epidermis of infants. They also noted that nude mouse skin has greater permeability than human skin, but it may be a good model for human facial skin, which has a 4-fold higher permeability than other sites. Pigs are an acceptable model for dermal absorption studies. The source of the animals was reported. Full-thickness skin can be used when properly justified and if the thickness is not excessive. However, OECD TG 156 specifies that full-thickness skin should not be used for calculating fluxes, which was the main outcome of this study. A flux for DEHP could not be determined because no test substance was detected in the receptor fluid, underscoring that full-thickness skin was likely, not appropriate. Viable skin was used, but no details of its preparation prior to being placed in the diffusion cells were provided. Skin integrity was not tested. The skin thickness was not reported. There was no information on storage, but it appears that the skin was used immediately. The missing details are likely to have a substantial impact on the study results.
Continued on next page ...				

...continued from previous page

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Mouse-DEHP			
Domain	Metric	Rating	Comments	
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was not explicitly reported in the study methods and there is some lack of clarity. The data results indicate an n = 4, suggesting that there were at least four samples/ replicates of the single concentration tested. This is the minimum number of replicates required as per OECD TG 428. However, there were 4 replicates in which the entire skin sample was homogenized for analysis, yet there are also data measuring concentrations in hair follicles following tape stripping (also noted as 4 replicates). It is presumed that these are two separate experiments, but the reporting details were not clear.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Low	The outcome assessment methodology deviated significantly from OECD TG 428 recommendations. This study did not determine a mean mass balance recovery. This study analyzed concentrations of the test substance in the receptor fluid and in the skin at the end of the study using HPLC. Based on the figures provided, receptor fluid was sampled every 2 hours for the duration of the study. Flux was specified as an outcome, but Kp was not reported. The study did not report total absorption or percentage applied. Insufficient information on dosing was provided to determine whether an appropriate (infinite) exposure condition was used. Possibly in a separate experiment, some samples were tape stripped 20 times to remove the stratum corneum, to allow concentration analysis in hair follicles. The missing details make it difficult to determine the appropriateness of the outcome assessments and have a substantial impact on results.
	Metric 15:	Consistency of outcome assessment	High	Based on the available information, the same duration of exposure, and receptor fluid collection times were applied to all of the replicates. All replicates had the same donor vehicle and receptor fluid. Based on the information provided, there is no indication that there were significant differences between replicates.
	Metric 16:	Sampling adequacy and sensitivity	Medium	All data were derived from an n = 4. Details of HLPC, including the LOD were reported. It is unclear whether the sampling intervals for the receptor fluid were sufficient to allow at least 4 data points at steady state for calculation of flux.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Low	Insufficient information was provided to determine confounding. The number of donors, skin integrity, and skin thicknesses were not reported. % Recovery was not assessed.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	There were no reported differences among the study replicates that were unrelated to exposure. It was not specified whether the test substance was soluble in the receptor fluid; however, a 50% ethanol solution (this study used 40%) is common and acceptable, particularly for lipophilic compounds.
Domain 7: Data Presentation and Analysis				
Continued on next page ...				

...continued from previous page

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Mouse-DEHP			
Domain	Metric	Rating	Comments	
	Metric 19: Data analysis	Low	Limited details on data analysis were provided. Flux was calculated from the slope of the permeated amount vs. time. It was not specified that it was a linear portion of the curve. No data were reported as percentage estimates. The coefficient of variation for skin accumulation was 40% for mouse and 27% for pig, which are higher than the guideline requirement of <25%; however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results. Flux was not determined because no measurements were observed in the receptor fluid.	
	Metric 20: Data interpretation	Medium	The lack of exposure details, and deviations from guideline in outcome assessments make this study difficult to interpret. The authors do not make any unreasonable claims, but also do not report standard outcomes (e.g., % recovery, % absorption, Kp). It is also unclear if dosing was infinite or finite.	
	Metric 21: Reporting of data	Low	Data for some specified outcomes were adequately reported as means \pm SD. The study did not report concentrations in receptor fluid by time. A figure showing the plotted data with a corresponding linear slope was not provided. The text indicated that DEHP showed negligible accumulation in the receptor fluid.	

Overall Quality Determination**Uninformative**

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Pig-DEHP			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was identified as DEHP. A CASRN was not provided. The test substance was not radiolabeled.
	Metric 2:	Test substance source	High	The test substance was obtained from Sigma-Aldrich (St. Louis, MO). The batch/lot number was not provided. Test substance identity was not certified by the in the publication but could be verified on manufacturer’s website.
	Metric 3:	Test substance purity	High	The reported purity was >99%
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Medium	Tests were conducted in a Franz diffusion cell, presumably under static conditions. Freshly excised skin samples were mounted onto the cells (n = 4) leaving a diffusion area of 0.785 cm2. The exposure solution in the donor chamber contained a 5.4mM concentration of the test substance in 40% ethanol, and the receptor fluid was 40% ethanol. It is unclear if this was intended to be an infinite or finite exposure. A magnetic stir bar was used and the receptor was maintained at 37 degrees C. The pH was 7.4. Humidity was not reported, and it was not stated whether the chambers were left open or closed. The exposure duration was 12 hours. During exposure, receptor fluid aliquots were taken every 3 hours, and the volume was replaced with fresh fluid. HPLC was used to analyze the test substance in the receptor fluid and also in homogenized skin at the end of the exposure period. The limits of detection were reported. Tape stripping was mentioned, but may be part of a separate experiment in which concentrations in hair follicles were measured.
	Metric 6:	Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use, and percent recovery was not determined. The variation across replicates for the reported endpoints can be determined from the data available see metric 19 for more details. There is no text indicating the test met pre-established criteria. Inadequate data were provided in the results to demonstrate that the test conformed to current standards or guidelines.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details on the test substance preparation were provided. No details on stability, homogeneity, mixing, or storage conditions were reported. The test substance was delivered in 40% ethanol, which is often used, and is appropriate as a receptor fluid for lipophilic test substances, but it is unclear if it is also appropriate in the donor chamber. Solubility was not confirmed. Generally, the use of radiolabeled test substances is preferred for penetration studies. This study only used unlabeled test substances. The lack of details could substantially impact the study results.

Continued on next page ...

...continued from previous page

Study Citation: Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 2219803 Unique ID: Pig-DEHP				
Domain		Metric	Rating	Comments
	Metric 8:	Consistency of exposure administration	Low	The diffusion area was reported (0.785 cm ²) and consistent across replicates. Each replicate was exposed to a 5.4 mM concentration of the test substance; however, the volume added to each donor chamber and the skin thicknesses were not reported. These missing details could have a substantial impact on the study results.
	Metric 9:	Reporting of concentrations	Low	Insufficient information on dosing was provided. The reported concentration was 5.4 mM, which is presumed to be nominal. There is no indication that the exposure concentration was analytically verified. The mass per skin area (mg/cm ²) or volume per area (mL/cm ²) were not reported. It is unclear if conditions were met for an infinite exposure.
	Metric 10:	Exposure frequency	Low	The exposure duration of 12 hours was not justified by the study authors. It may reflect an appropriate 'in-use' practice; however, no test substance was measured in the receptor fluid indicating that the exposure duration may have been too short, especially when full-thickness skin is used. Longer times (sometimes >24 hrs) are often required for highly lipophilic substances.
	Metric 11:	Number of exposure groups and concentration spacing	Low	Fewer than three concentrations were tested. This study only tested one concentration and it was not justified by the study authors.
Domain 4: Test Model	Metric 12:	Test model (skin)	Low	Full-thickness skin was excised from the dorsal regions of 8-week-old mice and 1-week-old pigs. It was not specified how many animals were used to obtain the samples. Mice are not a typical species for dermal absorption studies, and it is unclear whether this species is appropriate for this study type. However, the authors justified the use of nude mouse skin by indicating that it has a similar number of layers (3-4 cell layers) as the epidermis of infants. They also noted that nude mouse skin has greater permeability than human skin, but it may be a good model for human facial skin, which has a 4-fold higher permeability than other sites. Pigs are an acceptable model for dermal absorption studies. The source of the animals was reported. Full-thickness skin can be used when properly justified and if the thickness is not excessive. However, OECD TG 156 specifies that full-thickness skin should not be used for calculating fluxes, which was the main outcome of this study. A flux for DEHP could not be determined because no test substance was detected in the receptor fluid, underscoring that full-thickness skin was likely, not appropriate. Viable skin was used, but no details of its preparation prior to being placed in the diffusion cells were provided. Skin integrity was not tested. The skin thickness was not reported. There was no information on storage, but it appears that the skin was used immediately. The missing details are likely to have a substantial impact on the study results.
Continued on next page ...				

...continued from previous page

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Pig-DEHP			
Domain	Metric	Rating	Comments	
	Metric 13: Number/Replicates per group	Medium	The number of replicates was not explicitly reported in the study methods and there is some lack of clarity. The data results indicate an n = 4, suggesting that there were at least four samples/ replicates of the single concentration tested. This is the minimum number of replicates required as per OECD TG 428. However, there were 4 replicates in which the entire skin sample was homogenized for analysis, yet there are also data measuring concentrations in hair follicles following tape stripping (also noted as 4 replicates). It is presumed that these are two separate experiments, but the reporting details were not clear.	
Domain 5: Outcome Assessment				
	Metric 14: Outcome assessment methodology	Low	The outcome assessment methodology deviated significantly from OECD TG 428 recommendations. This study did not determine a mean mass balance recovery. This study analyzed concentrations of the test substance in the receptor fluid and in the skin at the end of the study using HPLC. Based on the figures provided, receptor fluid was sampled every 2 hours for the duration of the study. Flux was specified as an outcome, but Kp was not reported. The study did not report total absorption or percentage applied. Insufficient information on dosing was provided to determine whether an appropriate (infinite) exposure condition was used. Possibly in a separate experiment, some samples were tape stripped 20 times to remove the stratum corneum, to allow concentration analysis in hair follicles. The missing details make it difficult to determine the appropriateness of the outcome assessments and have a substantial impact on results.	
	Metric 15: Consistency of outcome assessment	High	Based on the available information, the same duration of exposure, and receptor fluid collection times were applied to all of the replicates. All replicates had the same donor vehicle and receptor fluid. Based on the information provided, there is no indication that there were significant differences between replicates.	
	Metric 16: Sampling adequacy and sensitivity	Medium	All data were derived from an n = 4. Details of HLPC, including the LOD were reported. It is unclear whether the sampling intervals for the receptor fluid were sufficient to allow at least 4 data points at steady state for calculation of flux.	
Domain 6: Confounding/Variable Control				
	Metric 17: Confounding variables in test design and procedures	Low	Insufficient information was provided to determine confounding. The number of donors, skin integrity, and skin thicknesses were not reported. % Recovery was not assessed.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	There were no reported differences among the study replicates that were unrelated to exposure. It was not specified whether the test substance was soluble in the receptor fluid; however, a 50% ethanol solution (this study used 40%) is common and acceptable, particularly for lipophilic compounds.	
Domain 7: Data Presentation and Analysis				
Continued on next page ...				

...continued from previous page

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Pig-DEHP			
Domain	Metric	Rating	Comments	
	Metric 19: Data analysis	Low	Limited details on data analysis were provided. Flux was calculated from the slope of the permeated amount vs. time. It was not specified that it was a linear portion of the curve. No data were reported as percentage estimates. The coefficient of variation for skin accumulation was 40% for mouse and 27% for pig, which are higher than the guideline requirement of <25%; however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results. Flux was not determined because no measurements were observed in the receptor fluid.	
	Metric 20: Data interpretation	Medium	The lack of exposure details, and deviations from guideline in outcome assessments make this study difficult to interpret. The authors do not make any unreasonable claims, but also do not report standard outcomes (e.g., % recovery, % absorption, Kp). It is also unclear if dosing was infinite or finite.	
	Metric 21: Reporting of data	Low	Data for some specified outcomes were adequately reported as means \pm SD. The study did not report concentrations in receptor fluid by time. A figure showing the plotted data with a corresponding linear slope was not provided. The text indicated that DEHP showed negligible accumulation in the receptor fluid.	

Overall Quality Determination**Uninformative**

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Strat-M membrane-DEHP			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was identified as DEHP. A CASRN was not provided. The test substance was not radiolabeled.
	Metric 2:	Test substance source	High	The test substance was obtained from Sigma-Aldrich (St. Louis, MO). The batch/lot number was not provided. Test substance identity was not certified by the in the publication but could be verified on manufacturer’s website.
	Metric 3:	Test substance purity	High	The reported purity was >99%
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Medium	Tests were conducted in a Franz diffusion cell, presumably under static conditions. Freshly excised skin samples were mounted onto the cells (n = 4) leaving a diffusion area of 0.785 cm2. The exposure solution in the donor chamber contained a 5.4mM concentration of the test substance in 40% ethanol, and the receptor fluid was 40% ethanol. It is unclear if this was intended to be an infinite or finite exposure. A magnetic stir bar was used and the receptor was maintained at 37 degrees C. The pH was 7.4. Humidity was not reported, and it was not stated whether the chambers were left open or closed. The exposure duration was 12 hours. During exposure, receptor fluid aliquots were taken every 2 hours, and the volume was replaced with fresh fluid. HPLC was used to analyze the test substance in the receptor fluid and also in homogenized skin at the end of the exposure period. The limits of detection were reported.
	Metric 6:	Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use, and percent recovery was not determined. The variation across replicates for the reported endpoints was acceptable (SD <25%). There is no text indicating the test met pre-established criteria. Inadequate data were provided in the results to demonstrate that the test conformed to current standards or guidelines.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details on the test substance preparation were provided. No details on stability, homogeneity, mixing, or storage conditions were reported. The test substance was delivered in 40% ethanol, which is often used, and is appropriate as a receptor fluid for lipophilic test substances, but it is unclear if it is also appropriate in the donor chamber. Solubility was not confirmed. Generally, the use of radiolabeled test substances is preferred for penetration studies. This study only used unlabeled test substances. The lack of details could substantially impact the study results.
	Metric 8:	Consistency of exposure administration	Low	The diffusion area was reported (0.785 cm2) and consistent across replicates. Each replicate was exposed to a 5.4 mM concentration of the test substance; however, the volume added to each donor chamber and the skin thicknesses were not reported. These missing details could have a substantial impact on the study results.

Continued on next page ...

...continued from previous page

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Strat-M membrane-DEHP			
Domain	Metric	Rating	Comments	
	Metric 9:	Reporting of concentrations	Low	Insufficient information on dosing was provided. The reported concentration was 5.4 mM, which is presumed to be nominal. There is no indication that the exposure concentration was analytically verified. The mass per skin area (mg/cm2) or volume per area (mL/cm2) were not reported. It is unclear if conditions were met for an infinite exposure.
	Metric 10:	Exposure frequency	High	The exposure duration of 12 hours was not justified by the study authors. It may reflect an appropriate 'in-use' practice and is acceptable according to OECD test guidelines. The timepoint was used for flux measurements.
	Metric 11:	Number of exposure groups and concentration spacing	Low	Fewer than three concentrations were tested. This study only tested one concentration and it was not justified by the study authors.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Uninformative	Experiments were conducted on Strat-M membrane, a synthetic, non-animal-based model for transdermal diffusion testing. The membrane was obtained from Merk-Millipore, although these standardized membranes are sold by multiple vendors. The thickness was not reported in the study. Some vendors note the thickness to be 25 mm. All vendors provide additional information and certificates of quality upon request. These certificates are likely to assure integrity. Integrity was not confirmed by the performing laboratory. Synthetic membranes are not mentioned in the current OECD TG, and although these are sold as an adequate model for humans, it is not clear they are currently accepted as an appropriate model system. There was no information on storage. The missing details are likely to have a substantial impact on the study results.
	Metric 13:	Number/Replicates per group	Medium	The number of replicates was not explicitly reported in the study methods and there is some lack of clarity. The data results indicate an n = 4, suggesting that there were at least four samples/replicates of the single concentration tested. This is the minimum number of replicates required as per OECD TG 428.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Low	The outcome assessment methodology deviated significantly from OECD TG 428 recommendations. This study did not determine a mean mass balance recovery. This study analyzed concentrations of the test substance in the receptor fluid using HPLC. Based on the figures provided, receptor fluid was sampled every 2 hours for the duration of the study. Flux was specified as an outcome, but Kp was not reported. Insufficient information on dosing was provided to determine whether an appropriate (infinite) exposure condition was used. The missing details make it difficult to determine the appropriateness of the outcome assessments and have a substantial impact on results.
	Metric 15:	Consistency of outcome assessment	High	Based on the available information, the same duration of exposure, and receptor fluid collection times were applied to all of the replicates. All replicates had the same donor vehicle and receptor fluid. Based on the information provided, there is no indication that there were significant differences between replicates.
Continued on next page ...				

...continued from previous page

Study Citation:	Pan, T. L., Wang, P. W., Aljuffali, I. A., Hung, Y. Y., Lin, C. F., Fang, J. Y. (2014). Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. Food and Chemical Toxicology 65:105-114.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	2219803			
Unique ID:	Strat-M membrane-DEHP			
Domain	Metric	Rating	Comments	
	Metric 16:	Sampling adequacy and sensitivity	Medium	All data were derived from an n = 4. Details of HPLC, including the LOD were reported. It is unclear whether the sampling intervals for the receptor fluid were sufficient to allow at least 4 data points at steady state for calculation of flux.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Insufficient information was provided to determine confounding; however, the use of a standardized synthetic membrane removes several confounding factors related to donors, donor sites, thickness, integrity, etc..
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	There were no reported differences among the study replicates that were unrelated to exposure. It was not specified whether the test substance was soluble in the receptor fluid; however, a 50% ethanol solution (this study used 40%) is common and acceptable, particularly for lipophilic compounds.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	Limited details on data analysis were provided. Flux was calculated from the slope of the permeated amount vs. time. It was not specified that it was a linear portion of the curve. No data were reported as percentage estimates. A Kp was not reported. DEHP was not able to penetrate the Strat-M membrane, which was consistent with the results from ex vivo skin samples. Standard deviations were plotted on a graphical representation of the data and appeared to be appropriate (<25%)
	Metric 20:	Data interpretation	Medium	A quantitative flux value was not actually reported, it was only stated in the study text that the data were consistent with what was observed in ex vivo model systems. Based on the data provided, the authors correctly interpreted the results, and due to the lack of penetration of DEHP through the membrane, a flux cannot be calculated.
	Metric 21:	Reporting of data	High	Data were graphically reported showing permeated amount vs. time. Points represented means \pm SD.
Overall Quality Determination			Uninformative	

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - 50% ethanol			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C [di(2-ethylhexyl) phthalate; DEHP. A structure showing the site of the radiolabel was not provided.
	Metric 2:	Test substance source	Low	The DEHP test substance was prepared in the performing laboratory from [7-14C]phthalic anhydride purchased from Amersham International plc, Bucks, UK. The production process was cited to: Albro P. W., Thomas R. and Fishbein L. (1973)Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of urinary metabolites. Journal of Chromatography 76, 321 - 33. The test substance was not analytically verified by the performing laboratory.
	Metric 3:	Test substance purity	High	The purity of the radiolabeled phthalic anhydride was not specified. The specific activity and radiochemical purity of DEHP were determined by thin-layer chromatography and were 1.24 mCi/mmol and >98%, respectively. Impurities were not identified, but the chemical of interest was the main component.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Medium	The study included tests using the reference compound Benzoic acid. Benzoic acid is a hydrophilic compound and DEHP is lipophilic. A Lipophilic reference compound would have been more appropriate.
	Metric 5:	Assay procedures	Medium	Details of the assay procedure were adequately described. Skin samples were placed in a flow-through diffusion cell system. The flow rate was set to 1.9 mL/min. Skin temperature was maintained at 31.5 ± 1 degrees C. Humidity was not reported. Two different receptor fluids were tested; 0.9% saline (PBS) containing ampicillin and streptomycin, or 50% (v/v) aqueous ethanol. The study was examining the differences between the two receptor fluids; the aqueous ethanol fluid was expected to be more appropriate for lipophilic compounds. The receptor fluids were maintained at a temperature of 40 degrees C and were gassed with helium to minimize air bubble accumulation. Absorption tests were conducted in open compartments on skin samples with a surface area of 0.64 cm2. The radiolabeled test material (0.25 uCi) was applied in 50 uL acetone. Receptor fluid fractions were collected hourly for the first 6 hours, and then more extended (unspecified) intervals for up to 72 hours; radioactivity was counted. After exposure, the donor chambers were rinsed twice with 2% Lipsol detergent, and the radioactivity of the donor chamber and surface of the skin samples was measured. The epidermal and dermal membranes were dissolved and radioactivity was measured. Any residual radioactivity in the diffusion cell after soaking for 24 hours was counted. The study did not perform tape stripping, or utilize a carbon trap. However, the test substance is not volatile in nature. The lack of humidity reporting is not expected to have a significant impact on the study results.
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - 50% ethanol			
Domain	Metric	Rating	Comments	
	Metric 6: Standards for tests	Medium	The integrity of the skin samples was tested using tritiated H20 in saline and both of the receptor fluids. Integrity was not tested using acetone, the vehicle used for delivery of the test substance. Kp values and Kp ratio dermis/epidermis values were reported along with the percent of 3H recovered in each receptor fluid at 1 hour, and up to 4 hours. Any epidermal membranes with Kp > 1.5 x 10^-2 and dermal layers with a Kp > 8.5 x 10^-2 were rejected. Skin samples that had been processed with sodium bromide and subjected to physical separation were also histologically compared to sodium bromide-treated skin without physical separation and to untreated skin. The examinations showed some differences in epithelial thickness, folding, and displacement of hair follicles and sebaceous glands. The method used to prepare the intact skin was selected due to previous reports that use of the sodium bromide was superior. However, the study noted that histopathological analysis had not been conducted. This information is helpful in the interpretation of the study results. The coefficients of variation can be determined from the information provided (see Metric 19 for more details).	
Domain 3: Exposure Characterization				
	Metric 7: Preparation and storage of test substance (chemical)	Low	Details on the preparation of the test substance were limited. The study cited Albro et al. 1973 (HERO ID 63429) for the process of preparing DEHP from phthalic anhydride, but other details including test substance stability, homogeneity, mixing temperature, stock concentration, stirring methods, and storage conditions were not reported. No physical/chemical properties of the test substance were provided in the study. The test substance is lipophilic, suggesting that solubility may be an issue. The test substance was delivered in acetone. It is unclear whether acetone contributed to any damage to the skin.	
	Metric 8: Consistency of exposure administration	Medium	Details of exposure administration were incompletely reported. The epidermal and dermal membranes were 16 mm in diameter with a surface area of 0.64 cm2; however, the thickness of the membranes was not specified. The study exposed chemically separated epidermal and dermal membranes, therefore large differences in thickness are not expected. The text indicates a total volume of 50uL was applied, which would be equivalent to 78 uL/cm2, indicating an infinite exposure. Based on the information available, exposures were administered consistently across groups.	
	Metric 9: Reporting of concentrations	Uninformative	This study did not report doses or concentrations. Only the volume applied, the specific activity, and the surface area of the skin were reported.	
	Metric 10: Exposure frequency	Low	The total duration of exposure was longer than recommended (OECD TG 428). Receptor fluid collections were conducted for up to 72 hours. For test substances that penetrate more slowly, durations longer than the recommended 24 hours may be justified; however, the study authors did not explicitly provide justification for the longer duration. One concern for longer-duration studies is the deterioration of skin integrity. This study only tested skin integrity (as determined by Kp) for 5 hours, and did histopathology examinations at 24 hours. Integrity was not assessed after longer durations.	
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - 50% ethanol			
Domain	Metric	Rating	Comments	
	Metric 11: Number of exposure groups and concentration spacing	Low	The purpose of the study was to compare penetration through different skin layers (epidermal and dermal membranes) and also to assess differences between receptor fluids, and compare a lipophilic to a hydrophilic compound. The study did not report the dose/concentration used, but it is presumed that there was a single exposure concentration tested.	
Domain 4: Test Model				
	Metric 12: Test model (skin)	High	Dosal skin was excised from 4-5 week-old male Ola: Sprague-Dawley rats. The animal source was not specified. The skin was stored at 2-8 degrees C, and sealed in polythene until used. The samples were used within 1 week. This study separately tested absorption through the epidermal membrane and the residual stripped dermis. Therefore, the thawed samples were soaked for 24 hours in 2M sodium bromide so that the two layers could be separated. Each layer was cut into 16-mm diameter discs and used immediately. Typically, split-thickness skin is preferred to single epidermal or dermal membranes, but testing of the single membranes was justified by the study authors. The surface area for absorption was 0.64 cm2. Skin integrity was tested before exposure to the test.	
	Metric 13: Number/Replicates per group	Medium	The number of replicates was reported and was appropriate. There were 8-11 replicates per membrane type (epidermis or dermis) per receptor fluid type (PBS or 50% ethanol).	
Domain 5: Outcome Assessment				
	Metric 14: Outcome assessment methodology	Medium	The outcome assessment methodology addressed the intended outcome of interest and was sensitive for the outcome, but there were minor uncertainties. This was a non-guideline study with specific goals and therefore had some deviations from standard practices. Kp was derived from what was presumed to be an infinite dose (78uL/cm2), this is lower than the guideline recommendation of 100 ul/cm2.	
	Metric 15: Consistency of outcome assessment	High	Based on the data available, there is no indication of inconsistencies across replicates in the outcome assessment protocols. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed.	
	Metric 16: Sampling adequacy and sensitivity	Low	The study did not specify whether a steady state was reached. Scintillation counts/sample and/or duration of radioactivity detection, and whether there was adequate signal-to-noise [i.e., background] ratio for detection were not reported. It is unclear whether these missing details would have a significant impact on the results.	
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - 50% ethanol			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Skin integrity was measured by a less desirable method (tritiated water). The study used limit values that were much higher than recommended (1.5×10^{-2} for the epidermis and 8.5×10^{-2} for the dermis compared to the guideline recommendation of 4.5×10^{-3}). The percent absorption (as measured in receptor fluid) at 1 hour was also significantly $> 0.6\%$. Although the purpose of the study was to look at absorption separately in the epidermal and dermal layers, the reliability/biological significance of the results is unclear. The chemical separation process resulted in both histological changes and skin integrity results that are lower than the recommended standards.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	The study did not explicitly demonstrate the solubility of the test substance in the receptor fluids, but the 50% aqueous ethanol fluid used was compatible with lipophilic substances. There were no reported differences among the replicates that were unrelated to exposure. There was no evidence that the test substance interfered with the assay.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	The Kp was derived from a linear regression as described by Dugard et al. 1984 using the steepest part of an absorption curve. Percent recovery was calculated from radioactivity in the receptor fluid, membrane washings, diffusion cell washings, and skin membranes. Statistical analysis was performed using appropriate methods. Percent absorption were presented across a time series for the receptor fluid, but not for the other compartments of the test system. It is not clear whether there were any outliers, and if calculations accounted for outliers. More than half of the CV values within an individual scenario were $>25\%$ and $<50\%$ or $>50\%$; however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results.	
	Metric 20: Data interpretation	Medium	Absorption estimates were calculated incompletely. The study did not report total % recovery (from all compartments). Recovery into the receptor fluids and recovery in the skin membranes were reported, but recovery from washes was not specified. The recoveries in the receptor fluids plus the reported recoveries in skin. Based on recoveries in the receptor fluid and skin, the recovery was less than $100\% \pm 10\%$, but the estimations are not accurate due to the missing recovery data for the washes. Kp was derived from what is presumed to be an infinite dose.	
	Metric 21: Reporting of data	Low	There are discrepancies between the data tables and the study text. Table 2 shows the % recovery into the different receptor fluids for each test (using epidermis and dermis) at 1 hour; however, the study text references the same table indicating it reports the total recovery in the receptor fluids at 72 hours. Based on the absorption plotted over time figures, the data from Table 2 appears to represent 72 hours, not 1 hour. Recovery data for the washes were not reported.	

Overall Quality Determination**Uninformative**

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - 50% ethanol			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C [di(2-ethylhexyl) phthalate; DEHP. A structure showing the site of the radiolabel was not provided.
	Metric 2:	Test substance source	Low	The DEHP test substance was prepared in the performing laboratory from [7-14C]phthalic anhydride purchased from Amersham International plc, Bucks, UK. The production process was cited to: Albro P. W., Thomas R. and Fishbein L. (1973)Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of urinary metabolites. Journal of Chromatography 76, 321 - 33. The test substance was not analytically verified by the performing laboratory.
	Metric 3:	Test substance purity	High	The purity of the radiolabeled phthalic anhydride was not specified. The specific activity and radiochemical purity of DEHP were determined by thin-layer chromatography and were 1.24 mCi/mmol and >98%, respectively. Impurities were not identified, but the chemical of interest was the main component.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Medium	The study included tests using the reference compound Benzoic acid. Benzoic acid is a hydrophilic compound and DEHP is lipophilic. A Lipophilic reference compound would have been more appropriate.
	Metric 5:	Assay procedures	Medium	Details of the assay procedure were adequately described. Skin samples were placed in a flow-through diffusion cell system. The flow rate was set to 1.9 mL/min. Skin temperature was maintained at 31.5 ± 1 degrees C. Humidity was not reported. Two different receptor fluids were tested; 0.9% saline (PBS) containing ampicillin and streptomycin, or 50% (v/v) aqueous ethanol. The study was examining the differences between the two receptor fluids; the aqueous ethanol fluid was expected to be more appropriate for lipophilic compounds. The receptor fluids were maintained at a temperature of 40 degrees C and were gassed with helium to minimize air bubble accumulation. Absorption tests were conducted in open compartments on skin samples with a surface area of 0.64 cm2. The radiolabeled test material (0.25 uCi) was applied in 50 uL acetone. Receptor fluid fractions were collected hourly for the first 6 hours, and then more extended (unspecified) intervals for up to 72 hours; radioactivity was counted. After exposure, the donor chambers were rinsed twice with 2% Lipsol detergent, and the radioactivity of the donor chamber and surface of the skin samples was measured. The epidermal and dermal membranes were dissolved and radioactivity was measured. Any residual radioactivity in the diffusion cell after soaking for 24 hours was counted. The study did not perform tape stripping, or utilize a carbon trap. However, the test substance is not volatile in nature. The lack of humidity reporting is not expected to have a significant impact on the study results.
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - 50% ethanol			
Domain	Metric	Rating	Comments	
	Metric 6: Standards for tests	Medium	The integrity of the skin samples was tested using tritiated H20 in saline and both of the receptor fluids. Integrity was not tested using acetone, the vehicle used for delivery of the test substance. Kp values and Kp ratio dermis/epidermis values were reported along with the percent of 3H recovered in each receptor fluid at 1 hour, and up to 4 hours. Any epidermal membranes with Kp > 1.5 x 10^-2 and dermal layers with a Kp > 8.5 x 10^-2 were rejected. Skin samples that had been processed with sodium bromide and subjected to physical separation were also histologically compared to sodium bromide-treated skin without physical separation and to untreated skin. The examinations showed some differences in epithelial thickness, folding, and displacement of hair follicles and sebaceous glands. The method used to prepare the intact skin was selected due to previous reports that use of the sodium bromide was superior. However, the study noted that histopathological analysis had not been conducted. This information is helpful in the interpretation of the study results. The coefficients of variation can be determined from the information provided (see Metric 19 for more details).	
Domain 3: Exposure Characterization				
	Metric 7: Preparation and storage of test substance (chemical)	Low	Details on the preparation of the test substance were limited. The study cited Albro et al. 1973 (HERO ID 63429) for the process of preparing DEHP from phthalic anhydride, but other details including test substance stability, homogeneity, mixing temperature, stock concentration, stirring methods, and storage conditions were not reported. No physical/chemical properties of the test substance were provided in the study. The test substance is lipophilic, suggesting that solubility may be an issue. The test substance was delivered in acetone. It is unclear whether acetone contributed to any damage to the skin.	
	Metric 8: Consistency of exposure administration	Medium	Details of exposure administration were incompletely reported. The epidermal and dermal membranes were 16 mm in diameter with a surface area of 0.64 cm2; however, the thickness of the membranes was not specified. The study exposed chemically separated epidermal and dermal membranes, therefore large differences in thickness are not expected. The text indicates a total volume of 50uL was applied, which would be equivalent to 78 uL/cm2, indicating an infinite exposure. Based on the information available, exposures were administered consistently across groups.	
	Metric 9: Reporting of concentrations	Uninformative	This study did not report doses or concentrations. Only the volume applied, the specific activity, and the surface area of the skin were reported.	
	Metric 10: Exposure frequency	Low	The total duration of exposure was longer than recommended (OECD TG 428). Receptor fluid collections were conducted for up to 72 hours. For test substances that penetrate more slowly, durations longer than the recommended 24 hours may be justified; however, the study authors did not explicitly provide justification for the longer duration. One concern for longer-duration studies is the deterioration of skin integrity. This study only tested skin integrity (as determined by Kp) for 5 hours, and did histopathology examinations at 24 hours. Integrity was not assessed after longer durations.	
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - 50% ethanol			
Domain	Metric	Rating	Comments	
	Metric 11: Number of exposure groups and concentration spacing	Low	The purpose of the study was to compare penetration through different skin layers (epidermal and dermal membranes) and also to assess differences between receptor fluids, and compare a lipophilic to a hydrophilic compound. The study did not report the dose/concentration used, but it is presumed that there was a single exposure concentration tested.	
Domain 4: Test Model				
	Metric 12: Test model (skin)	High	Dorsal skin was excised from 4-5 week-old male Ola: Sprague-Dawley rats. The animal source was not specified. The skin was stored at 2-8 degrees C, and sealed in polythene until used. The samples were used within 1 week. This study separately tested absorption through the epidermal membrane and the residual stripped dermis. Therefore, the thawed samples were soaked for 24 hours in 2M sodium bromide so that the two layers could be separated. Each layer was cut into 16-mm diameter discs and used immediately. Typically, split-thickness skin is preferred to single epidermal or dermal membranes, but testing of the single membranes was justified by the study authors. The surface area for absorption was 0.64 cm2. Skin integrity was tested before exposure to the test.	
	Metric 13: Number/Replicates per group	Medium	The number of replicates was reported and was appropriate. There were 8-11 replicates per membrane type (epidermis or dermis) per receptor fluid type (PBS or 50% ethanol).	
Domain 5: Outcome Assessment				
	Metric 14: Outcome assessment methodology	Medium	The outcome assessment methodology addressed the intended outcome of interest and was sensitive for the outcome, but there were minor uncertainties. This was a non-guideline study with specific goals and therefore had some deviations from standard practices. Kp was derived from what was presumed to be an infinite dose (78uL/cm2), this is lower than the guideline recommendation of 100 ul/cm2.	
	Metric 15: Consistency of outcome assessment	High	Based on the data available, there is no indication of inconsistencies across replicates in the outcome assessment protocols. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed.	
	Metric 16: Sampling adequacy and sensitivity	Low	The study did not specify whether a steady state was reached. Scintillation counts/sample and/or duration of radioactivity detection, and whether there was adequate signal-to-noise [i.e., background] ratio for detection were not reported. It is unclear whether these missing details would have a significant impact on the results.	
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - 50% ethanol			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Skin integrity was measured by a less desirable method (tritiated water). The study used limit values that were much higher than recommended (1.5×10^{-2} for the epidermis and 8.5×10^{-2} for the dermis compared to the guideline recommendation of 4.5×10^{-3}). The percent absorption (as measured in receptor fluid) at 1 hour was also significantly $> 0.6\%$. Although the purpose of the study was to look at absorption separately in the epidermal and dermal layers, the reliability/biological significance of the results is unclear. The chemical separation process resulted in both histological changes and skin integrity results that are lower than the recommended standards.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	The study did not explicitly demonstrate the solubility of the test substance in the receptor fluids, but the 50% aqueous ethanol fluid used was compatible with lipophilic substances. There were no reported differences among the replicates that were unrelated to exposure. There was no evidence that the test substance interfered with the assay.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	The Kp was derived from a linear regression as described by Dugard et al. 1984 using the steepest part of an absorption curve. Percent recovery was calculated from radioactivity in the receptor fluid, membrane washings, diffusion cell washings, and skin membranes. Statistical analysis was performed using appropriate methods. Percent absorption were presented across a time series for the receptor fluid, but not for the other compartments of the test system. It is not clear whether there were any outliers, and if calculations accounted for outliers. More than half of the CV values within an individual scenario were $>25\%$ and $<50\%$ or $>50\%$; however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results.	
	Metric 20: Data interpretation	Medium	Absorption estimates were calculated incompletely. The study did not report total % recovery (from all compartments). Recovery into the receptor fluids and recovery in the skin membranes were reported, but recovery from washes was not specified. The recoveries in the receptor fluids plus the reported recoveries in skin. Based on recoveries in the receptor fluid and skin, the recovery was less than $100\% \pm 10\%$, but the estimations are not accurate due to the missing recovery data for the washes. Kp was derived from what is presumed to be an infinite dose.	
	Metric 21: Reporting of data	Low	There are discrepancies between the data tables and the study text. Table 2 shows the % recovery into the different receptor fluids for each test (using epidermis and dermis) at 1 hour; however, the study text references the same table indicating it reports the total recovery in the receptor fluids at 72 hours. Based on the absorption plotted over time figures, the data from Table 2 appears to represent 72 hours, not 1 hour. Recovery data for the washes were not reported.	

Overall Quality Determination**Uninformative**

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - PBS			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C [di(2-ethylhexyl) phthalate; DEHP. A structure showing the site of the radiolabel was not provided.
	Metric 2:	Test substance source	Low	The DEHP test substance was prepared in the performing laboratory from [7-14C]phthalic anhydride purchased from Amersham International plc, Bucks, UK. The production process was cited to: Albro P. W., Thomas R. and Fishbein L. (1973)Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of urinary metabolites. Journal of Chromatography 76, 321 - 33. The test substance was not analytically verified by the performing laboratory.
	Metric 3:	Test substance purity	High	The purity of the radiolabeled phthalic anhydride was not specified. The specific activity and radiochemical purity of DEHP were determined by thin-layer chromatography and were 1.24 mCi/mmol and >98%, respectively. Impurities were not identified, but the chemical of interest was the main component.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Medium	The study included tests using the reference compound Benzoic acid. Benzoic acid is a hydrophilic compound and DEHP is lipophilic. A Lipophilic reference compound would have been more appropriate.
	Metric 5:	Assay procedures	Medium	Details of the assay procedure were adequately described. Skin samples were placed in a flow-through diffusion cell system. The flow rate was set to 1.9 mL/min. Skin temperature was maintained at 31.5 ± 1 degrees C. Humidity was not reported. Two different receptor fluids were tested; 0.9% saline (PBS) containing ampicillin and streptomycin, or 50% (v/v) aqueous ethanol. The study was examining the differences between the two receptor fluids; the aqueous ethanol fluid was expected to be more appropriate for lipophilic compounds. The receptor fluids were maintained at a temperature of 40 degrees C and were gassed with helium to minimize air bubble accumulation. Absorption tests were conducted in open compartments on skin samples with a surface area of 0.64 cm2. The radiolabeled test material (0.25 uCi) was applied in 50 uL acetone. Receptor fluid fractions were collected hourly for the first 6 hours, and then more extended (unspecified) intervals for up to 72 hours; radioactivity was counted. After exposure, the donor chambers were rinsed twice with 2% Lipsol detergent, and the radioactivity of the donor chamber and surface of the skin samples was measured. The epidermal and dermal membranes were dissolved and radioactivity was measured. Any residual radioactivity in the diffusion cell after soaking for 24 hours was counted. The study did not perform tape stripping, or utilize a carbon trap. However, the test substance is not volatile in nature. The lack of humidity reporting is not expected to have a significant impact on the study results.
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - PBS			
Domain	Metric	Rating	Comments	
	Metric 6: Standards for tests	Medium	The integrity of the skin samples was tested using tritiated H20 in saline and both of the receptor fluids. Integrity was not tested using acetone, the vehicle used for delivery of the test substance. Kp values and Kp ratio dermis/epidermis values were reported along with the percent of 3H recovered in each receptor fluid at 1 hour, and up to 4 hours. Any epidermal membranes with Kp > 1.5 x 10^-2 and dermal layers with a Kp > 8.5 x 10^-2 were rejected. Skin samples that had been processed with sodium bromide and subjected to physical separation were also histologically compared to sodium bromide-treated skin without physical separation and to untreated skin. The examinations showed some differences in epithelial thickness, folding, and displacement of hair follicles and sebaceous glands. The method used to prepare the intact skin was selected due to previous reports that use of the sodium bromide was superior. However, the study noted that histopathological analysis had not been conducted. This information is helpful in the interpretation of the study results. Coefficients of variation can be determined from the information provided (see Metric 19 for more details).	
Domain 3: Exposure Characterization				
	Metric 7: Preparation and storage of test substance (chemical)	Low	Details on the preparation of the test substance were limited. The study cited Albro et al. 1973 (HERO ID 63429) for the process of preparing DEHP from phthalic anhydride, but other details including test substance stability, homogeneity, mixing temperature, stock concentration, stirring methods, and storage conditions were not reported. No physical/chemical properties of the test substance were provided in the study. The test substance is lipophilic, suggesting that solubility may be an issue. The test substance was delivered in acetone. It is unclear whether acetone contributed to any damage to the skin.	
	Metric 8: Consistency of exposure administration	Medium	Details of exposure administration were incompletely reported. The epidermal and dermal membranes were 16 mm in diameter with a surface area of 0.64 cm2; however, the thickness of the membranes was not specified. The study exposed chemically separated epidermal and dermal membranes, therefore large differences in thickness are not expected. The text indicates a total volume of 50uL was applied, which would be equivalent to 78 uL/cm2, indicating an infinite exposure. Based on the information available, exposures were administered consistently across groups.	
	Metric 9: Reporting of concentrations	Uninformative	This study did not report doses or concentrations. Only the volume applied, the specific activity, and the surface area of the skin were reported.	
	Metric 10: Exposure frequency	Low	The total duration of exposure was longer than recommended (OECD TG 428). Receptor fluid collections were conducted for up to 72 hours. For test substances that penetrate more slowly, durations longer than the recommended 24 hours may be justified; however, the study authors did not explicitly provide justification for the longer duration. One concern for longer-duration studies is the deterioration of skin integrity. This study only tested skin integrity (as determined by Kp) for 5 hours, and did histopathology examinations at 24 hours. Integrity was not assessed after longer durations.	
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - PBS			
Domain	Metric	Rating	Comments	
	Metric 11: Number of exposure groups and concentration spacing	Low	The purpose of the study was to compare penetration through different skin layers (epidermal and dermal membranes) and also to assess differences between receptor fluids, and compare a lipophilic to a hydrophilic compound. The study did not report the dose/concentration used, but it is presumed that there was a single exposure concentration tested.	
Domain 4: Test Model				
	Metric 12: Test model (skin)	High	Dosal skin was excised from 4-5 week-old male Ola: Sprague-Dawley rats. The animal source was not specified. The skin was stored at 2-8 degrees C, and sealed in polythene until used. The samples were used within 1 week. This study separately tested absorption through the epidermal membrane and the residual stripped dermis. Therefore, the thawed samples were soaked for 24 hours in 2M sodium bromide so that the two layers could be separated. Each layer was cut into 16-mm diameter discs and used immediately. Typically, split-thickness skin is preferred to single epidermal or dermal membranes, but testing of the single membranes was justified by the study authors. The surface area for absorption was 0.64 cm2. Skin integrity was tested before exposure to the test.	
	Metric 13: Number/Replicates per group	Medium	The number of replicates was reported and was appropriate. There were 8-11 replicates per membrane type (epidermis or dermis) per receptor fluid type (PBS or 50% ethanol).	
Domain 5: Outcome Assessment				
	Metric 14: Outcome assessment methodology	Medium	The outcome assessment methodology addressed the intended outcome of interest and was sensitive for the outcome, but there were minor uncertainties. This was a non-guideline study with specific goals and therefore had some deviations from standard practices. Kp was derived from what was presumed to be an infinite dose (78uL/cm2), this is lower than the guideline recommendation of 100 ul/cm2.	
	Metric 15: Consistency of outcome assessment	High	Based on the data available, there is no indication of inconsistencies across replicates in the outcome assessment protocols. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed.	
	Metric 16: Sampling adequacy and sensitivity	Low	The study did not specify whether a steady state was reached. Scintillation counts/sample and/or duration of radioactivity detection, and whether there was adequate signal-to-noise [i.e., background] ratio for detection were not reported. It is unclear whether these missing details would have a significant impact on the results.	
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Epidermis - PBS			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Skin integrity was measured by a less desirable method (tritiated water). The study used limit values that were much higher than recommended (1.5×10^{-2} for the epidermis and 8.5×10^{-2} for the dermis compared to the guideline recommendation of 4.5×10^{-3}). The percent absorption (as measured in receptor fluid) at 1 hour was also significantly $> 0.6\%$. Although the purpose of the study was to look at absorption separately in the epidermal and dermal layers, the reliability/biological significance of the results is unclear. The chemical separation process resulted in both histological changes and skin integrity results that are lower than the recommended standards.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Low	The study did not explicitly demonstrate the solubility of the test substance in the receptor fluids, and PBS is likely not appropriate for lipophilic substances. There were no reported differences among the replicates that were unrelated to exposure. There was no evidence that the test substance interfered with the assay.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	The Kp was derived from a linear regression as described by Dugard et al. 1984 using the steepest part of an absorption curve. Percent recovery was calculated from radioactivity in the receptor fluid, membrane washings, diffusion cell washings, and skin membranes. Statistical analysis was performed using appropriate methods. Percent absorption was presented across a time series for the receptor fluid, but not for the other compartments of the test system. It is not clear whether there were any outliers, and if calculations accounted for outliers. More than half of the CV values within an individual scenario were $>25\%$ and $<50\%$ or $>50\%$; however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results.	
	Metric 20: Data interpretation	Medium	Absorption estimates were calculated incompletely. The study did not report total % recovery (from all compartments). Recovery into the receptor fluids and recovery in the skin membranes were reported, but recovery from washes was not specified. The recoveries in the receptor fluids plus the reported recoveries in skin. Based on recoveries in the receptor fluid and skin, the recovery was less than $100\% \pm 10\%$, but the estimations are not accurate due to the missing recovery data for the washes. Kp was derived from what is presumed to be an infinite dose.	
	Metric 21: Reporting of data	Low	There are discrepancies between the data tables and the study text. Table 2 shows the % recovery into the different receptor fluids for each test (using epidermis and dermis) at 1 hour; however, the study text references the same table indicating it reports the total recovery in the receptor fluids at 72 hours. Based on the absorption plotted over time figures, the data from Table 2 appears to represent 72 hours, not 1 hour. Recovery data for the washes were not reported.	

Overall Quality Determination**Uninformative**

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - PBS			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C [di(2-ethylhexyl) phthalate; DEHP. A structure showing the site of the radiolabel was not provided.
	Metric 2:	Test substance source	Low	The DEHP test substance was prepared in the performing laboratory from [7-14C]phthalic anhydride purchased from Amersham International plc, Bucks, UK. The production process was cited to: Albro P. W., Thomas R. and Fishbein L. (1973)Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of urinary metabolites. Journal of Chromatography 76, 321 - 33. The test substance was not analytically verified by the performing laboratory.
	Metric 3:	Test substance purity	High	The purity of the radiolabeled phthalic anhydride was not specified. The specific activity and radiochemical purity of DEHP were determined by thin-layer chromatography and were 1.24 mCi/mmol and >98%, respectively. Impurities were not identified, but the chemical of interest was the main component.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Medium	The study included tests using the reference compound Benzoic acid. Benzoic acid is a hydrophilic compound and DEHP is lipophilic. A Lipophilic reference compound would have been more appropriate.
	Metric 5:	Assay procedures	Medium	Details of the assay procedure were adequately described. Skin samples were placed in a flow-through diffusion cell system. The flow rate was set to 1.9 mL/min. Skin temperature was maintained at 31.5 ± 1 degrees C. Humidity was not reported. Two different receptor fluids were tested; 0.9% saline (PBS) containing ampicillin and streptomycin, or 50% (v/v) aqueous ethanol. The study was examining the differences between the two receptor fluids; the aqueous ethanol fluid was expected to be more appropriate for lipophilic compounds. The receptor fluids were maintained at a temperature of 40 degrees C and were gassed with helium to minimize air bubble accumulation. Absorption tests were conducted in open compartments on skin samples with a surface area of 0.64 cm2. The radiolabeled test material (0.25 uCi) was applied in 50 uL acetone. Receptor fluid fractions were collected hourly for the first 6 hours, and then more extended (unspecified) intervals for up to 72 hours; radioactivity was counted. After exposure, the donor chambers were rinsed twice with 2% Lipsol detergent, and the radioactivity of the donor chamber and surface of the skin samples was measured. The epidermal and dermal membranes were dissolved and radioactivity was measured. Any residual radioactivity in the diffusion cell after soaking for 24 hours was counted. The study did not perform tape stripping, or utilize a carbon trap. However, the test substance is not volatile in nature. The lack of humidity reporting is not expected to have a significant impact on the study results.
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - PBS			
Domain	Metric	Rating	Comments	
	Metric 6: Standards for tests	Medium	The integrity of the skin samples was tested using tritiated H20 in saline and both of the receptor fluids. Integrity was not tested using acetone, the vehicle used for delivery of the test substance. Kp values and Kp ratio dermis/epidermis values were reported along with the percent of 3H recovered in each receptor fluid at 1 hour, and up to 4 hours. Any epidermal membranes with Kp > 1.5 x 10^-2 and dermal layers with a Kp > 8.5 x 10^-2 were rejected. Skin samples that had been processed with sodium bromide and subjected to physical separation were also histologically compared to sodium bromide-treated skin without physical separation and to untreated skin. The examinations showed some differences in epithelial thickness, folding, and displacement of hair follicles and sebaceous glands. The method used to prepare the intact skin was selected due to previous reports that use of the sodium bromide was superior. However, the study noted that histopathological analysis had not been conducted. This information is helpful in the interpretation of the study results. Coefficients of variation can be determined from the information provided (see Metric 19 for more details).	
Domain 3: Exposure Characterization				
	Metric 7: Preparation and storage of test substance (chemical)	Low	Details on the preparation of the test substance were limited. The study cited Albro et al. 1973 (HERO ID 63429) for the process of preparing DEHP from phthalic anhydride, but other details including test substance stability, homogeneity, mixing temperature, stock concentration, stirring methods, and storage conditions were not reported. No physical/chemical properties of the test substance were provided in the study. The test substance is lipophilic, suggesting that solubility may be an issue. The test substance was delivered in acetone. It is unclear whether acetone contributed to any damage to the skin.	
	Metric 8: Consistency of exposure administration	Medium	Details of exposure administration were incompletely reported. The epidermal and dermal membranes were 16 mm in diameter with a surface area of 0.64 cm2; however, the thickness of the membranes was not specified. The study exposed chemically separated epidermal and dermal membranes, therefore large differences in thickness are not expected. The text indicates a total volume of 50uL was applied, which would be equivalent to 78 uL/cm2, indicating an infinite exposure. Based on the information available, exposures were administered consistently across groups.	
	Metric 9: Reporting of concentrations	Uninformative	This study did not report doses or concentrations. Only the volume applied, the specific activity, and the surface area of the skin were reported.	
	Metric 10: Exposure frequency	Low	The total duration of exposure was longer than recommended (OECD TG 428). Receptor fluid collections were conducted for up to 72 hours. For test substances that penetrate more slowly, durations longer than the recommended 24 hours may be justified; however, the study authors did not explicitly provide justification for the longer duration. One concern for longer-duration studies is the deterioration of skin integrity. This study only tested skin integrity (as determined by Kp) for 5 hours, and did histopathology examinations at 24 hours. Integrity was not assessed after longer durations.	
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - PBS			
Domain	Metric	Rating	Comments	
	Metric 11: Number of exposure groups and concentration spacing	Low	The purpose of the study was to compare penetration through different skin layers (epidermal and dermal membranes) and also to assess differences between receptor fluids, and compare a lipophilic to a hydrophilic compound. The study did not report the dose/concentration used, but it is presumed that there was a single exposure concentration tested.	
Domain 4: Test Model				
	Metric 12: Test model (skin)	High	Dorsal skin was excised from 4-5 week-old male Ola: Sprague-Dawley rats. The animal source was not specified. The skin was stored at 2-8 degrees C, and sealed in polythene until used. The samples were used within 1 week. This study separately tested absorption through the epidermal membrane and the residual stripped dermis. Therefore, the thawed samples were soaked for 24 hours in 2M sodium bromide so that the two layers could be separated. Each layer was cut into 16-mm diameter discs and used immediately. Typically, split-thickness skin is preferred to single epidermal or dermal membranes, but testing of the single membranes was justified by the study authors. The surface area for absorption was 0.64 cm2. Skin integrity was tested before exposure to the test.	
	Metric 13: Number/Replicates per group	Medium	The number of replicates was reported and was appropriate. There were 8-11 replicates per membrane type (epidermis or dermis) per receptor fluid type (PBS or 50% ethanol).	
Domain 5: Outcome Assessment				
	Metric 14: Outcome assessment methodology	Medium	The outcome assessment methodology addressed the intended outcome of interest and was sensitive for the outcome, but there were minor uncertainties. This was a non-guideline study with specific goals and therefore had some deviations from standard practices. Kp was derived from what was presumed to be an infinite dose (78uL/cm2), this is lower than the guideline recommendation of 100 ul/cm2.	
	Metric 15: Consistency of outcome assessment	High	Based on the data available, there is no indication of inconsistencies across replicates in the outcome assessment protocols. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed. Some information was not specified, for example, how soon after the collection of receptor fluids was scintillation counts performed.	
	Metric 16: Sampling adequacy and sensitivity	Low	The study did not specify whether a steady state was reached. Scintillation counts/sample and/or duration of radioactivity detection, and whether there was adequate signal-to-noise [i.e., background] ratio for detection were not reported. It is unclear whether these missing details would have a significant impact on the results.	
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Pelling, D., Phillips, J. C., Cunninghame, M. E. (1998). Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology In Vitro 12(1):47-55.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1333950			
Unique ID:	Dermis - PBS			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Skin integrity was measured by a less desirable method (tritiated water). The study used limit values that were much higher than recommended (1.5×10^{-2} for the epidermis and 8.5×10^{-2} for the dermis compared to the guideline recommendation of 4.5×10^{-3}). The percent absorption (as measured in receptor fluid) at 1 hour was also significantly $> 0.6\%$. Although the purpose of the study was to look at absorption separately in the epidermal and dermal layers, the reliability/biological significance of the results is unclear. The chemical separation process resulted in both histological changes and skin integrity results that are lower than the recommended standards.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Low	The study did not explicitly demonstrate the solubility of the test substance in the receptor fluids, and PBS is likely not appropriate for lipophilic substances. There were no reported differences among the replicates that were unrelated to exposure. There was no evidence that the test substance interfered with the assay.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	The Kp was derived from a linear regression as described by Dugard et al. 1984 using the steepest part of an absorption curve. Percent recovery was calculated from radioactivity in the receptor fluid, membrane washings, diffusion cell washings, and skin membranes. Statistical analysis was performed using appropriate methods. Percent absorption was presented across a time series for the receptor fluid, but not for the other compartments of the test system. It is not clear whether there were any outliers, and if calculations accounted for outliers. More than half of the CV values within an individual scenario were $>25\%$ and $<50\%$ or $>50\%$; however, standard deviations were provided which will allow for EPA to calculate an alternate upper end value to account for variability in the results.	
	Metric 20: Data interpretation	Medium	Absorption estimates were calculated incompletely. The study did not report total % recovery (from all compartments). Recovery into the receptor fluids and recovery in the skin membranes were reported, but recovery from washes was not specified. The recoveries in the receptor fluids plus the reported recoveries in skin. Based on recoveries in the receptor fluid and skin, the recovery was less than $100\% \pm 10\%$, but the estimations are not accurate due to the missing recovery data for the washes. Kp was derived from what is presumed to be an infinite dose.	
	Metric 21: Reporting of data	Low	There are discrepancies between the data tables and the study text. Table 2 shows the % recovery into the different receptor fluids for each test (using epidermis and dermis) at 1 hour; however, the study text references the same table indicating it reports the total recovery in the receptor fluids at 72 hours. Based on the absorption plotted over time figures, the data from Table 2 appears to represent 72 hours, not 1 hour. Recovery data for the washes were not reported.	

Overall Quality Determination**Uninformative**

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	674473			
Unique ID:	DEHP-Human skin			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	The test substance was identified as [14C]DEHP. No CASRN, structure, or position of the radiolabel were provided. The study included a table reporting physical/chemical properties. Unlabeled DEHP was also used.
	Metric 2:	Test substance source	Low	The source of the radiolabeled test substance was Amersham International PLC; the unlabeled test substance was obtained from Aldrich Chemical Co. The identity of the test substance was not verified by the performing laboratory and certificates of analysis were not provided. The chemicals used in this 1987 study cannot be verified on the manufacturer’s website.
	Metric 3:	Test substance purity	High	The purity of the unlabeled compound was 99% and the purity of the radiolabeled compound was >99%.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	A concurrent reference compound was not tested along with the test substance and the authors did not specify previous experience with dermal absorption studies. One of the papers cited for preparation of epidermal layers was conducted by the same group of authors.
	Metric 5:	Assay procedures	Medium	In this study, epidermal membrane samples were placed in a static glass diffusion cell; details of the setup were cited to Dugard et al. 1984, which is open access and was viewed for this review. The number of donors or samples was not reported in the methods. After permeability testing using tritiated water (day 1), receptor chambers were filled with 4.5 mL of 50% v/v aqueous ethanol. The authors did not provide justification for the receptor fluid used, but aqueous ethanol is considered to be appropriate (OECD 156). A 0.5mL volume of DEHP (specific activity of ~35 uCi/mL) was applied neat to the donor compartment; the loading rate (mg/cm2) was not specified; however, the skin diameter was given (3 cm), and the area was calculated for this review to be 7.07 cm2. Diffusions cells were maintained at 30 ± 1°C. They system was left uncovered; humidity was not reported. Samples of receptor fluid (50uL) were “taken frequently” (number and frequency not specified) and replaced with equal volumes of fresh receptor fluid. Scintillation counting was used. The LOD and number of counts were not specified. At the end of the experiment, the skin was washed (washing method not specified), and a second permeability test was conducted. This allowed the determination of a damage ratio.
Continued on next page ...				

...continued from previous page

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	674473			
Unique ID:	DEHP-Human skin			
Domain	Metric	Rating	Comments	
	Metric 6:	Standards for tests	Medium	The skin integrity was determined using tritiated water. Integrity was determined before the application of the test substance. Membranes with permeability constants $>1.5 \times 10^{-3}$ (human), and $>2.5 \times 10^{-2}$ (rat) were rejected. Another tritiated water permeability test was conducted at the end of the study and a damage ratio was calculated. A slight increase in human skin permeability occurred over the course of the study. The damage ratio was 2.3.Percent recovery was not reported, but this is not expected for an infinite exposure study. Coefficients of variation for Kp and steady-state flux could be calculated using the data provided. CV values were $>25\%$ and an adjustment should be applied.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Medium	Storage of test substance was not adequately reported; however, this is not likely to substantially impact results. The substance is non-volatile. The test substance was used neat; therefore, a discussion of preparation was not necessary.
	Metric 8:	Consistency of exposure administration	Medium	Epidermal membranes 3cm in diameter were used (7.07 cm2). The thickness of the heat-isolated epidermis is not typically reported. The application volume was reported as approximately 0.5mL; given the application is an infinite dose slight deviation in volume are unlikely to substantially impact results. The available information suggests consistency of application across replicates.
	Metric 9:	Reporting of concentrations	Medium	The test substance was studied neat. The specific activity of the test substance (mixture of radiolabeled and non-radiolabeled) was 35 uCi/g. The dose (mg/cm2) was not reported, only a volume of 0.5mL. It is possible the density of DEHP could be used to calculate an approximate dose (mg). Based on the application area (7.07 cm2, based on a given diameter of 3 cm), the loading rate was 70 uL/cm2.
	Metric 10:	Exposure frequency	Low	Human epidermis studies were continued for up to 72 hours. The authors noted a lag time of 3.1 hours, but it is unclear why exposure beyond 24 hours was needed. Absorption curves were not provided to visualize what time-frames a linear portion of the curve was obtained.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study only tested a single dose (neat).
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Human abdominal skin was obtained from cadavers. Donors were reported as mostly females aged ≥ 55 years. The number of donors and number of skin samples per donor were not reported. The epidermis was separated from the dermis after immersing the skin into water at 60 degrees C for 40 to 45 seconds. The skin was stored at 4 degrees C; it was used within 7 days of preparation. The integrity of the skin was evaluated by measuring the permeability of tritiated water at both the beginning and end of the experiment. Split-thickness skin is preferred because the use of epidermal membranes may overestimate human in vivo skin absorption (OECD 156).
Continued on next page ...				

...continued from previous page

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	674473			
Unique ID:	DEHP-Human skin			
Domain	Metric	Rating	Comments	
	Metric 13:	Number/Replicates per group	Medium	The number of replicates at the start of the study was not reported; absorption data were obtained from an n = 9, which was sufficient.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Medium	The study used approximately 70 uL/cm2 (calculated for this review, based on skin diameter and volume applied). This is less than the 100 uL/cm2 recommended for infinite dose scenarios. Steady-state absorption rates were calculated from linear portions of the curve. The study cited Dugard et al. (1984) for details on calculating permeability constants.
	Metric 15:	Consistency of outcome assessment	Medium	Details regarding the execution of the study protocol were mostly reported. The duration of exposure and the same receptor fluid composition were used across replicates. The same volume of receptor fluid was removed at each collection, although the frequency of collections was not specified (see metric 16).
	Metric 16:	Sampling adequacy and sensitivity	Low	Details regarding sampling were insufficiently reported. Samples of receptor fluid (50uL) were “taken frequently” (number and frequency not specified).
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	The study did not report the thickness of the skin studied; therefore, it is unclear how much variation existed between the samples. Skin integrity was evaluated prior to the start of the study using tritiated water. Human membranes with values greater than 1.5 x 10-3 were excluded which is acceptable. Actual measurements were not reported. The authors noted that human skin epidermal layers prepared and stored as reported “have been shown to maintain their permeability parameters.” Integrity was also measured at the end of the study. The number of donors and skin samples per donor was not reported.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility in the receptor fluid was not demonstrated, although solubility in water was reported. OECD guidelines recommend that with very lipophilic substances, such as DEHP, that BSA or 6% polyethylene glycol 20 oleyl ether be added to the receptor fluid to overcome solubility restrictions. This study used 50% v/v aqueous ethanol which is also acceptable according to OECD TG 28.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	Methods for the calculation of the permeability constant was cited to Dugard et al. (1984; available open access). The coefficients of variation, calculated for this review, were 63% for both Kp and the steady-state absorption rate. Sufficient information is available for EPA to calculate an upper-end value to account for variability in the results.
	Metric 20:	Data interpretation	High	Data were properly interpreted. Permeability (Kp) was calculated using infinite concentration. Recovery was not reported, but this is not an important endpoint for infinite exposures.
	Metric 21:	Reporting of data	Medium	Calculated absorption rate and permeability constant are reported. The study does not graphically show the permeation curves.

Continued on next page ...

...continued from previous page

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.
Chemical:	Diethylhexyl Phthalate
Exposure Type:	Parent compound
HERO ID:	674473
Unique ID:	DEHP-Human skin

Domain	Metric	Rating	Comments
--------	--------	--------	----------

Overall Quality Determination	Medium
-------------------------------	--------

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	674473			
Unique ID:	DEHP-Rat skin			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	Medium	The test substance was identified as [14C]DEHP. No CASRN, structure, or position of the radiolabel were provided. The study included a table reporting physical/chemical properties. Unlabeled DEHP was also used.	
Metric 2:	Test substance source	Low	The source of the radiolabeled test substance was Amersham International PLC; the unlabeled test substance was obtained from Aldrich Chemical Co. The identity of the test substance was not verified by the performing laboratory and certificates of analysis were not provided. The chemicals used in this 1987 study cannot be verified on the manufacturer's website.	
Metric 3:	Test substance purity	High	The purity of the unlabeled compound was 99% and the purity of the radiolabeled compound was >99%.	
Domain 2: Test Design				
Metric 4:	Reference compounds	Low	A concurrent reference compound was not tested along with the test substance and the authors did not specify previous experience with dermal absorption studies. One of the papers cited for preparation of epidermal layers was conducted by the same group of authors.	
Metric 5:	Assay procedures	Medium	In this study, epidermal membrane samples were placed in a static glass diffusion cell; details of the setup were cited to Dugard et al. 1984, which is open access and was viewed for this review. The number of donors or samples was not reported in the methods. After permeability testing using tritiated water (day 1), receptor chambers were filled with 4.5 mL of 50% v/v aqueous ethanol. The authors did not provide justification for the receptor fluid used, but aqueous ethanol is considered to be appropriate (OECD 156). A 0.5mL volume of DEHP (specific activity of ~35 uCi/mL) was applied neat to the donor compartment; the loading rate (mg/cm2) was not specified; however, the skin diameter was given (3 cm), and the area was calculated for this review to be 7.07 cm2. Diffusion cells were maintained at 30 ± 1°C. The system was left uncovered; humidity was not reported. Samples of receptor fluid (50uL) were "taken frequently" (number and frequency not specified) and replaced with equal volumes of fresh receptor fluid. Scintillation counting was used. The LOD and number of counts were not specified. At the end of the experiment, the skin was washed (washing method not specified), and a second permeability test was conducted. This allowed the determination of a damage ratio.	
Metric 6:	Standards for tests	Medium	The skin integrity was determined using tritiated water. Integrity was determined before the application of the test substance. Membranes with permeability constants >1.5 x 10^-3 (human), and >2.5 x 10^-2 (rat) were rejected. Another tritiated water permeability test was conducted at the end of the study and a damage ratio was calculated. Rat skin showed large changes in permeability between the start and end of the study. The authors stated that contact with the test substance resulted in "irreversibly alteration of the membrane." The damage ratio was 9.5 .Percent recovery was not reported, but this is not expected for an infinite exposure study. Coefficients of variation for Kp and steady-state flux could be calculated using the data provided. CV values were >25% and an adjustment should be applied.	

Continued on next page ...

...continued from previous page

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.		
Chemical:	Diethylhexyl Phthalate		
Exposure Type:	Parent compound		
HERO ID:	674473		
Unique ID:	DEHP-Rat skin		
Domain	Metric	Rating	Comments
Domain 3: Exposure Characterization			
Metric 7:	Preparation and storage of test substance (chemical)	Medium	Storage of test substance was not adequately reported; however, this is not likely to substantially impact results. The substance is non-volatile. The test substance was used neat; therefore, a discussion of preparation was not necessary.
Metric 8:	Consistency of exposure administration	Medium	Epidermal membranes 3cm in diameter were used (7.07 cm ²). The thickness of the heat-isolated epidermis is not typically reported. The application volume was 0.5mL. The available information suggests consistency of application across replicates.
Metric 9:	Reporting of concentrations	Medium	The test substance was studied neat. The specific activity of the test substance (mixture of radiolabeled and non-radiolabeled) was 35 uCi/g. The dose (mg/cm ²) was not reported, only a volume of 0.5mL. It is possible the density of DEHP could be used to calculate an approximate dose (mg). Based on the application area (7.07 cm ² , based on a given diameter of 3 cm), the loading rate was 70 uL/cm ² .
Metric 10:	Exposure frequency	Low	Rat epidermis studies were continued for up to 53 hours. The authors noted a lag time of 3.9 hours, but it is unclear why exposure beyond 24 hours was needed. Absorption curves were not provided to visualize what time-frames a linear portion of the curve was obtained.
Metric 11:	Number of exposure groups and concentration spacing	Low	The study only tested a single dose (neat).
Domain 4: Test Model			
Metric 12:	Test model (skin)	Low	The dorsal skin from Wistar rats (number, sex, and age not reported) was removed and placed in 2M NaBr for up to 24 hours. The epidermis was then peeled from the dermis and stored at 4 degrees C; it was used within 7 days of preparation. The integrity of the skin was evaluated by measuring the permeability of tritiated water at both the beginning and end of the experiment. Split-thickness skin is preferred because the use of epidermal membranes may overestimate human in vivo skin absorption (OECD 156).
Metric 13:	Number/Replicates per group	Medium	The number of replicates at the start of the study was not reported; absorption data were obtained from an n = 8- 9, which was sufficient.
Domain 5: Outcome Assessment			
Metric 14:	Outcome assessment methodology	Medium	The study used approximately 70 uL/cm ² (calculated for this review, based on skin diameter and volume applied). This is less than the 100 uL/cm ² recommended for infinite dose scenarios. Steady-state absorption rates were calculated from linear portions of the curve. The study cited Dugard et al. (1984) for details on calculating permeability constants.
Metric 15:	Consistency of outcome assessment	Medium	Details regarding the execution of the study protocol were mostly reported. The duration of exposure and the same receptor fluid composition were used across replicates. The same volume of receptor fluid was removed at each collection, although the frequency of collections was not specified (see metric 16).
Continued on next page ...			

...continued from previous page

Study Citation:	Scott, R. C., Dugard, P. H., Ramsey, J. D., Rhodes, C. (1987). In vitro absorption of some o-phthalate diesters through human and rat skin. Environmental Health Perspectives 74(0):223-227.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	674473			
Unique ID:	DEHP-Rat skin			
Domain	Metric	Rating	Comments	
	Metric 16:	Sampling adequacy and sensitivity	Low	Details regarding sampling were insufficiently reported. Samples of receptor fluid (50uL) were “taken frequently” (number and frequency not specified).
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Low	The study did not report the thickness of the skin studied; therefore, it is unclear how much variation existed between the samples. Skin integrity was evaluated prior to the start of the study using tritiated water. Rat membranes with values greater than 2.5 x 10-3 were excluded which is acceptable. Actual measurements were not reported. Integrity was also measured at the end of the study. The authors described Large changes in permeability during the experiment as “irreversible alterations of the membrane” following contact with the test substance. It is unclear how this impacted the study results. The number of animals and skin samples per animal was not reported.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	Solubility in the receptor fluid was not demonstrated, although solubility in water was reported. OECD guidelines recommend that with very lipophilic substances, such as DEHP, that BSA should be added to the receptor fluid to overcome solubility restrictions. This study used 50% v/v aqueous ethanol.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	Methods for the calculation of the permeability constant was cited to Dugard et al. (1984; available open access). The coefficients of variation, calculated for this review, were 63% for both Kp and the steady-state absorption rate. Sufficient information is available for EPA to calculate an upper-end value to account for variability in the results.
	Metric 20:	Data interpretation	High	Data were properly interpreted. Permeability (Kp) was calculated using infinite concentration. Recovery was not reported, but this is not an important endpoint for infinite exposures.
	Metric 21:	Reporting of data	Medium	Calculated absorption rate and permeability constant are reported. The study does not graphically show the permeation curves.
Overall Quality Determination			Low	

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -stripped (human)			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was identified as Di (2-ethylhexyl phthalate (DEHP). A CASRN and structure were provided. The test substance was not radiolabeled.
	Metric 2:	Test substance source	High	The test substance was purchased from Wako Pure Chemical Industries, Ltd. Certificates of analysis are available on the supplier website for the current listings of this chemical. The performing laboratory did not analytically verify the test substance.
	Metric 3:	Test substance purity	Medium	The purity was not reported in the study. The product currently listed on the website of the supplier has a purity of >97%. Other chemicals used in the study were of special or HPLC grade. It is assumed the purity is high, but due to the lack of listing in the main study report, there is some uncertainty.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Medium	The assay procedures were clearly described, with only a few missing details. Skin samples (presumably n = 4-5/group) were mounted on static diffusion cells yielding a surface area of 0.95 cm2. It was not specified whether the chambers were open or closed, but the test substance has low volatility. The donor and receiver chambers were held at 32 degrees C; humidity was not reported. Prior to exposure, the skin was hydrated with PBS. At exposure time, the receptor chamber was filled with 2.5 mL of fresh PBS + 10% DMSO, and the donor chamber was filled with 2.5 mL of PBS + 10% DMSO containing 0.256 mM DEHP. All of the solutions were stirred with a magnetic stir bar. In some cases, 0.53mM DFP (an esterase inhibitor) was added to the receptor fluid for metabolism tests. Receptor fluid samples were taken periodically and replenished with an equal volume of the receptor fluid. The collected fractions were held at 4 degrees C until analysis. At the end of exposure (24 hours), the skin was washed 3 times with receptor fluid and then homogenized for analysis via HPLC. The lower limits of quantitation were reported.
	Metric 6:	Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use. Percent recovery was not determined; however, recovery determination is not relevant for infinite dose applications determining a Kp. The results for measurements of DEHP in the receptor fluid and in skin samples were not quantitatively reported, and it is unclear whether coefficients of variation were assessed.
Domain 3: Exposure Characterization				
Continued on next page ...				

...continued from previous page

Study Citation: Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.				
Chemical: Diethylhexyl Phthalate				
Exposure Type: Parent compound				
HERO ID: 3859042				
Unique ID: DEHP and its EHP metabolite -stripped (human)				
Domain		Metric	Rating	Comments
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details of the test substance preparation were provided. The test substance (0.256 mM) was diluted in PBS +10% DMSO. No details on stability, or mixing to assure homogeneity, mixing temperature, stirring methods, or storage conditions were reported. The solubility of the test substance in the receptor fluid was not specified. DEHP is lipophilic and it is assumed DMSO was added to increase solubility.
	Metric 8:	Consistency of exposure administration	Low	Details of exposure administration were insufficiently reported. The study included a single exposure group. The number of replicates was not explicitly reported. Stripped human skin thicknesses were not reported. Tests were conducted using a consistent test solution volume of 2.5 mL, and a skin surface area of 0.95 cm ² . The missing information could have a significant impact on the results.
	Metric 9:	Reporting of concentrations	Medium	A nominal 0.256 mM concentration was added to the donor chamber. The volume applied and skin surface area was reported. It is unclear whether the test concentration was analytically verified. The selected concentration was not justified by the study authors.
	Metric 10:	Exposure frequency	Low	The duration of exposure was dependent on when permeation reached a steady state. The samples were exposed for 48 hours before termination; no penetration was observed.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study used a single exposure concentration; however, the purpose of the study was to compare data from multiple skin types (full-thickness and stripped), chemicals, and species (rat and human), and also to assess metabolic parameters. It is not clear that testing multiple concentrations was in line with the goals of the study, although OECD guidelines recommend at least three exposure groups.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Frozen abdominal skin (presumably 4 pieces total per group) from two Caucasian females aged 51 and 55 yrs old, was purchased from Biopredic International. The skin was stored at -50 degrees C and thawed just prior to the experiments. The samples were tape stripped 20 times to remove the stratum corneum. No information on thickness or skin integrity was provided.
	Metric 13:	Number/Replicates per group	Low	The number of replicates per group was not clearly specified in the methods. A sample size of 4 is the minimum sample size required, as specified in OECD 428.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	High	Use of a guideline was not specified. The study was focused on determining K _p and flux, which should be obtained using an infinite dose scenario. A volume of 2.5 mL was applied to the donor chamber (area 0.95 cm ²), so the applied volume was ~ 2.6 mL/cm ² which is appropriate for infinite exposure to a liquid, regardless of concentration. A molar concentration of 0.256mM was reported. Based on a molecular weight of 390.55, and the 2.5 mL volume, approximately 0.249 mg of the test substance was added to the donor chamber with a 0.95 cm ² surface area (equivalent to ~0.263 mg/cm ²).

Continued on next page ...

...continued from previous page

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.				
Chemical:	Diethylhexyl Phthalate				
Exposure Type:	Parent compound				
HERO ID:	3859042				
Unique ID:	DEHP and its EHP metabolite -stripped (human)				
Domain	Metric	Rating	Comments		
	Metric 15:	Consistency of outcome assessment	Low	Limited information was provided to determine whether there were inconsistencies across groups. The same vehicle and receptor fluids were used for each replicate, and the exposure duration was 48 hours. The receptor fluid collection times were not specified.	
	Metric 16:	Sampling adequacy and sensitivity	Low		Details regarding the sampling of outcomes were not reported.
Domain 6: Confounding/Variable Control					
	Metric 17:	Confounding variables in test design and procedures	Low	The study included only a single group. The skin was excised from two donors. The skin was tape stripped, and the thickness was not reported. Integrity/quality was not assessed making it difficult to determine whether there were any confounding differences across replicates. The missing details could have a substantial impact on the study results.	
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Low		There was insufficient information to determine whether there were confounding variables between replicates that were unrelated to exposure. The study did not indicate whether the test substance was soluble in the receptor fluid (PBS + 10% DMSO) although DMSO is expected to enhance the solubility of lipophilic substances. The study noted significant metabolism occurred and concentrations of two metabolites were assessed. This could cause additional variation across replicates.
Domain 7: Data Presentation and Analysis					
	Metric 19:	Data analysis	Uninformative	The data analysis methods were generally reported but since only a qualitative statement on the study results was provided, it is unclear whether any statistical methods were applied to these data, and the data were not provided to conduct an independent analysis. Infinite exposure conditions were used to derive Kp and flux. This study did not determine % recovery; however, for infinite dose applications in which Kp or flux are determined, recovery is not relevant (OECD TG 28).	
	Metric 20:	Data interpretation	High		A qualitative statement saying "tape stripping did not affect the skin permeation properties of DEHP, " and "no permeation was found after applying DEHP" were the only results reported.
	Metric 21:	Reporting of data	Uninformative		
Overall Quality Determination			Uninformative		

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -Full-thickness (human)			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was identified as Di (2-ethylhexyl phthalate (DEHP). A CASRN and structure were provided. The test substance was not radiolabeled.
	Metric 2:	Test substance source	High	The test substance was purchased from Wako Pure Chemical Industries, Ltd. Certificates of analysis are available on the supplier website for the current listings of this chemical. The performing laboratory did not analytically verify the test substance.
	Metric 3:	Test substance purity	Medium	The purity was not reported in the study. The product currently listed on the website of the supplier has a purity of >97%. Other chemicals used in the study were of special or HPLC grade. It is assumed the purity is high, but due to the lack of listing in the main study report, there is some uncertainty.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Medium	The assay procedures were clearly described, with only a few missing details. Skin samples (presumably n = 4-5/group) were mounted on static diffusion cells yielding a surface area of 0.95 cm2. It was not specified whether the chambers were open or closed, but the test substance has low volatility. The donor and receiver chambers were held at 32 degrees C; humidity was not reported. Prior to exposure, the skin was hydrated with PBS. At exposure time, the receptor chamber was filled with 2.5 mL of fresh PBS + 10% DMSO, and the donor chamber was filled with 2.5 mL of PBS + 10% DMSO containing 0.256 mM DEHP. All of the solutions were stirred with a magnetic stir bar. In some cases, 0.53mM DFP (an esterase inhibitor) was added to the receptor fluid for metabolism tests. Receptor fluid samples were taken periodically and replenished with an equal volume of the receptor fluid. The collected fractions were held at 4 degrees C until analysis. At the end of exposure (24 hours), the skin was washed 3 times with receptor fluid and then homogenized for analysis via HPLC. The lower limits of quantitation were reported.
	Metric 6:	Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use. Percent recovery was not determined; however, recovery determination is not relevant for infinite dose applications determining a Kp. The results for measurements of DEHP in the receptor fluid and in skin samples were not quantitatively reported, and it is unclear whether coefficients of variation were assessed.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details of the test substance preparation were provided. The test substance (0.256 mM) was diluted in PBS +10% DMSO. No details on stability, or mixing to assure homogeneity, mixing temperature, stirring methods, or storage conditions were reported. The solubility of the test substance in the receptor fluid was not specified. DEHP is lipophilic and it is assumed DMSO was added to increase solubility.

Continued on next page ...

...continued from previous page

Study Citation: Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 3859042 Unique ID: DEHP and its EHP metabolite -Full-thickness (human)				
Domain		Metric	Rating	Comments
	Metric 8:	Consistency of exposure administration	Low	Details of exposure administration were insufficiently reported. The study included a single exposure group. The number of replicates was not explicitly reported. Full-thickness human skin samples had a thickness of 500 and 550 um. Tests were conducted using a consistent test solution volume of 2.5 mL, and a skin surface area of 0.95 cm ² . The missing information could have a significant impact on the results.
	Metric 9:	Reporting of concentrations	Medium	A nominal 0.256 mM concentration was added to the donor chamber. The volume applied and skin surface area was reported. It is unclear whether the test concentration was analytically verified. The selected concentration was not justified by the study authors.
	Metric 10:	Exposure frequency	Low	The duration of exposure was dependent on when permeation reached a steady state. The samples were exposed for 48 hours before termination; no penetration was observed.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study used a single exposure concentration; however, the purpose of the study was to compare data from multiple skin types (full-thickness and stripped), chemicals, and species (rat and human), and also to assess metabolic parameters. It is not clear that testing multiple concentrations was in line with the goals of the study, although OECD guidelines recommend at least three exposure groups.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Frozen abdominal skin (presumably 4 pieces total per group) from two Caucasian females aged 51 and 55 yrs old, with thicknesses of 500 and 550 um, respectively were purchased from Biopredic International. The skin was stored at -50 degrees C and thawed just prior to the experiments. No information on skin integrity was provided. According to OECD guidelines, full-thickness skin should not be used to calculate flux, which was the main endpoint in this study, but results were purportedly the same regardless of skin type used (stripped or full-thickness).
	Metric 13:	Number/Replicates per group	Low	The number of replicates per group was not clearly specified in the methods. A sample size of 4 is the minimum sample size required, as specified in OECD 428.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	High	Use of a guideline was not specified. The study was focused on determining Kp and flux, which should be obtained using an infinite dose scenario. A volume of 2.5 mL was applied to the donor chamber (area 0.95 cm ²), so the applied volume was ~ 2.6 mL/cm ² which is appropriate for infinite exposure to a liquid, regardless of concentration. A molar concentration of 0.256mM was reported. Based on a molecular weight of 390.55, and the 2.5 mL volume, approximately 0.249 mg of the test substance was added to the donor chamber with a 0.95 cm ² surface area (equivalent to ~0.263 mg/cm ²).
	Metric 15:	Consistency of outcome assessment	Low	Limited information was provided to determine whether there were inconsistencies across groups. The same vehicle and receptor fluids were used for each replicate, and the exposure duration was 48 hours. The receptor fluid collection times were not specified.
	Metric 16:	Sampling adequacy and sensitivity	Low	Details regarding the sampling of outcomes were not reported.

Continued on next page ...

...continued from previous page

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.		
Chemical:	Diethylhexyl Phthalate		
Exposure Type:	Parent compound		
HERO ID:	3859042		
Unique ID:	DEHP and its EHP metabolite -Full-thickness (human)		
Domain	Metric	Rating	Comments
Domain 6: Confounding/Variable Control			
	Metric 17: Confounding variables in test design and procedures	Medium	The study included only a single group. The skin was excised from two donors with thicknesses of 500 and 550 um, respectively. Integrity/quality was not assessed making it difficult to determine whether there were any confounding differences across replicates. The missing details could have a substantial impact on the study results.
	Metric 18: Confounding variables in outcomes unrelated to exposure	Low	There was insufficient information to determine whether there were confounding variables between replicates that were unrelated to exposure. The study did not indicate whether the test substance was soluble in the receptor fluid (PBS + 10% DMSO) although DMSO is expected to enhance the solubility of lipophilic substances. The study noted significant metabolism occurred and concentrations of two metabolites were assessed. This could cause additional variation across replicates.
Domain 7: Data Presentation and Analysis			
	Metric 19: Data analysis	Uninformative	The data analysis methods were generally reported but since only a qualitative statement on the study results was provided, it is unclear whether any statistical methods were applied to these data, and the data were not provided to conduct an independent analysis.
	Metric 20: Data interpretation	High	Infinite exposure conditions were used to derive Kp and flux. This study did not determine % recovery; however, for infinite dose applications in which Kp or flux are determined, recovery is not relevant (OECD TG 28).
	Metric 21: Reporting of data	Uninformative	A qualitative statement saying "Neither DEHP nor its monoester metabolite, EHP, was transported through the skin when DEHP was applied to the full-thickness skin" were the only result reported.
Overall Quality Determination		Uninformative	

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -Full-thickness (rat)			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was identified as Di (2-ethylhexyl phthalate (DEHP). A CASRN and structure were provided. The test substance was not radiolabeled.
	Metric 2:	Test substance source	High	The test substance was purchased from Wako Pure Chemical Industries, Ltd. Certificates of analysis are available on the supplier website for the current listings of this chemical. The performing laboratory did not analytically verify the test substance.
	Metric 3:	Test substance purity	Medium	The purity was not reported in the study. The product currently listed on the website of the supplier has a purity of >97%. Other chemicals used in the study were of special or HPLC grade. It is assumed the purity is high, but due to the lack of listing in the main study report, there is some uncertainty.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Medium	The assay procedures were clearly described, with only a few missing details. Skin samples (presumably n = 4-5/group) were mounted on static diffusion cells yielding a surface area of 0.95 cm2. It was not specified whether the chambers were open or closed, but the test substance has low volatility. The donor and receiver chambers were held at 32 degrees C; humidity was not reported. Prior to exposure, the skin was hydrated with PBS. At exposure time, the receptor chamber was filled with 2.5 mL of fresh PBS + 10% DMSO, and the donor chamber was filled with 2.5 mL of PBS + 10% DMSO containing 0.256 mM DEHP. All of the solutions were stirred with a magnetic stir bar. In some cases, 0.53mM DFP (an esterase inhibitor) was added to the receptor fluid for metabolism tests. Receptor fluid samples were taken periodically and replenished with an equal volume of the receptor fluid. The collected fractions were held at 4 degrees C until analysis. At the end of exposure (24 hours), the skin was washed 3 times with receptor fluid and then homogenized for analysis via HPLC. The lower limits of quantitation were reported.
	Metric 6:	Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use. Percent recovery was not determined; however, recovery determination is not relevant for infinite dose applications determining a Kp. The results for measurements of DEHP in the receptor fluid and in skin samples were not quantitatively reported, and it is unclear whether coefficients of variation were assessed.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details of the test substance preparation were provided. The test substance (0.256 mM) was diluted in PBS +10% DMSO. No details on stability, or mixing to assure homogeneity, mixing temperature, stirring methods, or storage conditions were reported. The solubility of the test substance in the receptor fluid was not specified. DEHP is lipophilic and it is assumed DMSO was added to increase solubility.

Continued on next page ...

...continued from previous page

Study Citation: Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 3859042 Unique ID: DEHP and its EHP metabolite -Full-thickness (rat)				
Domain		Metric	Rating	Comments
	Metric 8:	Consistency of exposure administration	Low	Details of exposure administration were insufficiently reported. The study included a single exposure group. The number of replicates and skin thicknesses was not reported. Skin Tests were conducted using a consistent test solution volume of 2.5 mL, and a skin surface area of 0.95 cm ² . The missing information could have a significant impact on the results.
	Metric 9:	Reporting of concentrations	Medium	A nominal 0.256 mM concentration was added to the donor chamber. The volume applied and skin surface area was reported. It is unclear whether the test concentration was analytically verified. The selected concentration was not justified by the study authors.
	Metric 10:	Exposure frequency	Low	The duration of exposure was dependent on when permeation reached a steady state. The samples were exposed for 48 hours before termination; no penetration was observed.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study used a single exposure concentration; however, the purpose of the study was to compare data from multiple skin types (full-thickness and stripped), chemicals, and species (rat and human), and also to assess metabolic parameters. It is not clear that testing multiple concentrations was in line with the goals of the study, although OECD guidelines recommend at least three exposure groups.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Abdominal skin was excised from 8-10 week-old male hairless rats (WBN/Ita-Ht) obtained from either the Life Science Research Center in Josai University or Ishikawa Experimental Animals Laboratories (Fukaya, Saitama). It is unclear how many donors were used, and if samples from animals from the different sites were randomly distributed. The skin samples were not stored. Skin integrity was not assessed and thickness was not reported. The samples were used either as full-thickness with the fat trimmed off or as stripped skin. According to OECD guidelines, full-thickness skin should not be used to calculate flux, which was the main endpoint in this study, but results were purportedly the same regardless of skin type used (stripped or full-thickness).
	Metric 13:	Number/Replicates per group	Low	The number of replicates per group was not clearly specified in the methods. A sample size of 4 is the minimum sample size required, as specified in OECD 428.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	High	Use of a guideline was not specified. The study was focused on determining Kp and flux, which should be obtained using an infinite dose scenario. A volume of 2.5 mL was applied to the donor chamber (area 0.95 cm ²), so the applied volume was ~ 2.6 mL/cm ² which is appropriate for infinite exposure to a liquid, regardless of concentration. A molar concentration of 0.256mM was reported. Based on a molecular weight of 390.55, and the 2.5 mL volume, approximately 0.249 mg of the test substance was added to the donor chamber with a 0.95 cm ² surface area (equivalent to ~0.263 mg/cm ²).
	Metric 15:	Consistency of outcome assessment	Low	Limited information was provided to determine whether there were inconsistencies across groups. The same vehicle and receptor fluids were used for each replicate, and the exposure duration was 48 hours. The receptor fluid collection times were not specified.

Continued on next page ...

...continued from previous page

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -Full-thickness (rat)			
Domain	Metric	Rating	Comments	
	Metric 16:	Sampling adequacy and sensitivity	Low	Details regarding the sampling of outcomes were not reported.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	The study included only a single group. The skin was excised from animals from two different facilities, and skin thicknesses were not reported. Integrity/quality was not assessed making it difficult to determine whether there were any confounding differences across replicates. The missing details could have a substantial impact on the study results.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Low	There was insufficient information to determine whether there were confounding variables between replicates that were unrelated to exposure. The study did not indicate whether the test substance was soluble in the receptor fluid (PBS + 10% DMSO) although DMSO is expected to enhance the solubility of lipophilic substances. The study noted significant metabolism occurred and concentrations of two metabolites were assessed. This could cause additional variation across replicates.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Uninformative	The data analysis methods were generally reported but since only a qualitative statement on the study results was provided, it is unclear whether any statistical methods were applied to these data, and the data were not provided to conduct an independent analysis.
	Metric 20:	Data interpretation	High	Infinite exposure conditions were used to derive Kp and flux. This study did not determine % recovery; however, for infinite dose applications in which Kp or flux are determined, recovery is not relevant (OECD TG 28).
	Metric 21:	Reporting of data	Uninformative	Qualitative statements indicating there was no DEHP penetration in any sample, were the only results reported.
Overall Quality Determination			Uninformative	

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -stripped (rat)			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was identified as Di (2-ethylhexyl phthalate (DEHP). A CASRN and structure were provided. The test substance was not radiolabeled.
	Metric 2:	Test substance source	High	The test substance was purchased from Wako Pure Chemical Industries, Ltd. Certificates of analysis are available on the supplier website for the current listings of this chemical. The performing laboratory did not analytically verify the test substance.
	Metric 3:	Test substance purity	Medium	The purity was not reported in the study. The product currently listed on the website of the supplier has a purity of >97%. Other chemicals used in the study were of special or HPLC grade. It is assumed the purity is high, but due to the lack of listing in the main study report, there is some uncertainty.
Domain 2: Test Design				
	Metric 4:	Reference compounds	Low	No reference compound was used and no history of test performance in the laboratory was reported.
	Metric 5:	Assay procedures	Medium	The assay procedures were clearly described, with only a few missing details. Skin samples (presumably n = 4-5/group) were mounted on static diffusion cells yielding a surface area of 0.95 cm2. It was not specified whether the chambers were open or closed, but the test substance has low volatility. The donor and receiver chambers were held at 32 degrees C; humidity was not reported. Prior to exposure, the skin was hydrated with PBS. At exposure time, the receptor chamber was filled with 2.5 mL of fresh PBS + 10% DMSO, and the donor chamber was filled with 2.5 mL of PBS + 10% DMSO containing 0.256 mM DEHP. All of the solutions were stirred with a magnetic stir bar. In some cases, 0.53mM DFP (an esterase inhibitor) was added to the receptor fluid for metabolism tests. Receptor fluid samples were taken periodically and replenished with an equal volume of the receptor fluid. The collected fractions were held at 4 degrees C until analysis. At the end of exposure (24 hours), the skin was washed 3 times with receptor fluid and then homogenized for analysis via HPLC. The lower limits of quantitation were reported.
	Metric 6:	Standards for tests	Uninformative	This study did not conduct the typical standard tests to determine the validity, reliability, or quality of the experiments. Skin integrity was not tested prior to use. Percent recovery was not determined; however, recovery determination is not relevant for infinite dose applications determining a Kp. The results for measurements of DEHP in the receptor fluid and in skin samples were not quantitatively reported, and it is unclear whether coefficients of variation were assessed.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and storage of test substance (chemical)	Low	Limited details of the test substance preparation were provided. The test substance (0.256 mM) was diluted in PBS +10% DMSO. No details on stability, or mixing to assure homogeneity, mixing temperature, stirring methods, or storage conditions were reported. The solubility of the test substance in the receptor fluid was not specified. DEHP is lipophilic and it is assumed DMSO was added to increase solubility.

Continued on next page ...

...continued from previous page

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -stripped (rat)			
Domain	Metric	Rating	Comments	
	Metric 8:	Consistency of exposure administration	Low	Details of exposure administration were insufficiently reported. The study included a single exposure group. The number of replicates and skin thicknesses was not reported. Skin Tests were conducted using a consistent test solution volume of 2.5 mL, and a skin surface area of 0.95 cm2. The missing information could have a significant impact on the results.
	Metric 9:	Reporting of concentrations	Medium	A nominal 0.256 mM concentration was added to the donor chamber. The volume applied and skin surface area was reported. It is unclear whether the test concentration was analytically verified. The selected concentration was not justified by the study authors.
	Metric 10:	Exposure frequency	Low	The duration of exposure was dependent on when permeation reached a steady state. The samples were exposed for 48 hours before termination; no penetration was observed.
	Metric 11:	Number of exposure groups and concentration spacing	Low	The study used a single exposure concentration; however, the purpose of the study was to compare data from multiple skin types (full-thickness and stripped), chemicals, and species (rat and human), and also to assess metabolic parameters. It is not clear that testing multiple concentrations was in line with the goals of the study, although OECD guidelines recommend at least three exposure groups.
Domain 4: Test Model				
	Metric 12:	Test model (skin)	Low	Abdominal skin was excised from 8-10 week-old male hairless rats (WBN/Ita-Ht) obtained from either the Life Science Research Center in Josai University or Ishikawa Experimental Animals Laboratories (Fukaya, Saitama). It is unclear how many donors were used, and if samples from animals from the different sites were randomly distributed. The skin samples were not stored. Skin integrity was not assessed and thickness was not reported. The samples were used either as full-thickness with the fat trimmed off or as stripped skin. According to OECD guidelines, full-thickness skin should not be used to calculate flux, which was the main endpoint in this study, but results were purportedly the same regardless of skin type used (stripped or full-thickness).
	Metric 13:	Number/Replicates per group	Low	The number of replicates per group was not clearly specified in the methods. A sample size of 4 is the minimum sample size required, as specified in OECD 428.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	High	Use of a guideline was not specified. The study was focused on determining Kp and flux, which should be obtained using an infinite dose scenario. A volume of 2.5 mL was applied to the donor chamber (area 0.95 cm2), so the applied volume was ~ 2.6 mL/cm2 which is appropriate for infinite exposure to a liquid, regardless of concentration. A molar concentration of 0.256mM was reported. Based on a molecular weight of 390.55, and the 2.5 mL volume, approximately 0.249 mg of the test substance was added to the donor chamber with a 0.95 cm2 surface area (equivalent to ~0.263 mg/cm2).
	Metric 15:	Consistency of outcome assessment	Low	Limited information was provided to determine whether there were inconsistencies across groups. The same vehicle and receptor fluids were used for each replicate, and the exposure duration was 48 hours. The receptor fluid collection times were not specified.

Continued on next page ...

...continued from previous page

Study Citation:	Sugino, M., Hatanaka, T., Todo, H., Mashimo, Y., Suzuki, T., Kobayashi, M., Hosoya, O., Jinno, H., Juni, K., Sugibayashi, K. (2017). Safety evaluation of dermal exposure to phthalates: Metabolism-dependent percutaneous absorption. Toxicology and Applied Pharmacology 328(Elsevier):10-17.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	3859042			
Unique ID:	DEHP and its EHP metabolite -stripped (rat)			
Domain	Metric	Rating	Comments	
	Metric 16:	Sampling adequacy and sensitivity	Low	Details regarding the sampling of outcomes were not reported.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	The study included only a single group. The skin was excised from animals from two different facilities, and skin thicknesses were not reported. Integrity/quality was not assessed making it difficult to determine whether there were any confounding differences across replicates. The missing details could have a substantial impact on the study results.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Low	There was insufficient information to determine whether there were confounding variables between replicates that were unrelated to exposure. The study did not indicate whether the test substance was soluble in the receptor fluid (PBS + 10% DMSO) although DMSO is expected to enhance the solubility of lipophilic substances. The study noted significant metabolism occurred and concentrations of two metabolites were assessed. This could cause additional variation across replicates.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Uninformative	The data analysis methods were generally reported but since only a qualitative statement on the study results was provided, it is unclear whether any statistical methods were applied to these data, and the data were not provided to conduct an independent analysis.
	Metric 20:	Data interpretation	High	Infinite exposure conditions were used to derive Kp and flux. This study did not determine % recovery; however, for infinite dose applications in which Kp or flux are determined, recovery is not relevant (OECD TG 28).
	Metric 21:	Reporting of data	Uninformative	Qualitative statements indicating there was no DEHP penetration in any sample, were the only results reported.
Overall Quality Determination			Uninformative	

Study Citation:	Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335670			
Unique ID:	24-hour exposure. Immediate sacrifice			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	Medium	The test chemical was identified as a PVC film plasticized with [14C-carbonyl] DEHP. The DEHP concentration was 40.37% w/w or 26.9 mg/sq cm of the film. However, other components or contaminants in the PVC film were not specified.	
Metric 2:	Test substance source	High	PVC film plasticized with [14C-carbonyl] DEHP was obtained from A.D. Little, Inc., Cambridge, MA. The content of DEHP in the film was analytically verified by the performing laboratory.	
Metric 3:	Test substance purity	High	The DEHP chemical purity was reported as 98.9% and 14C-DEHP radiochemical purity was 99.5%.	
Domain 2: Test Design				
Metric 4:	Randomized allocation of animals	Medium	Four animals were randomly selected from a pool of 6 animals for the study. The method of randomization was not specified.	
Metric 5:	Standards for Tests	Medium	This is a guideline study. The study reports the percent total recovery as 103.5%. The authors did not report variance or coefficients of variation; however, individual animal data were available and these values could be independently determined. There was a significant amount of variability across individuals. The coefficient of variation for the absorption rate and other measurements (e.g. % recovered in total wash and rinse, skin, feces, urine were >25%). The CV for total recovery was acceptable ~1%). The appropriateness of the CV calculations is addressed in Metric 19. The study conducted separate tests to determine the efficiency of different wash methods.	
Domain 3: Exposure Characterization				
Metric 6:	Preparation and storage of test substance (chemical)	High	The test substance was used as a plasticizer of a PVC film that was placed on the rat’s skin. No preparation was needed. Storage of the plastic film was not reported; however, pieces of the film were tested for radioactivity level prior to use.	
Metric 7:	Consistency of exposure administration	High	The test substance was delivered consistently across study animals. A 15 cm2 area of film that was 20 mL thick was placed on the clipped dorsal surface of the animal and covered with aluminum foil and bandage. After 24 hours of exposure, the skin was washed and rinsed three times with a 40% aqueous solution of pHidoDerm (R) at 45oC.	
Metric 8:	Reporting of concentrations	Low	The dose applied via the film was approximately 400 mg/15 cm2 or 26.9 mg/cm2 (nominal). The nominal DEHP dose level was reported to be 2,000 mg/kg. Specific radioactivity levels were measured, but no mean analytical measurements were provided. However, no experiments were conducted to indicate the actual bioavailability of DEHP. Data indicating ~103% recovery from the film after removal suggests low transfer of DEHP from the film. There was considerable variation in the ranges of absorbed radioactivity. The authors considered this to be an indicator of variability in the amount of DEHP transferred from the film. There is significant ambiguity regarding the actual bioavailable dose of DEHP.	
Continued on next page ...				

...continued from previous page

Study Citation:	Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335670			
Unique ID:	24-hour exposure. Immediate sacrifice			
Domain	Metric	Rating	Comments	
	Metric 9:	Exposure duration	High	The 24-hr duration of exposure was appropriate and consistent with OECD 427 guidelines.
	Metric 10:	Number of exposure groups and concentration spacing	Medium	Only one dose was studied; however, the purpose of the study was to learn about the possible transfer of DEHP from the film to the skin and the concentration of DEHP in the film used was higher than typically used in commercial formulations.
Domain 4: Test Model				
	Metric 11:	Test animal characteristics	Low	Male Fisher-344 rats weighing between 195 and 210 grams were used. Age and source of the animals were not reported.
	Metric 12:	Adequacy and consistency of animal husbandry conditions	Low	Husbandry conditions were not reported.
	Metric 13:	Number of animals per group	Medium	Four animals were studied which is in agreement with OECD guidelines.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Low	The outcomes assessment method mostly agreed with OECD 427 guidelines except that blood was not collected and analyzed. Carcass, urine, feces and skin were collected and analyzed. Skin was washed before analysis of the application site. Given that there was 103% recovery of the test material in the removed film, it is not expected that levels in the blood would be significant. The authors exposed a 15 cm ² surface area (4% of total animal) in an attempt to maximize the area of contact. The quantity of DEHP in the film (26.7 mg/cm ²) is suggestive of infinite dosing; however, since it is unclear how much of the material migrated from the film and was bioavailable, this may reflect more of a finite dosing scenario. Therefore, it is unclear if the applied dose was non-depletable.
	Metric 15:	Consistency of outcome assessment	High	Details of the outcome assessment protocols were adequately described and were consistently assessed across groups.
	Metric 16:	Sampling adequacy and sensitivity	Medium	Measurement sensitivity (signal:noise ratio) was not reported. The number of evaluations per exposure group was sufficient.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Although the study did not report all information to determine whether confounding bias may exist, reported information did not identify differences (or identified only minor differences) among animals. Body weights were within 10%. Exposure area was consistent across animals.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment. Skin irritation was not reported.
Domain 7: Data Presentation and Analysis				
Continued on next page ...				

...continued from previous page

Study Citation:		Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.		
Chemical:		Diethylhexyl Phthalate		
Exposure Type:		Parent compound		
HERO ID:		1335670		
Unique ID:		24-hour exposure. Immediate sacrifice		
Domain	Metric		Rating	Comments
	Metric 19:	Data analysis	Low	The coefficient of variation was >25% for multiple measurements including CVs of 50.7% (recovery in skin), 32% (urine), 79% (feces), 61-66% (cage washes), 43% (carcass), and the CV for the absorption rate was 39%. Sufficient information was provided to allow the EPA to calculate an alternate (upper end) value to account for variability in the results. The CV for total recovery was 1.2%, which was acceptable.
	Metric 20:	Data interpretation	Medium	Recovery was adequate +/- 10% of 100%, with most of the test material remaining in the film. The authors appropriately recognized and addressed the limitations of the study, and therefore, cautioned that the absorption rates determined were only estimates. These rates were calculated only based on the quantities absorbed, the exposure area, and duration, due to uncertainties in the actual doses available to the animals. Independent analysis cannot address the uncertainties.
	Metric 21:	Reporting of Data	High	Individual animal data were reported for all specified outcomes.
Overall Quality Determination			Medium	

Study Citation:	Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335670			
Unique ID:	24-hour exposure. Sacrifice at 168 hr			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	Medium	The test chemical was identified PVC film plasticized with [14C-carbonyl] DEHP. The DEHP concentration was 40.37% w/w or 26.9 mg/sq cm of the film; however, other components or contaminants in the PVC film were not specified.	
Metric 2:	Test substance source	High	PVC film plasticized with [14C-carbonyl]DEHP was obtained from A.D. Little, Inc., Cambridge, MA. The content of DEHP in the film was analytically verified by the performing laboratory.	
Metric 3:	Test substance purity	High	DEHP chemical purity was reported as 98.9% and 14C-DEHP radiochemical purity was 99.5%.	
Domain 2: Test Design				
Metric 4:	Randomized allocation of animals	Medium	Four animals were randomly selected from a pool of 7 animals for the study. The method of randomization was not specified.	
Metric 5:	Standards for Tests	Medium	The study reports percent recovery as 103.13% . There was a significant amount of variability across individuals. The authors did not report variance or coefficients of variation; however, individual animal data were available and these values could be independently determined. There was a significant amount of variability across individuals. The coefficient of variation for the absorption rate, and the % recovered in skin, feces, and carcass, and the acetone cage wash were >25%. The CV for recovery in urine and the water cage wash, and total recovery were <25%. The appropriateness of the CV calculations is addressed in Metric 19.	
Domain 3: Exposure Characterization				
Metric 6:	Preparation and storage of test substance (chemical)	High	The test substance was on a PVC plastic film that was placed on the rat’s skin. No preparation was needed. Storage of the plastic film was not reported; however, pieces of the film were tested for radioactivity level prior to use.	
Metric 7:	Consistency of exposure administration	High	The test substance was delivered consistently across study animals. A 15 cm2 area of film that was 20 mL thick was placed on the clipped dorsal surface of the animal and covered with aluminum foil and bandage. After 24 hours, the film was removed and a fresh (untreated) wrap was applied. The wraps were removed at 168 hours.	
Metric 8:	Reporting of concentrations	Uninformative	The dose applied via the film was approximately 400 mg/15 cm2 or 26.9 mg/cm2 (nominal). The nominal DEHP dose level was 2,000 mg/kg. Specific radioactivity levels were measured, but no mean analytical measurements were provided. No experiments were conducted to indicate the actual bioavailability of DEHP. Data indicating ~103% recovery from the film after removal suggests low transfer of DEHP from the film. There was considerable variation in the ranges of absorbed radioactivity. The authors considered this to be an indicator of variability in the amount of DEHP transferred from the film. This metric is scored critically deficient due to uncertainties in the actual bioavailable dose of DEHP.	
Metric 9:	Exposure duration	Low	The film was placed on the skin from 24 hour and then removed and replaced with a bandage. The study did not wash the application site therefore residual test chemical remained on the for 7 days.	

Continued on next page ...

...continued from previous page

Study Citation:	Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335670			
Unique ID:	24-hour exposure. Sacrifice at 168 hr			
Domain	Metric	Rating	Comments	
	Metric 10:	Number of exposure groups and concentration spacing	Medium	Only one dose was used; however, the purpose of the study was to learn about the possible transfer of DEHP from the film to the skin and the concentration of DEHP in the film used was higher than typically used
Domain 4: Test Model				
	Metric 11:	Test animal characteristics	Low	Male Fisher-344 rats weighing between 195 and 210 grams were used. Age and source of the animals were not reported.
	Metric 12:	Adequacy and consistency of animal husbandry conditions	Low	Husbandry conditions were not reported.
	Metric 13:	Number of animals per group	Medium	Four animals were studied which is in agreement with OECD guidelines.
Domain 5: Outcome Assessment				
	Metric 14:	Outcome assessment methodology	Uninformative	The outcomes assessment method was not consistent with OECD 427 guidelines. The skin was not washed after the film was removed or before the analysis of skin. It therefore cannot be determined what amount of test chemical was absorbed into the skin and what amount remained on the surface. Blood was not collected and analyzed. The authors exposed a 15 cm2 surface area (4% of total animal) in an attempt to maximize the area of contact. The quantity of DEHP in the film (26.7 mg/cm2) is suggestive of infinite dosing; however, since it is unclear how much of the material migrated from the film and was bioavailable (this was only determined indirectly), and the actual dose may reflect more of a finite dosing scenario. It is unclear if the applied dose was non-depletable, although the authors believed that the receiving site was saturated.
	Metric 15:	Consistency of outcome assessment	High	Details of the outcome assessment protocols were adequately described and were consistently assessed across groups.
	Metric 16:	Sampling adequacy and sensitivity	Medium	Measurement sensitivity (signal:noise ratio) was not reported. The number of evaluations per exposure group was sufficient. However, those limitations are unlikely to have a substantial impact on results.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	Although the study did not report all information to determine whether confounding bias may exist, reported information did not identify differences (or identified only minor differences) among animals Body weights were within 10%. Exposure area was consistent across animals.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment. Skin irritation was not reported.
Domain 7: Data Presentation and Analysis				
Continued on next page ...				

...continued from previous page

Study Citation:	Chemical Manufacturers Association, (1991). Chloride film in male Fischer 344 rats (final report) with attachments and cover sheet dated 042491.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	1335670			
Unique ID:	24-hour exposure. Sacrifice at 168 hr			
Domain	Metric		Rating	Comments
	Metric 19:	Data analysis	Low	Percent absorption estimates were measured across a time series for urine and fecal compartments. The coefficient of variation was >25% for multiple measurements. The CVs were 55.2% (recovery in skin), 37.7% (feces), 93.9% (carcass), 146.4 (acetone cage wash), 43% (carcass), and the CV for the absorption rate was 42.7%. Only the CV for recovery in urine (20.4%), and the water cage wash (10.7) were considered acceptable. Variation in the percent total recovery was low (1.26%) because most of the test material remained in the PVC film. Sufficient information is available to allow the EPA to calculate an alternate (upper end) value to account for variability in the results.
	Metric 20:	Data interpretation	Medium	Recovery was adequate +/- 10% of 100%, with most of the test material remaining in the film. The authors appropriately recognized and addressed the limitations of the study, and therefore, cautioned that the absorption rates determined were only estimates. These rates were calculated only based on the quantities absorbed, the exposure area, and duration, due to uncertainties in the actual doses available to the animals. Independent analysis cannot address the uncertainties.
	Metric 21:	Reporting of Data	High	Data were reported for individual animals and all timepoints where relevant.

Overall Quality Determination**Uninformative**

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	24 hours			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C-radiolabelled di(2-ethylhexyl) phthalate (DEHP). The CASRN, structure and chemical properties were not specified in the report. The location of the radiolabel was not reported.
	Metric 2:	Test substance source	High	The test substance was obtained from Sigma Chemical Co. (St Louis, MO, USA). The batch or lot number was not specified. The chemical identity was not confirmed analytically by the performing laboratory. Certificates of analysis can be obtained on the supplier’s website at the time of purchase.
	Metric 3:	Test substance purity	Medium	The radiochemical purity was reported to be >98%. Impurities were not reported.
Domain 2: Test Design	Metric 4:	Randomized allocation of animals	Low	The study does not report how animals were allocated into study groups. Also, no information indicates that normalization to body weight occurred (body weights were not reported).
	Metric 5:	Standards for Tests	Low	The authors do not report whether the test met any pre-established criteria. The percent of dose recovered was less than the recommended 100 +/- 10% for all time point: 24 hrs (85.2%, CV=3.4%), 48 hrs (71.0%, CV=13%), 7-days (86.4%, CV= 6%), and 14 days (83.4%, CV= 0.7%).
Domain 3: Exposure Characterization	Metric 6:	Preparation and storage of test substance (chemical)	Low	No information is provided on how the test chemical was stored after arrival from the supplier or after it was diluted in acetone. No information is provided on how the test substance was prepared for use, except that was dissolved in acetone. The concentration of the solution, mixing or stirring methods, how far in advance the solution was made, stability and solubility were not reported. The concentration of the test solution was not analytically verified. Since the vehicle was acetone, which is volatile, missing information on how far in advance the solution was made and stored could have a substantial impact on results.
	Metric 7:	Consistency of exposure administration	Medium	Application of test substance was mostly consistent across all animals. Briefly, two dosing sites (4 cm2 each) were marked with a marker. 50ul of radiolabeled test solution was applied to each of the two test sites (vehicle was acetone). After the acetone evaporated (time not reported and may not have been consistent), the application area was covered with a foam pad and gauze and fixed in place with a Vetrap bandage.
	Metric 8:	Reporting of concentrations	Medium	The dose applied to the skin was reported as ug/cm2. The body weight of the animals was not reported, therefore a dose in mg/kg cannot be calculated. The study reported nominal instead of analytical doses. The volume applied (50 ul) and skin surface area (two dosing sites of 4 cm2 dorsal skin, 2 cm apart) were reported.
	Metric 9:	Exposure duration	High	Animals were exposed for 24 hours, which is consistent with OECD 427 guidelines.
Continued on next page ...				

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	24 hours			
Domain	Metric	Rating	Comments	
	Metric 10: Number of exposure groups and concentration spacing	Medium	The study included 4 groups, each exposed to a different dose and sacrificed at a different time point (24 hrs, 48 hrs, 7 days and 14 days post-treatment). Each time point was evaluated independently. The justification for using higher doses for the longer post-treatment time points was to ensure radioactivity could be detected in the skin samples. It is unclear how relevant the doses selected are to human exposure scenarios.	
Domain 4: Test Model	Metric 11: Test animal characteristics	Medium	The study used female Hartley hairless guinea pigs sourced from Charles River, Wilmington, MA. Age and body weights were not reported. The species selection was appropriate for the study's aim.	
	Metric 12: Adequacy and consistency of animal husbandry conditions	Low	After application of the test substance animals were housed in separate glass metabolism cages and provided food and water ad libitum. No other husbandry conditions were reported (temperature, humidity, light cycle).	
	Metric 13: Number of animals per group	Medium	Four animals/timepoint were dosed and data were reported in the table for 4 animals/group. This agrees with OECD guidelines that recommend 4 animals per group.	
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	24 hours			
Domain	Metric	Rating	Comments	
	Metric 14: Outcome assessment methodology	Low	The outcome assessment methodology was sufficient to address the intentions of this non-guideline study but deviated from a traditional dermal absorption study as per OECD 427. The skin was washed with a 1% soap solution followed by water prior to the application of the test substance. OECD 427 guidelines state skin should be “gently wiped down with acetone to remove sebum. An additional soap and water wash is not recommended because any soap residue might promote test substance absorption.” The test substance was applied to two application sites (each 4 cm2) on each animal. The two sites were 2 cm apart from each other. This is a deviation from OECD guidelines which recommend the application area to be at least 10 cm2. 50 uL of test solution was applied to each site. This would result in an application of 12.5 uL/cm2, which is slightly higher than the recommended 10 uL/cm2. The test substance was dissolved in acetone. After application, the study authors allowed the acetone to evaporate off and then covered the application site with a foam pad and gauze. Animals were placed in metabolism cages to collect urine and feces. Each cage was rinsed daily with water, which was combined with the urine collection. The duration of exposure (24 hours) was acceptable. The study does not report if any clinical signs were observed or if skin irritation occurred at the site of application, which is recommended in OECD guidelines. After 24 hours of exposure, the skin was washed 3 times with soap and water. Radioactivity was determined using a TR2500 liquid scintillation counter. The following were analyzed for the amount of radioactivity: urine, feces, backwash, foam pad, carcass (minus the skin), and dosed skin (from one site). One application site was used for radioactivity measurements, the other site used to determine the distribution of the radiolabeled material using autoradiographic analysis. The percent absorbed was determined from a finite dose, which was appropriate. However, only measuring the amount of test substance in the skin of one application site and not the other will impact the percent calculated in the dosed skin and thus percent absorbed and percent recovered. The study did not measure the amount of test substance in the blood, rather the amount in the whole carcass was determined.	
	Metric 15: Consistency of outcome assessment	High	Outcomes were assessed consistently across study groups and replicates.	
	Metric 16: Sampling adequacy and sensitivity	Low	All four dosed animals were included in the analysis. The number of animals was consistent with OECD guidelines. The study authors reported that radioactivity was measured on a TR2500 liquid scintillation counter (Packard Instruments); however, the scintillation counts/sample or duration of radioactivity detection were not reported. The signal-to-noise ratio was not reported. It appears that the scintillation counts were taken immediately after material (urine, feces, foam pads, back washes) was collected, although this is not explicitly stated. The skin and carcass were frozen and analyzed at a later time point.	
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	24 hours			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Animal body weights at the start of exposure or throughout the study were not reported. It is not reported if any weight changes, clinical signs or irritation at the application site were observed. OECD guidelines state the weight variation of animals should not exceed 20% of the mean weight. Since the weight and the ages of the animals were not reported, this cannot be determined. Regarding the use of acetone as a vehicle, the study does not provide any information on the absorption characteristics or potential interaction with the test substance, which OECD guidelines recommend when the vehicle is not water. The authors also noted that volatility of the compounds may have confounded the dose absorbed, which was lower compared with other studies.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	High	Percent absorption estimates were based on the amount of radioactivity in the carcass and dosed skin at the time of sacrifice and the cumulative amount in urine, feces and cage washes (collected daily post-treatment). The CVs (calculated for this review) were 25% for the 48-hour timepoint and 18% for the 14-day timepoint. Sufficient information (mean, SD and sample size) was provided for EPA to calculate an alternate upper-end value to account for variability in the results. No statistical analysis was conducted.	
	Metric 20: Data interpretation	Uninformative	This study was not conducted according to OECD 427. The aim of the study was to see how the distribution of the test chemical changes over time post-treatment and to examine the distribution of the test substance in the skin using autoradiographic analysis. The study applied the test substance at two application sites. One site was collected to determine the amount of test substance that remained in the skin, the other site was used for autoradiographic analysis to determine the distribution of radioactivity. Since the amount of test substance that remained in the skin from the second application site was not taken into consideration for absorption measurements, the percent absorbed calculations are not accurate.	
	Metric 21: Reporting of Data	Medium	Data were reported for all of the outcomes specified in the methods. Urine and feces radioactivity levels were measured independently; however, the study reports them combined as excreta. Data were presented as means ± SD with an n=4. Individual animal data were not provided. CV values were not provided but could be calculated for those endpoints.	
Overall Quality Determination		Uninformative		

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	7 days			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C-radiolabelled di(2-ethylhexyl) phthalate (DEHP). The CASRN, structure and chemical properties were not specified in the report. The location of the radiolabel was not reported.
	Metric 2:	Test substance source	High	The test substance was obtained from Sigma Chemical Co. (St Louis, MO, USA). The batch or lot number was not specified. The chemical identity was not confirmed analytically by the performing laboratory. Certificates of analysis can be obtained on the supplier’s website at the time of purchase.
	Metric 3:	Test substance purity	Medium	The radiochemical purity was reported to be >98%. Impurities were not reported.
Domain 2: Test Design	Metric 4:	Randomized allocation of animals	Low	The study does not report how animals were allocated into study groups. Also, no information indicates that normalization to body weight occurred (body weights were not reported).
	Metric 5:	Standards for Tests	Low	The authors do not report whether the test met any pre-established criteria. The percent of dose recovered was less than the recommended 100 +/- 10% for all time point: 24 hrs (85.2%, CV=3.4%), 48 hrs (71.0%, CV=13%), 7-days (86.4%, CV= 6%), and 14 days (83.4%, CV= 0.7%).
Domain 3: Exposure Characterization	Metric 6:	Preparation and storage of test substance (chemical)	Low	No information is provided on how the test chemical was stored after arrival from the supplier or after it was diluted in acetone. No information is provided on how the test substance was prepared for use, except that was dissolved in acetone. The concentration of the solution, mixing or stirring methods, how far in advance the solution was made, stability and solubility were not reported. The concentration of the test solution was not analytically verified. Since the vehicle was acetone, which is volatile, missing information on how far in advance the solution was made and stored could have a substantial impact on results.
	Metric 7:	Consistency of exposure administration	Medium	Application of test substance was mostly consistent across all animals. Briefly, two dosing sites (4 cm2 each) were marked with a marker. 50ul of radiolabeled test solution was applied to each of the two test sites (vehicle was acetone). After the acetone evaporated (time not reported and may not have been consistent), the application area was covered with a foam pad and gauze and fixed in place with a Vetrap bandage.
	Metric 8:	Reporting of concentrations	Medium	The dose applied to the skin was reported as ug/cm2. The body weight of the animals was not reported, therefore a dose in mg/kg cannot be calculated. The study reported nominal instead of analytical doses. The volume applied (50 ul) and skin surface area (two dosing sites of 4 cm2 dorsal skin, 2 cm apart) were reported.
	Metric 9:	Exposure duration	High	Animals were exposed for 24 hours, which is consistent with OECD 427 guidelines.

Continued on next page ...

...continued from previous page

Study Citation: Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 673605 Unique ID: 7 days				
Domain		Metric	Rating	Comments
	Metric 10:	Number of exposure groups and concentration spacing	Medium	The study included 4 groups, each exposed to a different dose and sacrificed at a different time point (24 hrs, 48 hrs, 7 days and 14 days post-treatment). Each time point was evaluated independently. The justification for using higher doses for the longer post-treatment time points was to ensure radioactivity could be detected in the skin samples. It is unclear how relevant the doses selected are to human exposure scenarios.
Domain 4: Test Model				
	Metric 11:	Test animal characteristics	Medium	The study used female Hartley hairless guinea pigs sourced from Charles River, Wilmington, MA. Age and body weights were not reported. The species selection was appropriate for the study's aim.
	Metric 12:	Adequacy and consistency of animal husbandry conditions	Low	After application of the test substance animals were housed in separate glass metabolism cages and provided food and water ad libitum. No other husbandry conditions were reported (temperature, humidity, light cycle).
	Metric 13:	Number of animals per group	Medium	Four animals/timepoint were dosed and data were reported in the table for 4 animals/group. This agrees with OECD guidelines that recommend 4 animals per group.
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation: Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 673605 Unique ID: 7 days				
Domain	Metric	Rating	Comments	
	Metric 14: Outcome assessment methodology	Low	<p>The outcome assessment methodology was sufficient to address the intentions of this non-guideline study but deviated from a traditional dermal absorption study as per OECD 427. The skin was washed with a 1% soap solution followed by water prior to the application of the test substance. OECD 427 guidelines state skin should be "gently wiped down with acetone to remove sebum. An additional soap and water wash is not recommended because any soap residue might promote test substance absorption." The test substance was applied to two application sites (each 4 cm²) on each animal. The two sites were 2 cm apart from each other. This is a deviation from OECD guidelines which recommend the application area to be at least 10 cm². 50 uL of test solution was applied to each site. This would result in an application of 12.5 uL/cm², which is slightly higher than the recommended 10 uL/cm². The test substance was dissolved in acetone. After application, the study authors allowed the acetone to evaporate off and then covered the application site with a foam pad and gauze. Animals were placed in metabolism cages to collect urine and feces. Each cage was rinsed daily with water, which was combined with the urine collection. The duration of exposure (24 hours) was acceptable. The study does not report if any clinical signs were observed or if skin irritation occurred at the site of application, which is recommended in OECD guidelines. After 24 hours of exposure, the skin was washed 3 times with soap and water. Radioactivity was determined using a TR2500 liquid scintillation counter. The following were analyzed for the amount of radioactivity: urine, feces, backwash, foam pad, carcass (minus the skin), and dosed skin (from one site). One application site was used for radioactivity measurements, the other site used to determine the distribution of the radiolabeled material using autoradiographic analysis. The percent absorbed was determined from a finite dose, which was appropriate. However, only measuring the amount of test substance in the skin of one application site and not the other will impact the percent calculated in the dosed skin and thus percent absorbed and percent recovered. The study did not measure the amount of test substance in the blood, rather the amount in the whole carcass was determined.</p>	
	Metric 15: Consistency of outcome assessment	High	Outcomes were assessed consistently across study groups and replicates.	
	Metric 16: Sampling adequacy and sensitivity	Low	<p>All four dosed animals were included in the analysis. The number of animals was consistent with OECD guidelines. The study authors reported that radioactivity was measured on a TR2500 liquid scintillation counter (Packard Instruments); however, the scintillation counts/sample or duration of radioactivity detection were not reported. The signal-to-noise ratio was not reported. It appears that the scintillation counts were taken immediately after material (urine, feces, foam pads, back washes) was collected, although this is not explicitly stated. The skin and carcass were frozen and analyzed at a later time point.</p>	

Domain 6: Confounding/Variable Control

Continued on next page ...

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	7 days			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Animal body weights at the start of exposure or throughout the study were not reported. It is not reported if any weight changes, clinical signs or irritation at the application site were observed. OECD guidelines state the weight variation of animals should not exceed 20% of the mean weight. Since the weight and the ages of the animals were not reported, this cannot be determined. Regarding the use of acetone as a vehicle, the study does not provide any information on the absorption characteristics or potential interaction with the test substance, which OECD guidelines recommend when the vehicle is not water. The authors also noted that volatility of the compounds may have confounded the dose absorbed, which was lower compared with other studies.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	High	Percent absorption estimates were based on the amount of radioactivity in the carcass and dosed skin at the time of sacrifice and the cumulative amount in urine, feces and cage washes (collected daily post-treatment). The CVs (calculated for this review) were 25% for the 48-hour timepoint and 18% for the 14-day timepoint. Sufficient information (mean, SD and sample size) was provided for EPA to calculate an alternate upper-end value to account for variability in the results. No statistical analysis was conducted.	
	Metric 20: Data interpretation	Uninformative	This study was not conducted according to OECD 427. The aim of the study was to see how the distribution of the test chemical changes over time post-treatment and to examine the distribution of the test substance in the skin using autoradiographic analysis. The study applied the test substance at two application sites. One site was collected to determine the amount of test substance that remained in the skin, the other site was used for autoradiographic analysis to determine the distribution of radioactivity. Since the amount of test substance that remained in the skin from the second application site was not taken into consideration for absorption measurements, the percent absorbed calculations are not accurate.	
	Metric 21: Reporting of Data	Medium	Data were reported for all of the outcomes specified in the methods. Urine and feces radioactivity levels were measured independently; however, the study reports them combined as excreta. Data were presented as means ± SD with an n=4. Individual animal data were not provided. CV values were not provided but could be calculated for those endpoints.	
Overall Quality Determination		Uninformative		

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	48 hours			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C-radiolabelled di(2-ethylhexyl) phthalate (DEHP). The CASRN, structure and chemical properties were not specified in the report. The location of the radiolabel was not reported.
	Metric 2:	Test substance source	High	The test substance was obtained from Sigma Chemical Co. (St Louis, MO, USA). The batch or lot number were not specified. The chemical identity was not confirmed analytically by performing laboratory. Certificates of analysis can be obtained on the supplier website at the time of purchase.
	Metric 3:	Test substance purity	Medium	The radiochemical purity was reported to be >98%. Impurities were not reported.
Domain 2: Test Design	Metric 4:	Randomized allocation of animals	Low	The study does not report how animals were allocated into study groups. Also, no information indicates that normalization to body weight occurred (body weights were not reported).
	Metric 5:	Standards for Tests	Low	The authors do not report whether the test met any pre-established criteria. The percentage of the dose recovered was less than the recommended 100 +/- 10% for all time points: 24 hrs (85.2%, CV=3.4%), 48 hrs (71.0%, CV=13%), 7-days (86.4%, CV= 6%), and 14 days (83.4%, CV= 0.7%).
Domain 3: Exposure Characterization	Metric 6:	Preparation and storage of test substance (chemical)	Low	No information is provided on how the test chemical was stored after arrival from the supplier or after it was diluted in acetone. No information is provided on how the test substance was prepared for use, except that was dissolved in acetone. The concentration of the solution, mixing or stirring methods, how far in advance the solution was made, stability and solubility were not reported. The concentration of the test solution was not analytically verified. Since the vehicle was acetone, which is volatile, missing information on how far in advance the solution was made and stored could have a substantial impact on results.
	Metric 7:	Consistency of exposure administration	Medium	Application of test substance was mostly consistent across all animals. Briefly, two dosing sites (4 cm2 each) were marked with a marker. 50ul of radiolabeled test solution was applied to each of the two test sites (vehicle was acetone). After the acetone evaporated (time not reported and may not have been consistent), the application area was covered with a foam pad and gauze and fixed in place with a Vetrap bandage.
	Metric 8:	Reporting of concentrations	Medium	The dose applied to the skin was reported as ug/cm2. The body weight of the animals was not reported, therefore a dose in mg/kg cannot be calculated. The study reported nominal instead of analytical doses. The volume applied (50 ul) and skin surface area (two dosing sites of 4 cm2 dorsal skin, 2 cm apart) were reported.
	Metric 9:	Exposure duration	High	Animals were exposed for 24 hours, which is consistent with OECD 427 guidelines.

Continued on next page ...

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	48 hours			
Domain	Metric	Rating	Comments	
	Metric 10: Number of exposure groups and concentration spacing	Medium	The study included 4 groups, each exposed to a different dose and sacrificed at a different time point (24 hrs, 48 hrs, 7 days and 14 days post-treatment). Each time point was evaluated independently. The justification for using higher doses for the longer post-treatment time points was to ensure radioactivity could be detected in the skin samples. It is unclear how relevant the doses selected are to human exposure scenarios.	
Domain 4: Test Model	Metric 11: Test animal characteristics	Medium	The study used female Hartley hairless guinea pigs sourced from Charles River, Wilmington, MA. Age and animal body weights were not reported. The species selection was appropriate for the study's aim.	
	Metric 12: Adequacy and consistency of animal husbandry conditions	Low	After application of the test substance animals were housed in separate glass metabolism cages and provided food and water ad libitum. No other husbandry conditions were reported (temperature, humidity, light cycle).	
	Metric 13: Number of animals per group	Medium	Four animals/timepoint were dosed and data were reported in the table for 4 animals/group. This agrees with OECD guidelines that require 4 animals per group.	
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:		Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.		
Chemical:		Diethylhexyl Phthalate		
Exposure Type:		Parent compound		
HERO ID:		673605		
Unique ID:		48 hours		
Domain	Metric	Rating	Comments	
	Metric 14: Outcome assessment methodology	Low	The outcome assessment methodology was sufficient to address the intentions of this non-guideline study but deviated from a traditional dermal absorption study as per OECD 427. The skin was washed with a 1% soap solution followed by water prior to application of test substance. OECD 427 guidelines state skin should be "gently wiped down with acetone to remove sebum. An additional soap and water wash is not recommended because any soap residue might promote test substance absorption." The test substance was applied to two application sites (each 4 cm ²) on each animal. The two sites were 2 cm apart from each other. This is a deviation from OECD guidelines which recommend the application area to be at least 10 cm ² . 50 uL of test solution was applied to each site. This would result in an application of 12.5 uL/cm ² , which is slightly higher than the recommended 10 uL/cm ² . The test substance was dissolved in acetone. After application, the study authors allowed the acetone to evaporate off and then covered the application site with a foam pad and gauze. Animals were placed in metabolism cages to collect urine and feces. Each cage was rinsed daily with water, which was combined with the urine collection. The duration of exposure (24 hours) was acceptable. The study does not report if any clinical signs were observed or if skin irritation occurred at the site of application, which is recommended in OECD guidelines. After 24 hours of exposure, the skin was washed 3 times with soap and water. Radioactivity was determined using a TR2500 liquid scintillation counter. The following were analyzed for amount of radioactivity: urine, feces, backwash, foam pad, carcass (minus the skin), and dosed skin (from one site). One application site was used for radioactivity measurements, and the other site was used to determine the distribution of the radiolabeled material using autoradiographic analysis. The percent absorbed was determined from a finite dose, which was appropriate. However, only measuring the amount of test substance in the skin of one application site and not the other will impact the percent calculated in the dosed skin and thus percent absorbed and percent recovered. The study did not measure the amount of test substance in the blood, rather the amount in the whole carcass was determined.	
	Metric 15: Consistency of outcome assessment	High	Outcomes were assessed consistently across study groups.	
	Metric 16: Sampling adequacy and sensitivity	Low	All four dosed animals were included in the analysis. The number of animals was consistent with OECD guidelines. The study authors reported that radioactivity was measured on a TR2500 liquid scintillation counter (Packard Instruments), however, the scintillation counts/sample or duration of radioactivity detection were not reported. The signal-to-noise ratio was not reported. It appears that the scintillation counts were taken immediately after material (urine, feces, foam pads, back washes) was collected, although this is not explicitly stated. The skin and carcass were frozen and analyzed at a later time point.	

Domain 6: Confounding/Variable Control

Continued on next page ...

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	48 hours			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Animal body weights at the start of exposure or throughout the study were not reported. It is not reported if any weight changes, clinical signs or irritation at the application site were observed. OECD guidelines state the weight variation of animals should not exceed 20% of the mean weight. Since the weight and the ages of the animals were not reported, this cannot be determined. Regarding the use of acetone as a vehicle, the study does not provide any information on the absorption characteristics or potential interaction with the test substance, which OECD guidelines recommend when the vehicle is not water. The authors also noted that the volatility of the compounds may have confounded the dose absorbed, which was lower compared with other studies.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	Percent absorption estimates were based on the amount of radioactivity in the carcass and dosed skin at the time of sacrifice and the cumulative amount in urine, feces and cage washes (collected daily post-treatment). The CVs (calculated for this review) were 28% for the 24-hour timepoint and 28% for the 7-day timepoint. Sufficient information (mean, SD and sample size) was provided for EPA to calculate an alternate upper-end value to account for variability in the results. No statistical analysis was conducted.	
	Metric 20: Data interpretation	Uninformative	This study was not conducted according to OECD 427. The aim of the study was to see how the distribution of the test chemical changes over time post-treatment and to examine the distribution of the test substance in the skin using autoradiographic analysis. The study applied the test substance at two application sites. One site was collected to determine the amount of test substance that remained in the skin, the other site was used for autoradiographic analysis to determine the distribution of radioactivity. Since the amount of test substance that remained in the skin from the second application site was not taken into consideration for absorption measurements, the percent absorbed calculations are not accurate.	
	Metric 21: Reporting of Data	Medium	Data were reported for all of the outcomes specified in the methods. Urine and feces radioactivity was measured independently; however, the study reports them combined as excreta. Data were presented as means ± SD with an n=4. Individual animal data were not provided. CV values were not provided but could be for those endpoints.	
Overall Quality Determination		Uninformative		

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	14 days			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1:	Test substance identity	Medium	The test substance was identified as 14C-radiolabelled di(2-ethylhexyl) phthalate (DEHP). The CASRN, structure and chemical properties were not specified in the report. The location of the radiolabel was not reported.
	Metric 2:	Test substance source	High	The test substance was obtained from Sigma Chemical Co. (St Louis, MO, USA). The batch or lot number were not specified. The chemical identity was not confirmed analytically by performing laboratory. Certificates of analysis can be obtained on the supplier website at the time of purchase.
	Metric 3:	Test substance purity	Medium	The radiochemical purity was reported to be >98%. Impurities were not reported.
Domain 2: Test Design	Metric 4:	Randomized allocation of animals	Low	The study does not report how animals were allocated into study groups. Also, no information indicates that normalization to body weight occurred (body weights were not reported).
	Metric 5:	Standards for Tests	Low	The authors do not report whether the test met any pre-established criteria. The percentage of the dose recovered was less than the recommended 100 +/- 10% for all time points: 24 hrs (85.2%, CV=3.4%), 48 hrs (71.0%, CV=13%), 7-days (86.4%, CV= 6%), and 14 days (83.4%, CV= 0.7%).
Domain 3: Exposure Characterization	Metric 6:	Preparation and storage of test substance (chemical)	Low	No information is provided on how the test chemical was stored after arrival from the supplier or after it was diluted in acetone. No information is provided on how the test substance was prepared for use, except that was dissolved in acetone. The concentration of the solution, mixing or stirring methods, how far in advance the solution was made, stability and solubility were not reported. The concentration of the test solution was not analytically verified. Since the vehicle was acetone, which is volatile, missing information on how far in advance the solution was made and stored could have a substantial impact on results.
	Metric 7:	Consistency of exposure administration	Medium	Application of test substance was mostly consistent across all animals. Briefly, two dosing sites (4 cm2 each) were marked with a marker. 50ul of radiolabeled test solution was applied to each of the two test sites (vehicle was acetone). After the acetone evaporated (time not reported and may not have been consistent), the application area was covered with a foam pad and gauze and fixed in place with a Vetrap bandage.
	Metric 8:	Reporting of concentrations	Medium	The dose applied to the skin was reported as ug/cm2. The body weight of the animals was not reported, therefore a dose in mg/kg cannot be calculated. The study reported nominal instead of analytical doses. The volume applied (50 ul) and skin surface area (two dosing sites of 4 cm2 dorsal skin, 2 cm apart) were reported.
	Metric 9:	Exposure duration	High	Animals were exposed for 24 hours, which is consistent with OECD 427 guidelines.

Continued on next page ...

...continued from previous page

Study Citation: Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276. Chemical: Diethylhexyl Phthalate Exposure Type: Parent compound HERO ID: 673605 Unique ID: 14 days				
Domain		Metric	Rating	Comments
	Metric 10:	Number of exposure groups and concentration spacing	Medium	The study included 4 groups, each exposed to a different dose and sacrificed at a different time point (24 hrs, 48 hrs, 7 days and 14 days post-treatment). Each time point was evaluated independently. The justification for using higher doses for the longer post-treatment time points was to ensure radioactivity could be detected in the skin samples. It is unclear how relevant the doses selected are to human exposure scenarios.
Domain 4: Test Model				
	Metric 11:	Test animal characteristics	Medium	The study used female Hartley hairless guinea pigs sourced from Charles River, Wilmington, MA. Age and animal body weights were not reported. The species selection was appropriate for the study's aim.
	Metric 12:	Adequacy and consistency of animal husbandry conditions	Low	After application of the test substance animals were housed in separate glass metabolism cages and provided food and water ad libitum. No other husbandry conditions were reported (temperature, humidity, light cycle).
	Metric 13:	Number of animals per group	Medium	Four animals/timepoint were dosed and data were reported in the table for 4 animals/group. This agrees with OECD guidelines that require 4 animals per group.
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:		Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.		
Chemical:		Diethylhexyl Phthalate		
Exposure Type:		Parent compound		
HERO ID:		673605		
Unique ID:		14 days		
Domain	Metric	Rating	Comments	
	Metric 14: Outcome assessment methodology	Low	The outcome assessment methodology was sufficient to address the intentions of this non-guideline study but deviated from a traditional dermal absorption study as per OECD 427. The skin was washed with a 1% soap solution followed by water prior to application of test substance. OECD 427 guidelines state skin should be "gently wiped down with acetone to remove sebum. An additional soap and water wash is not recommended because any soap residue might promote test substance absorption." The test substance was applied to two application sites (each 4 cm ²) on each animal. The two sites were 2 cm apart from each other. This is a deviation from OECD guidelines which recommend the application area to be at least 10 cm ² . 50 uL of test solution was applied to each site. This would result in an application of 12.5 uL/cm ² , which is slightly higher than the recommended 10 uL/cm ² . The test substance was dissolved in acetone. After application, the study authors allowed the acetone to evaporate off and then covered the application site with a foam pad and gauze. Animals were placed in metabolism cages to collect urine and feces. Each cage was rinsed daily with water, which was combined with the urine collection. The duration of exposure (24 hours) was acceptable. The study does not report if any clinical signs were observed or if skin irritation occurred at the site of application, which is recommended in OECD guidelines. After 24 hours of exposure, the skin was washed 3 times with soap and water. Radioactivity was determined using a TR2500 liquid scintillation counter. The following were analyzed for amount of radioactivity: urine, feces, backwash, foam pad, carcass (minus the skin), and dosed skin (from one site). One application site was used for radioactivity measurements, and the other site was used to determine the distribution of the radiolabeled material using autoradiographic analysis. The percent absorbed was determined from a finite dose, which was appropriate. However, only measuring the amount of test substance in the skin of one application site and not the other will impact the percent calculated in the dosed skin and thus percent absorbed and percent recovered. The study did not measure the amount of test substance in the blood, rather the amount in the whole carcass was determined.	
	Metric 15: Consistency of outcome assessment	High	Outcomes were assessed consistently across study groups.	
	Metric 16: Sampling adequacy and sensitivity	Low	All four dosed animals were included in the analysis. The number of animals was consistent with OECD guidelines. The study authors reported that radioactivity was measured on a TR2500 liquid scintillation counter (Packard Instruments), however, the scintillation counts/sample or duration of radioactivity detection were not reported. The signal-to-noise ratio was not reported. It appears that the scintillation counts were taken immediately after material (urine, feces, foam pads, back washes) was collected, although this is not explicitly stated. The skin and carcass were frozen and analyzed at a later time point.	

Domain 6: Confounding/Variable Control

Continued on next page ...

...continued from previous page

Study Citation:	Chu, I., Dick, D., Bronaugh, R., Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology 34(3):267-276.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	673605			
Unique ID:	14 days			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Low	Animal body weights at the start of exposure or throughout the study were not reported. It is not reported if any weight changes, clinical signs or irritation at the application site were observed. OECD guidelines state the weight variation of animals should not exceed 20% of the mean weight. Since the weight and the ages of the animals were not reported, this cannot be determined. Regarding the use of acetone as a vehicle, the study does not provide any information on the absorption characteristics or potential interaction with the test substance, which OECD guidelines recommend when the vehicle is not water. The authors also noted that the volatility of the compounds may have confounded the dose absorbed, which was lower compared with other studies.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	Percent absorption estimates were based on the amount of radioactivity in the carcass and dosed skin at the time of sacrifice and the cumulative amount in urine, feces and cage washes (collected daily post-treatment). The CVs (calculated for this review) were 28% for the 24-hour timepoint and 28% for the 7-day timepoint. Sufficient information (mean, SD and sample size) was provided for EPA to calculate an alternate upper-end value to account for variability in the results. No statistical analysis was conducted.	
	Metric 20: Data interpretation	Uninformative	This study was not conducted according to OECD 427. The aim of the study was to see how the distribution of the test chemical changes over time post-treatment and to examine the distribution of the test substance in the skin using autoradiographic analysis. The study applied the test substance at two application sites. One site was collected to determine the amount of test substance that remained in the skin, the other site was used for autoradiographic analysis to determine the distribution of radioactivity. Since the amount of test substance that remained in the skin from the second application site was not taken into consideration for absorption measurements, the percent absorbed calculations are not accurate.	
	Metric 21: Reporting of Data	Medium	Data were reported for all of the outcomes specified in the methods. Urine and feces radioactivity was measured independently; however, the study reports them combined as excreta. Data were presented as means ± SD with an n=4. Individual animal data were not provided. CV values were not provided but could be for those endpoints.	
Overall Quality Determination		Uninformative		

Study Citation:	Elsisi, A. E., Carter, D. E., Sipes, I. G. (1989). Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology 12(1):70-77.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	675074			
Unique ID:	DEHP absorption in rat			
Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test substance identity	High	The test substance was clearly identified. Radiolabeled chemicals were synthesized by the study authors using 14C-radiolabeled phthalic acid (uniformly labeled on the ring).
	Metric 2:	Test substance source	High	The source of the test substance was reported. The lot/ batch number were not reported.
	Metric 3:	Test substance purity	High	The test substance was >96% pure.
Domain 2: Test Design				
	Metric 4:	Randomized allocation of animals	Low	The study did not report how animals were allocated into groups.
Continued on next page ...				

...continued from previous page

Study Citation:	Elsisi, A. E., Carter, D. E., Sipes, I. G. (1989). Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology 12(1):70-77.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	675074			
Unique ID:	DEHP absorption in rat			
Domain	Metric		Rating	Comments
	Metric 5:	Standards for Tests	Low	OECD 427 guidelines recommend clipping the skin approximately 24 hours prior to dosing. The area should then be gently wiped with acetone to remove sebum. The application area should be at least 10 cm ² for rats weighing 20-250 grams. This study did not adhere to these guidelines. The skin clipped one hour before compound application and was not wiped with acetone. The skin surface area used for application of test substance was 1.3 cm ² . These deficiencies are not considered critical deficiencies. Absorption could be enhanced if skin is recently abraded; however, study authors stated that "animals which had any visual signs of abrasions were eliminated from the study". Impact is expected to be negligible to slight overestimation of absorption. Actual application area is 13% of guideline recommended area of application. The application rate per surface area of 5-8 mg/cm ² likely represents an infinite (instead of finite) dose, which is also supported by the fact that 80% of DIDP remained unabsorbed at the end of 7-d exposure. Similar saturation of absorption would be expected over a larger surface area with the same loading rate. Impact is expected to be negligible. The study did not follow OECD 427 guidelines for determining amount of test substance that remained on the surface of the skin compared to the amount absorbed into the skin (stratum corneum). The test substance remained on the skin surface for 7 days. Feces and urine were collected and analyzed every 24 hours. At the end of the 7 days, the skin, at the application site, was collected and analyzed, however the study authors did not wash the remaining test solution off before analyzing the skin. This could slightly underestimate actual dermal absorption because the potentially absorbable dose (in stratum corneum) is excluded as unabsorbed. Given the fact that the exposure was 7 days, it is reasonable to conclude that the any amount in the skin at 7 days is negligible and/or not absorbable. Impact is expected to be negligible to slight underestimation of absorption. The study also did not collect blood samples at the time of sacrifice. The study also did not collect blood samples at the time of sacrifice. Recovery was within 10% of 100% (93-105%) for DBP, DEHP and DIBP. Recovery was 82% for DIDP and 86% for BBP. It is unlikely that the material unaccounted for was in any unanalyzed tissues (e.g., carcass), given that the %dose in the adipose tissue+muscle+skin accounted for 0.5-4.9% dose across the phthalates, and the "other tissues" were <0.5% and represented the sum of the % dose found in brain, lungs, liver, spleen, small intestine, kidneys, testes, spinal cord, and blood. It is possible the unaccounted test substance was lost to evaporation, given the fact that the study had a 7-day duration with partial occlusion.
Domain 3: Exposure Characterization	Metric 6:	Preparation and storage of test substance (chemical)	Medium	The test substance was dissolved in absolute alcohol (no other details are provided). It is unclear if the dissolved test substance was used immediately or may have been stored for days/weeks. The radioactivity in the dosing solution was measured after preparation and before application to the skin, therefore the lack of reporting storage conditions is not expected to substantially impact results.
Continued on next page ...				

...continued from previous page

Study Citation:	Elsisi, A. E., Carter, D. E., Sipes, I. G. (1989). Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology 12(1):70-77.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	675074			
Unique ID:	DEHP absorption in rat			
Domain		Metric	Rating	Comments
	Metric 7:	Consistency of exposure administration	Low	The skin surface used for application of test substance was consistent (1.3 cm diameter which is equivalent to an area of 1.69 cm ²). This is substantially smaller than the OECD recommended surface of 10 cm ² . The volume applied was not reported. Animals were exposed to a dose range of 5-8 mg/cm ² . Inconsistencies in exposure administration may have contributed to variation in the study results. The study also states the ethanol was allowed to evaporate before the skin was covered. It is not clear whether any evaporation of the test substance also occurred during this step.
	Metric 8:	Reporting of concentrations	Medium	The applied dose was reported in the abstract as 157 umol/kg. Later, the study indicated that the applied dose ranged from 30-40 mg/kg. The specific activity of the dosing solutions was determined before application to the skin using liquid scintillation counting.
	Metric 9:	Exposure duration	Low	The duration (7 days) was longer than OECD guidelines of 6-24 hours based on expected human exposure duration. The study did collect urine and feces daily to measure extracts.
	Metric 10:	Number of exposure groups and concentration spacing	Medium	Only one dose group was studied. The chosen concentration was justified as being approximately 0.01 times the reported oral or intraperitoneal LD50.
Domain 4: Test Model				
	Metric 11:	Test animal characteristics	Medium	Male Fisher 344 rats with weight ranging from 180-220 grams were used for this study. The age of the animals was not reported. The animals were obtained from the Division of Animal Resources of the University of Arizona Health Sciences Center.
	Metric 12:	Adequacy and consistency of animal husbandry conditions	Low	Husbandry conditions were not adequately reported. Temperature and humidity of the animal facility were not reported. Food and water were available ad lib and a 12-hour light/dark cycle was maintained.
	Metric 13:	Number of animals per group	Low	The number of animals per group was not specified in the study methods. Based on information in the data figures, three animals were tested. This is less than the OECD guideline recommendation of 4 animals.
Domain 5: Outcome Assessment				
Continued on next page ...				

...continued from previous page

Study Citation:	Elsisi, A. E., Carter, D. E., Sipes, I. G. (1989). Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology 12(1):70-77.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	675074			
Unique ID:	DEHP absorption in rat			
Domain	Metric		Rating	Comments
	Metric 14:	Outcome assessment methodology	Low	There were several deviations from OECD 427 guidelines. For finite dosing 1-5 mg/cm ² is recommended, this study reported an application rate of 5-8 mg/cm ² , which is at the upper end to slightly higher than recommendations, and may have approached an infinite exposure scenario. The study did not follow OECD 427 guidelines for determining amount of test substance that remained on the surface of the skin compared to the amount absorbed into the skin (stratum corneum); no skin washing or tape stripping was done and the test substance remained on the skin surface for 7 days. Since no penetration information was provided, it is unclear if the concentrations on the skin of the application site were considered to be absorbable. OECD 427 guidelines recommend clipping the skin approximately 24 hours prior to dosing. The area should then be gently wiped with acetone to remove sebum. In this study, the skin clipped one hour before compound application and was not wiped with acetone. These deficiencies are not considered critical deficiencies. Absorption could be enhanced if skin is recently abraded; however, study authors stated that "animals which had any visual signs of abrasions were eliminated from the study". Impact is expected to be negligible to slight overestimation of absorption. Concentrations in exhaled air were not measured. Urine and feces were collected every 24 hours over 7 days. At the end of the study duration, concentrations in adipose tissue, muscle, skin, application site, the plastic cap, and "other tissues" (brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord, and blood) were measured. Occluded conditions are recommended for finite exposures. In this study, the application sight was covered by a circular plastic cap that was perforated with needle holes to allow aeration."
	Metric 15:	Consistency of outcome assessment	High	Outcomes were assessed consistently across animals.
	Metric 16:	Sampling adequacy and sensitivity	Medium	Measurement sensitivity (signal:noise ratio) and the number of scintillation counts was not reported. The sampling interval (24 hours) was appropriate.
Domain 6: Confounding/Variable Control				
	Metric 17:	Confounding variables in test design and procedures	Medium	The study did not report all information to determine confounding, although minor differences are not expected to substantially impact results. Initial body weights were reported as a range (exact not reported). No gross changes in the appearance of the skin were seen.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 19:	Data analysis	Low	CV values were >25% in at least half of the samples for DEHP, BBP, and DIBP, and in 2/6 reported measurements for DBP and DIDP, and all chemicals had at least one CV value >50%. However, sufficient information is provided to conduct alternate calculations. Absorption estimates were presented across a time series (urine and feces). Statistical methods were described.
Continued on next page ...				

...continued from previous page

Study Citation:	Elsisi, A. E., Carter, D. E., Sipes, I. G. (1989). Dermal absorption of phthalate diesters in rats. Fundamental and Applied Toxicology 12(1):70-77.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	675074			
Unique ID:	DEHP absorption in rat			
Domain	Metric		Rating	Comments
	Metric 20:	Data interpretation	Low	There are major uncertainties regarding the interpretation of data. The test substance was not wiped off of the skin prior to collection and analysis of the skin sample. It cannot be determined how much of the test substance was on the surface of the skin (not absorbed) and how much was in the stratum corneum or deeper layers. The study does provide data on excreted amounts in urine and feces, amount of test substance in other organs, and amount of test substance on the cap used for occlusion.
	Metric 21:	Reporting of Data	Medium	Data for some outcomes specified were presented in figures as bar graphs with unspecified measures of variance, or no measures of variance (time-series excretion profiles). The percent recovery in various samples was quantitatively reported as means \pm SD. The sample size was only reported in 2 figures. The study did not report if skin at the application site appeared irritated. Blood measurements were not reported separately; however, it was lumped in with "other tissues" which accounted for <0.5% of the applied dose.
Overall Quality Determination			Medium	

Study Citation:	Melnick, R. L., Morrissey, R. E., Tomaszewski, K. E. (1987). Studies by the national toxicology program on di-2-ethylhexylphthalate. Toxicology and Industrial Health 3(2):99-118.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	682050			
Unique ID:	Dermal Absorption Study F344			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test substance identity	Medium	The test substance was identified by name as DEHP, and the form was characterized as radiolabeled DEHP, specifically 14C-DEHP. No CASRN was provided, and the specific structural location of the radiolabel on DEHP was not reported.	
Metric 2:	Test substance source	Low	Radiolabeled DEHP was prepared, presumably by the performing laboratory, from 14C-phthalic acid ”as described by Sipes et al., (1984). The source of the 14C-phthalic acid was not provided, and the authors do not report any analytical verification of test substance identity.	
Metric 3:	Test substance purity	Low	Test substance purity was not provided by the authors.	
Domain 2: Test Design				
Metric 4:	Randomized allocation of animals	Low	The authors did not report how animals were allocated into the study group.	
Metric 5:	Standards for Tests	Medium	Authors did not report on whether the test met pre-existing criteria and few or no QC criteria were reported. Enough information is reported to suggest that there were minor deviations from OECD criteria, particularly regarding the omission of tape stripping and washes.Percent recovery was reported and did meet the standard criteria of 100 ± 20%Coefficients of variation were not provided in the study report but could be determined using the data provided.	
Domain 3: Exposure Characterization				
Metric 6:	Preparation and storage of test substance (chemical)	Low	Test substance storage conditions were not reported. The physical-chemical properties of DEHP were not reported, but the test substance is not considered to be volatile. Solubility was not specified.	
Metric 7:	Consistency of exposure administration	Medium	Details of exposure administration were limited. There does not appear to be variation in the surface area for the exposure (application area had a diameter of 1.3 cm); however, the volume applied was not specified. It appears that body weights were adjusted for in dosing, so it is likely that animals all had the same administered dose.	
Metric 8:	Reporting of concentrations	Low	The test substance dose was reported as 30 mg/kg bw. The total mass (mg) and animal body weights were not reported, and the dose per surface area (mg/cm2) cannot be determined. The test substance was in an ethanol vehicle. Neither the percent in ethanol or the specific activity was specified. No analytical measurements were reported.	
Metric 9:	Exposure duration	High	The authors exposed animals for 120 hours (no skin washing was described) but took measurements every 24 hours during that exposure period. There was no discussion of whether the exposure duration was relevant to any conditions of use.	
Metric 10:	Number of exposure groups and concentration spacing	Low	The authors only tested one dose/concentration group and did not justify their decision to do so.	
Domain 4: Test Model				
Continued on next page ...				

Continued on next page ...

...continued from previous page

Study Citation:	Melnick, R. L., Morrissey, R. E., Tomaszewski, K. E. (1987). Studies by the national toxicology program on di-2-ethylhexylphthalate. Toxicology and Industrial Health 3(2):99-118.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	682050			
Unique ID:	Dermal Absorption Study F344			
Domain	Metric	Rating	Comments	
	Metric 11: Test animal characteristics	Low	Test animal species, strain, and sex were reported and are appropriate to measure end-points of interest. Starting body weights and age were not reported. The source of test animals was not reported.	
	Metric 12: Adequacy and consistency of animal husbandry conditions	Low	Animals were housed in metabolic cages. No details are reported regarding temperature, humidity, light/dark cycle or food/water availability were provided.	
	Metric 13: Number of animals per group	Low	The study only uses 3 animals per group, which fails to meet the minimum guideline requirement of 4 animals per group.	
Domain 5: Outcome Assessment				
	Metric 14: Outcome assessment methodology	Medium	Kp/flux determinations are not presented. The area of skin used in the dermal exposure (diameter of 1.3 cm; e.g., 2.6 cm2) was less than what is recommended by OECD guidelines. Details of outcome methodology were partially reported. Male F344 rats (n=3) had hair clipped from the middle of the dorsal surface prior to the application of the test substance. 30 mg/kg of the test substance dissolved in ethanol vehicle (% not reported) was applied to the skin (diameter of 1.3 cm) via a pipette The volume of the test substance applied was not reported. After ethanol evaporated (time not specified) the area was covered with a non-occluded perforated plastic cap glued onto the skin via cyanoacrylate. Animals were placed in metabolic cages to collect urine and feces every 24 hours for 120 hours post dosing. At the end of the 120-hour exposure duration, animals were sacrificed, and the test substance was measured in harvested brain, lung, liver, spleen, small intestine, kidney, testis, fat, muscle, skin, spinal cord and blood. Absorption of the test substance was also measured in the skin area of the application site and the plastic cap. Tape stripping was not performed, and no washes were described other than a urine wash of the cage which seems to be incorporated in the results for recovery in urine. Scintillation counting was used to measure radioactivity, but no additional details were provided. The outcome of interest was to determine percent absorption. The authors did not explicitly state whether the intention was to conduct a finite or infinite exposure scenario. Based on the available information and outcomes, this appears to be a finite exposure. The study did not report signs of toxicity per guidelines or report irritation of skin.	
	Metric 15: Consistency of outcome assessment	High	The outcomes appeared to be measured consistently across replicates (i.e., collection times were specified).	
	Metric 16: Sampling adequacy and sensitivity	Low	Details regarding sampling were not fully reported. The authors only specify that radioactivity in urine and urine wash were counted directly in the scintillation counter, whereas feces were oxidized and digested first, but the number of samples analyzed per animal, details of sample handling prior to measurements, number of scintillation counts etc., were not provided.	
Domain 6: Confounding/Variable Control				
Continued on next page ...				

...continued from previous page

Study Citation:	Melnick, R. L., Morrissey, R. E., Tomaszewski, K. E. (1987). Studies by the national toxicology program on di-2-ethylhexylphthalate. Toxicology and Industrial Health 3(2):99-118.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	682050			
Unique ID:	Dermal Absorption Study F344			
Domain	Metric	Rating	Comments	
	Metric 17: Confounding variables in test design and procedures	Medium	The authors did not provide enough information to determine if confounding occurred. Body weights were not provided, and the authors didn't mention if animals with existing abrasions were removed from the study. There is likely no difference in reference number of the test substance between animals as only one reference number was reported.	
	Metric 18: Confounding variables in outcomes unrelated to exposure	Medium	No attrition or issues with test substance solubility were reported. Solubility in ethanol may have been a minor concern, but the authors did not report whether or not any issues with solubility were observed.	
Domain 7: Data Presentation and Analysis				
	Metric 19: Data analysis	Low	Methods for statistical analysis was not reported, but the reported data is sufficient to conduct an independent statistical analysis. Authors report mean % recovery for each endpoint with SD, and individual animal data is not reported. More than half of the CV values are below 50%, but only a quarter of the CV values are below 25%. Sufficient data are available to conduct an independent statistical analysis.	
	Metric 20: Data interpretation	Medium	Total recovery % is within 10% of 100% and includes recovery of all terminal endpoints. No tape stripping was performed. The authors did not fully explain if washes occurred; they mentioned that a urine wash was performed, but there is no separate endpoint for recovery in urine wash, so that data may have been combined with the recovery in urine. Recovery from expired air was also not measured and is not reflected in the total recovery %.	
	Metric 21: Reporting of Data	Medium	All data for measured endpoints was reported, including data across timepoints for urine and feces (whereas all other endpoints were only measured terminally). Individual animal data is not reported. Total recovery is reported as mean +/- SD with % recovery calculated by the authors.	
Overall Quality Determination		Low		

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Cumulative excreta, skin wash, skin stripping			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test substance identity	Medium	Test substances were identified as radiolabeled [carbonyl-14C]di(2-ethylhexyl) phthalate (DEHP) and unlabeled DEHP. The chemical structure was reported, however the location of the radiolabel within the structure was not denoted.
	Metric 2:	Test substance source	High	The non-radiolabeled DEHP was sourced from Aldrich Chemical Co. (Milwaukee, WI). The radiolabeled DEHP was sourced from Sigma Chemical Co. (St. Louis, MO). The study did not include certificates of analyses. Lot numbers were not provided
	Metric 3:	Test substance purity	Medium	The radiochemical purity of the radiolabeled test substance was reported by the supplying company to be >98%; this was confirmed by TLC and HPLC by the study authors (data not shown). The purity of the non-radiolabeled substance was reported to be >99%. Impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Randomized allocation of animals	Low	The method of animal allocation was not specified, and there is no indication normalization to body weight occurred.
	Metric 5:	Standards for Tests	Medium	The percent recovery was acceptable (95.2%) which is within OECD TG 427 recommendations or 100 +/-10%. Coefficients of variation were not reported but could be determined using the data provided.
Domain 3: Exposure Characterization				
	Metric 6:	Preparation and storage of test substance (chemical)	Low	Preparation of the test substance was not adequately reported. The study authors report radioactive DEHP was applied in 50ul acetone solution to the skin at 53ug or 34 nmol/cm2. No details on the volume of radiolabeled material used for the dilution are provided. Homogeneity or method used for mixing solutions was not reported. It is unclear how far in advance the dilutions were made. Storage conditions of stock test substances were not reported.
	Metric 7:	Consistency of exposure administration	Low	A constant volume (50 ul) was applied to the upper dorsal area (4 cm2). The area was covered with a nonocclusive pad for 24 hours. The weight of the animals was not reported, and it is not reported if there were large weight variations between animals. OECD 427 specifies that the weight variation of animals should not exceed ± 20% of the mean weight. The age of the animals was reported to be 20-30 weeks. Large variations in weight would impact the consistency of the applied dose, not enough information is provided to determine this.
	Metric 8:	Reporting of concentrations	Medium	Animals were exposed to 53 ug of DEHP (34 nmol/cm2) in acetone. Only the nominal dose is reported.
	Metric 9:	Exposure duration	High	Animals were exposed for 24 hours, prior to skin washing, which is consistent with OECD 427 guidelines. Urine and feces were collected for 7-days post-treatment.
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.			
Chemical:	Diethylhexyl Phthalate			
Exposure Type:	Parent compound			
HERO ID:	67185			
Unique ID:	Cumulative excreta, skin wash, skin stripping			
Domain	Metric	Rating	Comments	
	Metric 10:	Number of exposure groups and concentration spacing	Low	Only one dose was studied. No justification was provided for why this dose was chosen; it was, however, the same dose used in an ex vivo experiment in the same publication.
Domain 4: Test Model	Metric 11:	Test animal characteristics	Medium	The study used female hairless guinea pigs [CrI:LAF/HA(hr/hr)BR] obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were 20-30 week of age. Body weights were not reported; the variation between groups is not clear. Rats are a more common species, but the authors cited other studies that also used the hairless guinea pig.
	Metric 12:	Adequacy and consistency of animal husbandry conditions	Low	After exposure animals were housed individually in glass metabolism cages with free access to food and water. No other housing information is provided (e.g. temperature, humidity, light cycle).
	Metric 13:	Number of animals per group	Medium	The number of animals per group was reported to be 5 in the methods, but the Table 1 legend reports data were from 5 or more animals/group. OECD guidelines only require 4 animals per group, so overall, the number was more than required.
Domain 5: Outcome Assessment	Metric 14:	Outcome assessment methodology	Medium	The outcome assessment methodology was partially sufficient to address the intentions. A finite dose of 53 ug (34 nmol/cm2) was used to determine absorption. The volume administered was 50 ul onto a 4 cm2 area on the upper dorsal region. This volume (12.5 ul/cm2) is slightly greater than the recommended 10 ul/cm2. The study collected and analyzed urine and faeces separately, Skin was tape stripped 10 times (seven days post-administration of test substance). Animals were not sacrificed and therefore blood was not collected. This is a deviation from OECD guidelines. The amount of test chemical in skin wash after the 24-hour exposure was analyzed and reported. The study combined all of the tape strips when reporting data. Exhaled air and cage washes were not collected. Acetone was used as the vehicle. It is unclear how the use of this volatile vehicle influenced absorption.
	Metric 15:	Consistency of outcome assessment	High	Outcomes were assessed consistently across animals.
	Metric 16:	Sampling adequacy and sensitivity	Medium	The sampling size was appropriate for the endpoint of interest. Radioactivity was measured with a Tri-Carb liquid scintillation counter. Sensitivity, scintillation counts/sample or signal to noise ratio were not reported.
Domain 6: Confounding/Variable Control	Metric 17:	Confounding variables in test design and procedures	Medium	Animal body weights at the start of exposure were not reported, so it is not known if these ranges fall within 20% of the mean. The application area (4 cm2) and volume applied (50 ul) were consistent.
	Metric 18:	Confounding variables in outcomes unrelated to exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition, health outcomes unrelated to exposure, or solubility that could influence the outcome assessment
Continued on next page ...				

...continued from previous page

Study Citation:	Ng, K. M. E., Chu, I., Bronaugh, R. L., Franklin (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: Comparison of in vitro and in vivo results in the hairless guinea pig. Toxicology and Applied Pharmacology 115(2):216-223.		
Chemical:	Diethylhexyl Phthalate		
Exposure Type:	Parent compound		
HERO ID:	67185		
Unique ID:	Cumulative excreta, skin wash, skin stripping		
Domain	Metric	Rating	Comments
Domain 7: Data Presentation and Analysis			
	Metric 19: Data analysis	Low	The study authors corrected for incomplete excretion by determining excretion of test substance in another group of animals following intramuscular injection. They used the following equation to correct for dermal absorption: (excretion after topical application/ excretion after intramuscular injection) X 100%. Cumulative excretion prior to correction (dermal route) was approximately 21%, following the correction it was reported to be 53.1%. OECD guidelines do not discuss these types of correction calculations and validity is uncertain. Calculated CV for corrected cumulative excreta was 26% and skin stripping was 18%.
	Metric 20: Data interpretation	Medium	Recovery of applied the dose was adequate (95.2%). The skin was tape-stripped 10 times seven days post-administration of the test substance. All tape strips were included in analysis. Blood levels were not measured. Absorption was properly calculated.
	Metric 21: Reporting of Data	Medium	Data were reported for all of the outcomes specified in the methods. Urine and feces were analyzed separately, however these data were combined and reported as "Cumulative excreta". Data were presented as means \pm SD. The number of samples was reported as five or more animals. Individual animal data were not provided. CV values were not reported but could be calculated by reported data.
Overall Quality Determination		Medium	